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512_3

ORG 512 Anti-Scl-70

INTENDED PURPOSE

Anti-Scl-70 is an ELISA test system for the quantitative measurement of IgG class autoantibodies against Scl-70 in human serum or plasma. This product is intended for professional in vitro diagnostic use only.

Antibodies against Scl-70 (DNA topoisomerase I) are an accepted marker for progressive systemic scleroderma. They contribute to the differential diagnosis of scleroderma. Evaluation of a test result should always take into account all clinical and laboratory diagnostic findings.

SYMBOLS USED ON LABELS

IVD	In vitro diagnostic medical device
	Manufacturer
REF	Catalogue number
Σ 96	Sufficient for 96 determinations
LOT	Batch code
	Use by
2°C 8°C	Temperature limitation
	Keep away from sunlight
	Do not reuse
	Date of manufacture
CE	CE marked according to 98/79/EC
	Consult instructions for use
512_3	Electronic Instruction For Use: version

MICROPLATE	Microplate
CALIBRATOR A	Calibrator
CALIBRATOR B	Calibrator
CALIBRATOR C	Calibrator
CALIBRATOR D	Calibrator
CALIBRATOR E	Calibrator
CALIBRATOR F	Calibrator
CONTROL +	Control positive
CONTROL −	Control negative
DILUENT	Sample Buffer P
CONJUGATE	Enzyme Conjugate
TMB	TMB Substrate
STOP	Stop solution
WASH	Wash Buffer
RTU	Ready to use

PRINCIPLE OF THE TEST

Highly purified Scl-70 is bound to microwells.
The determination is based on an indirect enzyme linked immune reaction with the following steps:
Specific antibodies in the patient sample bind to the antigen coated on the surface of the reaction wells. After incubation, a washing step removes unbound and unspecifically bound serum or plasma components. Subesquently added enzyme conjugate binds to the immobilized antibody-antigen-complexes. After incubation, a second washing step removes unbound enzyme conjugate. After addition of substrate solution the bound enzyme conjugate hydrolyses the substrate forming a blue coloured product. Addition of an acid stopps the reaction generating a yellow end-product. The intensity of the yellow color
correlates with the concentration of the antibody-antigen-complex and can be measured photometrically at 450 nm.

WARNINGS AND PRECAUTIONS

- All reagents of this kit are intended for professional in vitro diagnostic use only.
- Components containing human serum were tested and found negative for HBsAg, HCV, HIV1 and HIV2 by FDA approved methods. No test can guarantee the absence of HBsAg, HCV, HIV1 or HIV2, and so all human serum based reagents in this kit must be handled as though capable of transmitting infection.
- Bovine serum albumin (BSA) used in components has been tested for BSE and found negative.
- Avoid contact with the substrate TMB (3,3´,5,5´-Tetramethyl-benzidine).
- Stop solution contains acid, classifiaction is non-hazardous. Avoid contact with skin.
- Control, sample buffer and wash buffer contain sodium azide 0.09% as preservative. This concentration is classified as non-hazardous.
- Enzyme conjugate contains ProClin 300 0.05% as preservative. This concentration is classified as non-hazardous.

During handling of all reagents, controls and serum samples observe the existing regulations for laboratory safety regulations and good laboratory practice:

- First aid measures: In case of skin contact, immediately wash thoroughly with water and soap. Remove contaminated clothing and shoes and wash before reuse. If system fluid comes into contact with skin, wash thoroughly with water. After contact with the eyes carefully rinse the opened eye with running water for at least 10 minutes. Get medical attention if necessary.
- Personal precautions, protective equipment and emergency procedures:

Observe laboratory safety regulations. Avoid contact with skin and eyes. Do not swallow. Do not pipette by mouth. Do not eat, drink, smoke or apply makeup in areas where specimens or kit reagents are handled. When spilled, absorb with an inert material and put the spilled material in an appropriate waste disposal.

- Exposure controls / personal protection: Wear protective gloves of nitril rubber or natural latex. Wear protective glasses. Used according to intended use no dangerous reactions known.
- Conditions to avoid: Since substrate solution is light-sensitive. Store in the dark.
- For disposal of laboratory waste the national or regional legislation has to be observed.

Observe the guidelines for performing quality control in medical laboratories by assaying control sera.

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Location: Moldova

CONTENTS OF THE KIT

ORG 512	Σ 96	Sufficient for 96 determinations
MICROPLATE	1	One divisible microplate consisting of 12 modules of 8 wells each. Ready to use. Color code on module
CALIBRATOR A	1x 1.5 ml	Calibrator A 0 U/ml, containing serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use.
CALIBRATOR B	1x 1.5 ml	Calibrator B 12.5 U/ml, containing Scl-70 antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use.
CALIBRATOR C	1x 1.5 ml	Calibrator C 25 U/ml, containing Scl-70 antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use.
CALIBRATOR D	1x 1.5 ml	Calibrator D 50 U/ml, containing Scl-70 antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use.
CALIBRATOR E	1x 1.5 ml	Calibrator E 100 U/ml, containing Scl-70 antibodies in a serum/buffer matrix (PBS, BSA, NaN3 0.09%), yellow. Ready to use.
CALIBRATOR F	1x 1.5 ml	Calibrator F 200 U/ml, containing Scl-70 antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use.
CONTROL +	1x 1.5 ml	Control positive, containing Scl-70 antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use. The concentration is specified on the certificate of analysis.
CONTROL −	1x 1.5 ml	Control negative, containing Scl-70 antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use. The concentration is specified on the certificate of analysis.
DILUENT	20 ml	Sample Buffer P, containing PBS, BSA, detergent, preservative sodium azide 0.09%, yellow, concentrate (5 x).
CONJUGATE	15 ml	Enzyme Conjugate containing anti-human IgG antibodies, HRP labelled; PBS, BSA, detergent, preservative PROCLIN 0.05%, light red. Ready to use.
TMB	15 ml	TMB Substrate; containing 3,3', 5,5'- Tetramethylbenzidin, colorless. Ready to use.
STOP	15 ml	Stop solution; contains acid. Ready to use.
WASH	20 ml	Wash Buffer, containing Tris, detergent, preservative sodium azide 0.09%; 50 x conc.
	1	Certificate of Analysis

MATERIALS REQUIRED

- Microplate reader capable of endpoint measurements at 450 nm; optional: reference filter at 620 nm
- Data reduction software
- Multi-channel dispenser or repeatable pipette for 100 µl
- Vortex mixer
- Pipettes for 10 µl, 100 µl and 1000 µl
- Laboratory timing device
- Distilled or deionised water
- Measuring cylinder for 1000 ml and 100 ml
- Plastic container for storage of the wash solution

This ELISA assay is suitable for use on open automated ELISA processors. Each assay has to be validated on the respective automated system. Detailed information is provided upon request.

SPECIMEN COLLECTION, STORAGE AND HANDLING

- Collect whole blood specimens using acceptable medical techniques to avoid hemolysis.
- Allow blood to clot and separate the serum or plasma by centrifugation.
- Test serum should be clear and non-hemolyzed. Contamination by hemolysis or lipemia should be avoided, but does not interfere with this assay.
- Specimens may be refrigerated at 2-8°C for up to five days or stored at -20°C up to six months.
- Avoid repetitive freezing and thawing of serum or plasma samples. This may result in variable loss of antibody activity.
- Testing of heat-inactivated sera is not recommended.

STORAGE AND STABILITY

- Store test kit at 2-8°C in the dark.
- Do not expose reagents to heat, sun, or strong light during storage and usage.
- Store microplate sealed and dessicated in the clip bag provided.
- Shelf life of the unopended test kit is 18 months from day of production. Unopened reagents are stable until expiration of the kit. See labels for individual batch.
- Diluted Wash Buffer and Sample Buffer are stable for at least 30 days when stored at 2-8°C. We recommend consumption on the same day.

PROCEDURAL NOTES

- Do not use kit components beyond their expiration dates.
- Do not interchange kit components from different lots and products.
- All materials must be at room temperature (20-28°C) prior to use.
- Prepare all reagents and samples. Once started, performe the test without interruption.
- Double determinations may be done. By this means pipetting errors may become obvious.
- Perform the assay steps only in the order indicated.
- Always use fresh sample dilutions.
- Pipette all reagents and samples into the bottom of the wells.
- To avoid carryover or contamination, change the pipette tip between samples and different kit controls.
- Wash microwells thoroughly and remove the last droplets of wash buffer.
- All incubation steps must be accurately timed.
- Do not re-use microplate wells.

PREPARATION OF REAGENTS

WASH

Dilute the contents of one vial of the buffered wash solution concentrate (50x) with distilled or deionised water to a final volume of 1000 ml prior to use.

DILUENT

Sample Buffer P: Prior to use dilute the contents (20 ml) of one vial of sample buffer 5x concentrate with distilled or deionised water to a final volume of 100 ml.

Preparation of samples

Dilute patient samples 1:100 before the assay: Put 990 µl of prediluted sample buffer in a polystyrene tube and add 10 µl of sample. Mix well. Note: Calibrators / Controls are ready to use and need not be diluted.

TEST PROCEDURE

Prepare enough microplate modules for all calibrators / controls and patient samples.

1. Pipette **100 µl** of calibrators, controls and prediluted patient samples into the wells.
   Incubate for **30 minutes** at room temperature (20-28 °C).
   Discard the contents of the microwells and **wash 3 times** with **300 µl** of wash solution.
2. Dispense **100 µ**l of enzyme conjugate into each well.
   Incubate for **15 minutes** at room temperature.
   Discard the contents of the microwells and **wash 3 times** with **300 µl** of wash solution.
3. Dispense **100 µ**l of TMB substrate solution into each well.
   Incubate for **15 minutes** at room temperature
4. **Add 100 µl** of stop solution to each well of the modules
   Incubate for **5 minutes** at room temperature.
   Read the optical density at 450 nm (reference 600-690nm) and calculate the results.
   The developed colour is stable for at least 30 minutes. Read during this time.

Example for a pipetting scheme:

	1	2	3	4	5	6	7	8	9	10	11	12
A	A	P1										
B	B	P2										
C	C	P3										
D	D											
E	E											
F	F											
G	C+											
H	C-											

P1, ... patient sample A-F calibrators C+, C- controls

VALIDATION

Test results are valid if the optical densities at 450 nm for calibrators / controls and the results for controls comply with the reference ranges indicated on the Certificate of Analysis enclosed in each test kit.
If these quality control criteria are not met the assay run is invalid and should be repeated.

CALCULATION OF RESULTS

For quantitative results plot the optical density of each calibrator versus the calibrator concentration to create a calibration curve. The concentration of patient samples may then be estimated from the calibration curve by interpolation.
Using data reduction software a 4-Parameter-Fit with lin-log coordinates for optical density and concentration is the data reduction method of choice.

PERFORMANCE CHARACTERISTICS

Calibration

The assay system is calibrated against the internationally recognized reference sera from CDC, Atlanta USA.

Measuring range

The calculation range of this ELISA assay is 0 - 200 U/ml

Expected values

In a normal range study with samples from healthy blood donors the following ranges have been established with this ELISA assay: Cut-off 25 U/ml

Interpretation of results

Negative:	< 15 U/ml
Borderline:	15 - 25 U/ml
Positive:	> 25 U/ml

Linearity

Patient samples containing high levels of specific antibody were serially diluted in sample buffer to demonstrate the dynamic range of the assay and the upper / lower end of linearity. Activity for each dilution was calculated from the calibration curve using a 4-Parameter-Fit with lin-log coordinates.

Sample	Dilution	Observed U/ml	Expected U/ml	O/E [%]
1	1:100	146.9	146.9	100
	1:200	76.3	73.5	104
	1:400	38.1	36.7	104
	1:800	18.8	18.4	102
2	1:100	122.3	122.3	100
	1:200	60.4	61.2	99
	1:400	29.6	30.6	97
	1:800	14.8	15.3	97

Limit of detection

Functional sensitivity was determined to be: 1 U/ml

Reproducibility

Intra-assay precision: Coefficient of variation (CV) was calculated for each of three samples from the results of 24 determinations in a single run. Results for precision-within-assay are shown in the table below.
Inter-assay precision: Coefficient of variation (CV) was calculated for each of three samples from the results of 6 determinations in 5 different runs. Results for run-to-run precision are shown in the table below.

Intra-Assay		
Sample	Mean U/ml	CV %
1	45.7	4.0
2	90.4	3.2
3	184.1	3.4

Inter-Assay		
Sample	Mean U/ml	CV %
1	41.1	2.8
2	89.9	2.8
3	157.4	2.3

Interfering substances

No interference has been observed with haemolytic (up to 1000 mg/dl) or lipemic (up to 3 g/dl triglycerides) sera or plasma, or bilirubin (up to 40 mg/dl) containing sera or plasma. Nor have any interfering effects been observed with the use of anticoagulants (Citrate, EDTA, Heparine). However for practical reasons it is recommended that grossly hemolyzed or lipemic samples should be avoided.

Study results

Study population	n	n Pos	%
Scleroderma	25	19	76.0
Rheumatoid arthritis	20	0	0.0
Normal human sera	80	1	1.3

		Clinical Diagnosis POS	NEG	
ORG 512	POS	19	1	
	NEG	6	99	
		25	100	125

Sensitivity: 76.0 %
Specificity: 99.0 %
Overall agreement: 94.4 %

LIMITATIONS OF THE PROCEDURE

This assay is a diagnostic aid. A definite clinical diagnosis should not be based on the results of a single test, but

should be made by the physician after all clinical and laboratory findings have been evaluated concerning the entire clinical picture of the patient. Also every decision for therapy should be taken individually.
The above pathological and normal reference ranges for antibodies in patient samples should be regarded as recommendations only. Each laboratory should establishe its own ranges according to ISO 15189 or other applicable laboratory guidelines.

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Notice to the user (European Union):
Any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the EU Member State in which the user and/or the patient is established .

Change Control
Former version: *ORG 512_IFU_EN_QM113139_2013-12-16_1.2* Reason for revision: *Introduction electronic IFU on homepage*

1. Pipet **100 µl** calibrator, control or patient sample
   → Incubate for **30 minutes** at room temperature
   → Discard the contents of the wells and wash 3 times with **300 µl** wash solution
2. Pipet **100 µl** enzyme conjugate
   → Incubate for **15 minutes** at room temperature
   → Discard the contents of the wells and wash 3 times with **300 µl** wash solution
3. Pipet **100 µl** substrate solution
   → Incubate for **15 minutes** at room temperature
4. Add **100 µl** stop solution
   → Leave untouched for **5 minutes**
   → Read at **450 nm**

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509_3

ORG 509 Anti-SS-B

INTENDED PURPOSE

Anti-SS-B is an ELISA test system for the quantitative measurement of IgG class autoantibodies against SS-B (La) in human serum or plasma. This product is intended for professional in vitro diagnostic use only.

Antibodies against SS-B are used for the differential diagnosis of systemic inflammatory autoimmune diseases. Autoantibodies against the SS-B protein are usually found together with anti-SS-A in cases of Sjoegren's syndrome. Evaluation of a test result should always take into account all clinical and laboratory diagnostic findings.

SYMBOLS USED ON LABELS

IVD	In vitro diagnostic medical device
	Manufacturer
REF	Catalogue number
Σ 96	Sufficient for 96 determinations
LOT	Batch code
	Use by
2°C 8°C	Temperature limitation
	Keep away from sunlight
	Do not reuse
	Date of manufacture
CE	CE marked according to 98/79/EC
	Consult instructions for use
509_3	Electronic Instruction For Use: version

MICROPLATE	Microplate
CALIBRATOR A	Calibrator
CALIBRATOR B	Calibrator
CALIBRATOR C	Calibrator
CALIBRATOR D	Calibrator
CALIBRATOR E	Calibrator
CALIBRATOR F	Calibrator
CONTROL +	Control positive
CONTROL −	Control negative
DILUENT	Sample Buffer P
CONJUGATE	Enzyme Conjugate
TMB	TMB Substrate
STOP	Stop solution
WASH	Wash Buffer
RTU	Ready to use

PRINCIPLE OF THE TEST

Highly purified SS-B is bound to microwells.
The determination is based on an indirect enzyme linked immune reaction with the following steps:
Specific antibodies in the patient sample bind to the antigen coated on the surface of the reaction wells. After incubation, a washing step removes unbound and unspecifically bound serum or plasma components. Subesquently added enzyme conjugate binds to the immobilized antibody-antigen-complexes. After incubation, a second washing step removes unbound enzyme conjugate. After addition of substrate solution the bound enzyme conjugate hydrolyses the substrate forming a blue coloured product. Addition of an acid stopps the reaction generating a yellow end-product. The intensity of the yellow color
correlates with the concentration of the antibody-antigen-complex and can be measured photometrically at 450 nm.

WARNINGS AND PRECAUTIONS

- All reagents of this kit are intended for professional in vitro diagnostic use only.
- Components containing human serum were tested and found negative for HBsAg, HCV, HIV1 and HIV2 by FDA approved methods. No test can guarantee the absence of HBsAg, HCV, HIV1 or HIV2, and so all human serum based reagents in this kit must be handled as though capable of transmitting infection.
- Bovine serum albumin (BSA) used in components has been tested for BSE and found negative.
- Avoid contact with the substrate TMB (3,3´,5,5´-Tetramethyl-benzidine).
- Stop solution contains acid, classifiaction is non-hazardous. Avoid contact with skin.
- Control, sample buffer and wash buffer contain sodium azide 0.09% as preservative. This concentration is classified as non-hazardous.
- Enzyme conjugate contains ProClin 300 0.05% as preservative. This concentration is classified as non-hazardous.

During handling of all reagents, controls and serum samples observe the existing regulations for laboratory safety regulations and good laboratory practice:

- First aid measures: In case of skin contact, immediately wash thoroughly with water and soap. Remove contaminated clothing and shoes and wash before reuse. If system fluid comes into contact with skin, wash thoroughly with water. After contact with the eyes carefully rinse the opened eye with running water for at least 10 minutes. Get medical attention if necessary.
- Personal precautions, protective equipment and emergency procedures:

Observe laboratory safety regulations. Avoid contact with skin and eyes. Do not swallow. Do not pipette by mouth. Do not eat, drink, smoke or apply makeup in areas where specimens or kit reagents are handled. When spilled, absorb with an inert material and put the spilled material in an appropriate waste disposal.

- Exposure controls / personal protection: Wear protective gloves of nitril rubber or natural latex. Wear protective glasses. Used according to intended use no dangerous reactions known.
- Conditions to avoid: Since substrate solution is light-sensitive. Store in the dark.
- For disposal of laboratory waste the national or regional legislation has to be observed.

Observe the guidelines for performing quality control in medical laboratories by assaying control sera.

CONTENTS OF THE KIT

ORG 509	Σ 96	Sufficient for 96 determinations
MICROPLATE	1	One divisible microplate consisting of 12 modules of 8 wells each. Ready to use. Color code on module
CALIBRATOR A	1x 1.5 ml	Calibrator A 0 U/ml, containing serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use.
CALIBRATOR B	1x 1.5 ml	Calibrator B 12.5 U/ml, containing SS-B antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use.
CALIBRATOR C	1x 1.5 ml	Calibrator C 25 U/ml, containing SS-B antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use.
CALIBRATOR D	1x 1.5 ml	Calibrator D 50 U/ml, containing SS-B antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use.
CALIBRATOR E	1x 1.5 ml	Calibrator E 100 U/ml, containing SS-B antibodies in a serum/buffer matrix (PBS, BSA, NaN3 0.09%), yellow. Ready to use.
CALIBRATOR F	1x 1.5 ml	Calibrator F 200 U/ml, containing SS-B antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use.
CONTROL +	1x 1.5 ml	Control positive, containing SS-B antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use. The concentration is specified on the certificate of analysis.
CONTROL −	1x 1.5 ml	Control negative, containing SS-B antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use. The concentration is specified on the certificate of analysis.
DILUENT	20 ml	Sample Buffer P, containing PBS, BSA, detergent, preservative sodium azide 0.09%, yellow, concentrate (5 x).
CONJUGATE	15 ml	Enzyme Conjugate containing anti-human IgG antibodies, HRP labelled; PBS, BSA, detergent, preservative PROCLIN 0.05%, light red. Ready to use.
TMB	15 ml	TMB Substrate; containing 3,3', 5,5'- Tetramethylbenzidin, colorless. Ready to use.
STOP	15 ml	Stop solution; contains acid. Ready to use.
WASH	20 ml	Wash Buffer, containing Tris, detergent, preservative sodium azide 0.09%; 50 x conc.
	1	Certificate of Analysis

MATERIALS REQUIRED

- Microplate reader capable of endpoint measurements at 450 nm; optional: reference filter at 620 nm
- Data reduction software
- Multi-channel dispenser or repeatable pipette for 100 µl
- Vortex mixer
- Pipettes for 10 µl, 100 µl and 1000 µl
- Laboratory timing device
- Distilled or deionised water
- Measuring cylinder for 1000 ml and 100 ml
- Plastic container for storage of the wash solution

This ELISA assay is suitable for use on open automated ELISA processors. Each assay has to be validated on the respective automated system. Detailed information is provided upon request.

SPECIMEN COLLECTION, STORAGE AND HANDLING

- Collect whole blood specimens using acceptable medical techniques to avoid hemolysis.
- Allow blood to clot and separate the serum or plasma by centrifugation.
- Test serum should be clear and non-hemolyzed. Contamination by hemolysis or lipemia should be avoided, but does not interfere with this assay.
- Specimens may be refrigerated at 2-8°C for up to five days or stored at -20°C up to six months.
- Avoid repetitive freezing and thawing of serum or plasma samples. This may result in variable loss of antibody activity.
- Testing of heat-inactivated sera is not recommended.

STORAGE AND STABILITY

- Store test kit at 2-8°C in the dark.
- Do not expose reagents to heat, sun, or strong light during storage and usage.
- Store microplate sealed and dessicated in the clip bag provided.
- Shelf life of the unopended test kit is 18 months from day of production. Unopened reagents are stable until expiration of the kit. See labels for individual batch.
- Diluted Wash Buffer and Sample Buffer are stable for at least 30 days when stored at 2-8°C. We recommend consumption on the same day.

PROCEDURAL NOTES

- Do not use kit components beyond their expiration dates.
- Do not interchange kit components from different lots and products.
- All materials must be at room temperature (20-28°C) prior to use.
- Prepare all reagents and samples. Once started, performe the test without interruption.
- Double determinations may be done. By this means pipetting errors may become obvious.
- Perform the assay steps only in the order indicated.
- Always use fresh sample dilutions.
- Pipette all reagents and samples into the bottom of the wells.
- To avoid carryover or contamination, change the pipette tip between samples and different kit controls.
- Wash microwells thoroughly and remove the last droplets of wash buffer.
- All incubation steps must be accurately timed.
- Do not re-use microplate wells.

PREPARATION OF REAGENTS

WASH

Dilute the contents of one vial of the buffered wash solution concentrate (50x) with distilled or deionised water to a final volume of 1000 ml prior to use.

DILUENT

Sample Buffer P: Prior to use dilute the contents (20 ml) of one vial of sample buffer 5x concentrate with distilled or deionised water to a final volume of 100 ml.

Preparation of samples

Dilute patient samples 1:100 before the assay: Put 990 µl of prediluted sample buffer in a polystyrene tube and add 10 µl of sample. Mix well. Note: Calibrators / Controls are ready to use and need not be diluted.

TEST PROCEDURE

Prepare enough microplate modules for all calibrators / controls and patient samples.

1. Pipette **100 µl** of calibrators, controls and prediluted patient samples into the wells.
   Incubate for **30 minutes** at room temperature (20-28 °C).
   Discard the contents of the microwells and **wash 3 times** with **300 µl** of wash solution.
2. Dispense **100 µ**l of enzyme conjugate into each well.
   Incubate for **15 minutes** at room temperature.
   Discard the contents of the microwells and **wash 3 times** with **300 µl** of wash solution.
3. Dispense **100 µ**l of TMB substrate solution into each well.
   Incubate for **15 minutes** at room temperature
4. **Add 100 µl** of stop solution to each well of the modules
   Incubate for **5 minutes** at room temperature.
   Read the optical density at 450 nm (reference 600-690nm) and calculate the results.
   The developed colour is stable for at least 30 minutes. Read during this time.

Example for a pipetting scheme:

	1	2	3	4	5	6	7	8	9	10	11	12
A	A	P1										
B	B	P2										
C	C	P3										
D	D											
E	E											
F	F											
G	C+											
H	C-											

P1, ... patient sample A-F calibrators C+, C- controls

VALIDATION

Test results are valid if the optical densities at 450 nm for calibrators / controls and the results for controls comply with the reference ranges indicated on the Certificate of Analysis enclosed in each test kit.
If these quality control criteria are not met the assay run is invalid and should be repeated.

CALCULATION OF RESULTS

For quantitative results plot the optical density of each calibrator versus the calibrator concentration to create a calibration curve. The concentration of patient samples may then be estimated from the calibration curve by interpolation.
Using data reduction software a 4-Parameter-Fit with lin-log coordinates for optical density and concentration is the data reduction method of choice.

PERFORMANCE CHARACTERISTICS

Calibration

The assay system is calibrated against the internationally recognized reference sera from CDC, Atlanta USA.

Measuring range

The calculation range of this ELISA assay is 0 - 200 U/ml

Expected values

In a normal range study with samples from healthy blood donors the following ranges have been established with this ELISA assay: Cut-off 25 U/ml

Interpretation of results

Negative:	< 15 U/ml
Borderline:	15 - 25 U/ml
Positive:	> 25 U/ml

Linearity

Patient samples containing high levels of specific antibody were serially diluted in sample buffer to demonstrate the dynamic range of the assay and the upper / lower end of linearity. Activity for each dilution was calculated from the calibration curve using a 4-Parameter-Fit with lin-log coordinates.

Sample	Dilution	Observed U/ml	Expected U/ml	O/E [%]
1	1:100	124.2	127.1	98
	1:200	62.4	34.8	98
	1:400	33.2	17.4	104
	1:800	16.1	8.7	101
2	1:100	104.4	104.4	100
	1:200	53.1	52.2	102
	1:400	27.6	26.1	106
	1:800	13.9	13.1	107

Limit of detection

Functional sensitivity was determined to be: 1 U/ml

Reproducibility

Intra-assay precision: Coefficient of variation (CV) was calculated for each of three samples from the results of 24 determinations in a single run. Results for precision-within-assay are shown in the table below.
Inter-assay precision: Coefficient of variation (CV) was calculated for each of three samples from the results of 6 determinations in 5 different runs. Results for run-to-run precision are shown in the table below.

Intra-Assay		
Sample	Mean U/ml	CV %
1	28.8	5.6
2	67.1	5.8
3	143.2	5.2

Inter-Assay		
Sample	Mean U/ml	CV %
1	24.5	11.0
2	70.5	6.9
3	157.6	4.1

Interfering substances

No interference has been observed with haemolytic (up to 1000 mg/dl) or lipemic (up to 3 g/dl triglycerides) sera or plasma, or bilirubin (up to 40 mg/dl) containing sera or plasma. Nor have any interfering effects been observed with the use of anticoagulants (Citrate, EDTA, Heparine). However for practical reasons it is recommended that grossly hemolyzed or lipemic samples should be avoided.

Study results

Study population	n	n Pos	%
Sjoegren´s syndrome	70	43	61.4
Rheumatoid arthritis	20	1	5.0
Normal human sera	100	4	4.0

		Clinical Diagnosis POS	NEG	
ORG 509	POS	43	5	
	NEG	27	115	
		70	120	190

Sensitivity: 61.4 %
Specificity: 95.8 %
Overall agreement: 83.2 %

LIMITATIONS OF THE PROCEDURE

This assay is a diagnostic aid. A definite clinical diagnosis should not be based on the results of a single test, but

should be made by the physician after all clinical and laboratory findings have been evaluated concerning the entire clinical picture of the patient. Also every decision for therapy should be taken individually.
The above pathological and normal reference ranges for antibodies in patient samples should be regarded as recommendations only. Each laboratory should establishe its own ranges according to ISO 15189 or other applicable laboratory guidelines.

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Notice to the user (European Union):
Any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the EU Member State in which the user and/or the patient is established .

Change Control
Former version: *ORG 509_IFU_EN_QM113136_2013-12-16_1.2* Reason for revision: *Introduction electronic IFU on homepage*

1. Pipet **100 µl** calibrator, control or patient sample
   → Incubate for **30 minutes** at room temperature
   → Discard the contents of the wells and wash 3 times with **300 µl** wash solution
2. Pipet **100 µl** enzyme conjugate
   → Incubate for **15 minutes** at room temperature
   → Discard the contents of the wells and wash 3 times with **300 µl** wash solution
3. Pipet **100 µl** substrate solution
   → Incubate for **15 minutes** at room temperature
4. Add **100 µl** stop solution
   → Leave untouched for **5 minutes**
   → Read at **450 nm**

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601_3

ORG 601 Anti-CCP hs® (high sensitive)

INTENDED PURPOSE

Anti-CCP hs® (high sensitive) is an ELISA test system for the quantitative measurement of IgG class autoantibodies against cyclic citrullinated peptides (CCP) in human serum or plasma. This product is intended for professional in vitro diagnostic use only.

Measurement of anti-CCP antibodies may aid in the diagnosis of rheumatoid arthritis (RA), where anti-CCP antibody levels represent one parameter of a multi-criterion diagnostic process, encompassing both clinical and laboratory-based assessments.

SYMBOLS USED ON LABELS

IVD	In vitro diagnostic medical device
	Manufacturer
REF	Catalogue number
96	Sufficient for 96 determinations
LOT	Batch code
	Use by
2°C 8°C	Temperature limitation
	Keep away from sunlight
	Do not reuse
	Date of manufacture
CE	CE marked according to 98/79/EC
	Consult instructions for use
601_3	Electronic Instruction For Use: version

MICROPLATE	Microplate
CALIBRATOR A	Calibrator
CALIBRATOR B	Calibrator
CALIBRATOR C	Calibrator
CALIBRATOR D	Calibrator
CALIBRATOR E	Calibrator
CALIBRATOR F	Calibrator
CONTROL +	Control positive
CONTROL −	Control negative
DILUENT	Sample Buffer P
CONJUGATE	Enzyme Conjugate
TMB	TMB Substrate
STOP	Stop solution
WASH	Wash Buffer
RTU	Ready to use

PRINCIPLE OF THE TEST

Highly purified cyclic citrullinated vimentin peptides (CCP) is bound to microwells.
The determination is based on an indirect enzyme linked immune reaction with the following steps:
Specific antibodies in the patient sample bind to the antigen coated on the surface of the reaction wells. After incubation, a washing step removes unbound and unspecifically bound serum or plasma components. Subesquently added enzyme conjugate binds to the immobilized antibody-antigen-complexes. After incubation, a second washing step removes unbound enzyme conjugate. After addition of substrate solution the bound enzyme conjugate hydrolyses the substrate forming a blue coloured product. Addition of an acid stopps the reaction generating a yellow end-product. The intensity of the yellow color
correlates with the concentration of the antibody-antigen-complex and can be measured photometrically at 450 nm.

WARNINGS AND PRECAUTIONS

- All reagents of this kit are intended for professional in vitro diagnostic use only.
- Components containing human serum were tested and found negative for HBsAg, HCV, HIV1 and HIV2 by FDA approved methods. No test can guarantee the absence of HBsAg, HCV, HIV1 or HIV2, and so all human serum based reagents in this kit must be handled as though capable of transmitting infection.
- Bovine serum albumin (BSA) used in components has been tested for BSE and found negative.
- Avoid contact with the substrate TMB (3,3´,5,5´-Tetramethyl-benzidine).
- Stop solution contains acid, classifiaction is non-hazardous. Avoid contact with skin.
- Control, sample buffer and wash buffer contain sodium azide 0.09% as preservative. This concentration is classified as non-hazardous.
- Enzyme conjugate contains ProClin 300 0.05% as preservative. This concentration is classified as non-hazardous.

During handling of all reagents, controls and serum samples observe the existing regulations for laboratory safety regulations and good laboratory practice:

- First aid measures: In case of skin contact, immediately wash thoroughly with water and soap. Remove contaminated clothing and shoes and wash before reuse. If system fluid comes into contact with skin, wash thoroughly with water. After contact with the eyes carefully rinse the opened eye with running water for at least 10 minutes. Get medical attention if necessary.
- Personal precautions, protective equipment and emergency procedures:

Observe laboratory safety regulations. Avoid contact with skin and eyes. Do not swallow. Do not pipette by mouth. Do not eat, drink, smoke or apply makeup in areas where specimens or kit reagents are handled. When spilled, absorb with an inert material and put the spilled material in an appropriate waste disposal.

- Exposure controls / personal protection: Wear protective gloves of nitril rubber or natural latex. Wear protective glasses. Used according to intended use no dangerous reactions known.
- Conditions to avoid: Since substrate solution is light-sensitive. Store in the dark.
- For disposal of laboratory waste the national or regional legislation has to be observed.

Observe the guidelines for performing quality control in medical laboratories by assaying control sera.

CONTENTS OF THE KIT

ORG 601	Σ 96	Sufficient for 96 determinations
MICROPLATE	1	One divisible microplate consisting of 12 modules of 8 wells each. Ready to use. Product code on module: ***CCP***
CALIBRATOR A	1x 1.5 ml	Calibrator A 0 U/ml, containing serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use.
CALIBRATOR B	1x 1.5 ml	Calibrator B 20 U/ml, containing CCP antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use.
CALIBRATOR C	1x 1.5 ml	Calibrator C 40 U/ml, containing CCP antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use.
CALIBRATOR D	1x 1.5 ml	Calibrator D 100 U/ml, containing CCP antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use.
CALIBRATOR E	1x 1.5 ml	Calibrator E 300 U/ml, containing CCP antibodies in a serum/buffer matrix (PBS, BSA, NaN3 0.09%), yellow. Ready to use.
CALIBRATOR F	1x 1.5 ml	Calibrator F 1000 U/ml, containing CCP antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use.
CONTROL +	1x 1.5 ml	Control positive, containing CCP antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use. The concentration is specified on the certificate of analysis.
CONTROL −	1x 1.5 ml	Control negative, containing CCP antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use. The concentration is specified on the certificate of analysis.
DILUENT	20 ml	Sample Buffer P, containing PBS, BSA, detergent, preservative sodium azide 0.09%, yellow, concentrate (5 x).
CONJUGATE	15 ml	Enzyme Conjugate containing anti-human IgG antibodies, HRP labelled; PBS, BSA, detergent, preservative PROCLIN 0.05%, light red. Ready to use.
TMB	15 ml	TMB Substrate; containing 3,3', 5,5'- Tetramethylbenzidin, colorless. Ready to use.
STOP	15 ml	Stop solution; contains acid. Ready to use.
WASH	20 ml	Wash Buffer, containing Tris, detergent, preservative sodium azide 0.09%; 50 x conc.
	1	Certificate of Analysis

MATERIALS REQUIRED

- Microplate reader capable of endpoint measurements at 450 nm; optional: reference filter at 620 nm
- Data reduction software
- Multi-channel dispenser or repeatable pipette for 100 µl
- Vortex mixer
- Pipettes for 10 µl, 100 µl and 1000 µl
- Laboratory timing device
- Distilled or deionised water
- Measuring cylinder for 1000 ml and 100 ml
- Plastic container for storage of the wash solution

This ELISA assay is suitable for use on open automated ELISA processors. Each assay has to be validated on the respective automated system. Detailed information is provided upon request.

SPECIMEN COLLECTION, STORAGE AND HANDLING

- Collect whole blood specimens using acceptable medical techniques to avoid hemolysis.
- Allow blood to clot and separate the serum or plasma by centrifugation.
- Test serum should be clear and non-hemolyzed. Contamination by hemolysis or lipemia should be avoided, but does not interfere with this assay.
- Specimens may be refrigerated at 2-8°C for up to five days or stored at -20°C up to six months.
- Avoid repetitive freezing and thawing of serum or plasma samples. This may result in variable loss of antibody activity.
- Testing of heat-inactivated sera is not recommended.

STORAGE AND STABILITY

- Store test kit at 2-8°C in the dark.
- Do not expose reagents to heat, sun, or strong light during storage and usage.
- Store microplate sealed and dessicated in the clip bag provided.
- Shelf life of the unopended test kit is 18 months from day of production. Unopened reagents are stable until expiration of the kit. See labels for individual batch.
- Diluted Wash Buffer and Sample Buffer are stable for at least 30 days when stored at 2-8°C. We recommend consumption on the same day.

PROCEDURAL NOTES

- Do not use kit components beyond their expiration dates.
- Do not interchange kit components from different lots and products.
- All materials must be at room temperature (20-28°C) prior to use.
- Prepare all reagents and samples. Once started, performe the test without interruption.
- Double determinations may be done. By this means pipetting errors may become obvious.
- Perform the assay steps only in the order indicated.
- Always use fresh sample dilutions.
- Pipette all reagents and samples into the bottom of the wells.
- To avoid carryover or contamination, change the pipette tip between samples and different kit controls.
- Wash microwells thoroughly and remove the last droplets of wash buffer.
- All incubation steps must be accurately timed.
- Do not re-use microplate wells.

PREPARATION OF REAGENTS

WASH

Dilute the contents of one vial of the buffered wash solution concentrate (50x) with distilled or deionised water to a final volume of 1000 ml prior to use.

DILUENT

Sample Buffer P: Prior to use dilute the contents (20 ml) of one vial of sample buffer 5x concentrate with distilled or deionised water to a final volume of 100 ml.

Preparation of samples

Dilute patient samples 1:100 before the assay: Put 990 µl of prediluted sample buffer in a polystyrene tube and add 10 µl of sample. Mix well. Note: Calibrators / Controls are ready to use and need not be diluted.

TEST PROCEDURE

Prepare enough microplate modules for all calibrators / controls and patient samples.

1. Pipette **100 µl** of calibrators, controls and prediluted patient samples into the wells.
   Incubate for **30 minutes** at room temperature (20-28 °C).
   Discard the contents of the microwells and **wash 3 times** with **300 µl** of wash solution.
2. Dispense **100 µ**l of enzyme conjugate into each well.
   Incubate for **15 minutes** at room temperature.
   Discard the contents of the microwells and **wash 3 times** with **300 µl** of wash solution.
3. Dispense **100 µ**l of TMB substrate solution into each well.
   Incubate for **15 minutes** at room temperature
4. **Add 100 µl** of stop solution to each well of the modules
   Incubate for **5 minutes** at room temperature.
   Read the optical density at 450 nm (reference 600-690nm) and calculate the results.
   The developed colour is stable for at least 30 minutes. Read during this time.

Example for a pipetting scheme:

	1	2	3	4	5	6	7	8	9	10	11	12
A	A	P1										
B	B	P2										
C	C	P3										
D	D											
E	E											
F	F											
G	C+											
H	C-											

P1, ... patient sample A-F calibrators C+, C- controls

VALIDATION

Test results are valid if the optical densities at 450 nm for calibrators / controls and the results for controls comply with the reference ranges indicated on the Certificate of Analysis enclosed in each test kit.
If these quality control criteria are not met the assay run is invalid and should be repeated.

CALCULATION OF RESULTS

For quantitative results plot the optical density of each calibrator versus the calibrator concentration to create a calibration curve. The concentration of patient samples may then be estimated from the calibration curve by interpolation.
Using data reduction software a 4-Parameter-Fit with lin-log coordinates for optical density and concentration is the data reduction method of choice.

PERFORMANCE CHARACTERISTICS

Calibration

This assay system is calibrated in relative arbitrary units. It is calibrated against an external anti-CCP Assay, since no international reference sera for RA diagnostic are available so far.

Measuring range

The calculation range of this ELISA assay is 0 - 1000 U/ml

Expected values

In a normal range study with samples from healthy blood donors the following ranges have been established with this ELISA assay: Cut-off 20 U/ml

Interpretation of results

Negative: < 20 U/ml
Positive: ≥ 20 U/ml

Linearity

Patient samples containing high levels of specific antibody were serially diluted in sample buffer to demonstrate the dynamic range of the assay and the upper / lower end of linearity. Activity for each dilution was calculated from the calibration curve using a 4-Parameter-Fit with lin-log coordinates.

Sample	Dilution	Observed U/ml	Expected U/ml	O/E [%]
1	1:100	950.2	950.2	100
	1:200	467.3	475.1	98
	1:400	245.4	237.6	103
	1:800	115.6	118.8	97
2	1:100	120.0	120.0	100
	1:200	60.5	60.0	101
	1:400	31.4	30.0	105
	1:800	14.2	15.0	95
	1:1600	7.3	7.5	97
3	1:100	321.3	321.3	100
	1:200	157.9	160.7	98
	1:400	96.4	80.3	120
	1:800	48.2	40.2	120

Limit of detection

Functional sensitivity was determined to be: 1 U/ml

Reproducibility

Intra-assay precision: Coefficient of variation (CV) was calculated for each of three samples from the results of 24 determinations in a single run. Results for precision-within-assay are shown in the table below.
Inter-assay precision: Coefficient of variation (CV) was calculated for each of three samples from the results of 6 determinations in 5 different runs. Results for run-to-run precision are shown in the table below.

Intra-Assay		
Sample	Mean U/ml	CV %
1	13.0	7.8
2	144.5	9.9
3	250.6	13.6

Inter-Assay		
Sample	Mean U/ml	CV %
1	12.3	6.1
2	134.9	7.1
3	262.2	9.3

Interfering substances

No interference has been observed with haemolytic (up to 1000 mg/dl) or lipemic (up to 3 g/dl triglycerides) sera or plasma, or bilirubin (up to 40 mg/dl) containing sera or plasma. Nor have any interfering effects been observed with the use of anticoagulants (Citrate, EDTA, Heparine). However for practical reasons it is recommended that grossly hemolyzed or lipemic samples should be avoided.

Study results

Study population	n	n Pos	%
Rheumatoid arthritis	259	237	91.5
Other arthritis	22	6	27.3
Other rheumatic disease	37	1	2.7
Healthy controls	118	1	0.8

		Clinical Diagnosis		
		POS	NEG	
ORG 601	POS	237	8	
	NEG	22	169	
		259	177	436

Sensitivity: 91.5 %
Specificity: 95.5 %
Overall agreement: 93.1 %

LIMITATIONS OF THE PROCEDURE

This assay is a diagnostic aid. A definite clinical diagnosis should not be based on the results of a single test, but should be made by the physician after all clinical and laboratory findings have been evaluated concerning the entire clinical picture of the patient. Also every decision for therapy should be taken individually.
The above pathological and normal reference ranges for antibodies in patient samples should be regarded as recommendations only. Each laboratory should establishe its own ranges according to ISO 15189 or other applicable laboratory guidelines.

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Notice to the user (European Union):
Any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the EU Member State in which the user and/or the patient is established .

Change Control
Former version: *ORG 601_IFU_EN_QM113201_2016-04-18_2* Reason for revision: *Introduction electronic IFU on homepage*

1. Pipet **100 µl** calibrator, control or patient sample
   → Incubate for **30 minutes** at room temperature
   → Discard the contents of the wells and wash 3 times with **300 µl** wash solution
2. Pipet **100 µl** enzyme conjugate
   → Incubate for **15 minutes** at room temperature
   → Discard the contents of the wells and wash 3 times with **300 µl** wash solution
3. Pipet **100 µl** substrate solution
   → Incubate for **15 minutes** at room temperature
4. Add **100 µl** stop solution
   → Leave untouched for **5 minutes**
   → Read at **450 nm**

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508_3

ORG 508 Anti-SS-A

INTENDED PURPOSE

Anti-SS-A is an ELISA test system for the quantitative measurement of IgG class autoantibodies against SS-A (52 and 60 kDa) in human serum or plasma. This product is intended for professional in vitro diagnostic use only.

This test is useful for the differential diagnosis and monitoring of systemic rheumatic inflammatory autoimmune diseases. Autoantibodies against the two antigens SS-A 52 and SS-A 60 are predominantly found in cases of Sjoegren's syndrome. Evaluation of a test result should always take into account all clinical and laboratory diagnostic findings.

SYMBOLS USED ON LABELS

IVD	In vitro diagnostic medical device
	Manufacturer
REF	Catalogue number
Σ 96	Sufficient for 96 determinations
LOT	Batch code
	Use by
2°C 8°C	Temperature limitation
	Keep away from sunlight
	Do not reuse
	Date of manufacture
CE	CE marked according to 98/79/EC
	Consult instructions for use
508_3	Electronic Instruction For Use: version

MICROPLATE	Microplate
CALIBRATOR A	Calibrator
CALIBRATOR B	Calibrator
CALIBRATOR C	Calibrator
CALIBRATOR D	Calibrator
CALIBRATOR E	Calibrator
CALIBRATOR F	Calibrator
CONTROL +	Control positive
CONTROL −	Control negative
DILUENT	Sample Buffer P
CONJUGATE	Enzyme Conjugate
TMB	TMB Substrate
STOP	Stop solution
WASH	Wash Buffer
RTU	Ready to use

PRINCIPLE OF THE TEST

Highly purified SS-A (52 and 60 kDa) is bound to microwells.
The determination is based on an indirect enzyme linked immune reaction with the following steps:
Specific antibodies in the patient sample bind to the antigen coated on the surface of the reaction wells. After incubation, a washing step removes unbound and unspecifically bound serum or plasma components. Subesquently added enzyme conjugate binds to the immobilized antibody-antigen-complexes. After incubation, a second washing step removes unbound enzyme conjugate. After addition of substrate solution the bound enzyme conjugate hydrolyses the substrate forming a blue coloured product. Addition of an acid stopps the reaction generating a yellow end-product. The intensity of the yellow color
correlates with the concentration of the antibody-antigen-complex and can be measured photometrically at 450 nm.

WARNINGS AND PRECAUTIONS

- All reagents of this kit are intended for professional in vitro diagnostic use only.
- Components containing human serum were tested and found negative for HBsAg, HCV, HIV1 and HIV2 by FDA approved methods. No test can guarantee the absence of HBsAg, HCV, HIV1 or HIV2, and so all human serum based reagents in this kit must be handled as though capable of transmitting infection.
- Bovine serum albumin (BSA) used in components has been tested for BSE and found negative.
- Avoid contact with the substrate TMB (3,3´,5,5´-Tetramethyl-benzidine).
- Stop solution contains acid, classifiaction is non-hazardous. Avoid contact with skin.
- Control, sample buffer and wash buffer contain sodium azide 0.09% as preservative. This concentration is classified as non-hazardous.
- Enzyme conjugate contains ProClin 300 0.05% as preservative. This concentration is classified as non-hazardous.

During handling of all reagents, controls and serum samples observe the existing regulations for laboratory safety regulations and good laboratory practice:

- First aid measures: In case of skin contact, immediately wash thoroughly with water and soap. Remove contaminated clothing and shoes and wash before reuse. If system fluid comes into contact with skin, wash thoroughly with water. After contact with the eyes carefully rinse the opened eye with running water for at least 10 minutes. Get medical attention if necessary.
- Personal precautions, protective equipment and emergency procedures:

Observe laboratory safety regulations. Avoid contact with skin and eyes. Do not swallow. Do not pipette by mouth. Do not eat, drink, smoke or apply makeup in areas where specimens or kit reagents are handled. When spilled, absorb with an inert material and put the spilled material in an appropriate waste disposal.

- Exposure controls / personal protection: Wear protective gloves of nitril rubber or natural latex. Wear protective glasses. Used according to intended use no dangerous reactions known.
- Conditions to avoid: Since substrate solution is light-sensitive. Store in the dark.
- For disposal of laboratory waste the national or regional legislation has to be observed.

Observe the guidelines for performing quality control in medical laboratories by assaying control sera.

CONTENTS OF THE KIT

ORG 508	Σ 96	Sufficient for 96 determinations
MICROPLATE	1	One divisible microplate consisting of 12 modules of 8 wells each. Ready to use. Color code on module
CALIBRATOR A	1x 1.5 ml	Calibrator A 0 U/ml, containing serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use.
CALIBRATOR B	1x 1.5 ml	Calibrator B 12.5 U/ml, containing SS-A antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use.
CALIBRATOR C	1x 1.5 ml	Calibrator C 25 U/ml, containing SS-A antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use.
CALIBRATOR D	1x 1.5 ml	Calibrator D 50 U/ml, containing SS-A antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use.
CALIBRATOR E	1x 1.5 ml	Calibrator E 100 U/ml, containing SS-A antibodies in a serum/buffer matrix (PBS, BSA, NaN3 0.09%), yellow. Ready to use.
CALIBRATOR F	1x 1.5 ml	Calibrator F 200 U/ml, containing SS-A antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use.
CONTROL +	1x 1.5 ml	Control positive, containing SS-A antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use. The concentration is specified on the certificate of analysis.
CONTROL −	1x 1.5 ml	Control negative, containing SS-A antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use. The concentration is specified on the certificate of analysis.
DILUENT	20 ml	Sample Buffer P, containing PBS, BSA, detergent, preservative sodium azide 0.09%, yellow, concentrate (5 x).
CONJUGATE	15 ml	Enzyme Conjugate containing anti-human IgG antibodies, HRP labelled; PBS, BSA, detergent, preservative PROCLIN 0.05%, light red. Ready to use.
TMB	15 ml	TMB Substrate; containing 3,3', 5,5'- Tetramethylbenzidin, colorless. Ready to use.
STOP	15 ml	Stop solution; contains acid. Ready to use.
WASH	20 ml	Wash Buffer, containing Tris, detergent, preservative sodium azide 0.09%; 50 x conc.
	1	Certificate of Analysis

MATERIALS REQUIRED

- Microplate reader capable of endpoint measurements at 450 nm; optional: reference filter at 620 nm
- Data reduction software
- Multi-channel dispenser or repeatable pipette for 100 µl
- Vortex mixer
- Pipettes for 10 µl, 100 µl and 1000 µl
- Laboratory timing device
- Distilled or deionised water
- Measuring cylinder for 1000 ml and 100 ml
- Plastic container for storage of the wash solution

This ELISA assay is suitable for use on open automated ELISA processors. Each assay has to be validated on the respective automated system. Detailed information is provided upon request.

SPECIMEN COLLECTION, STORAGE AND HANDLING

- Collect whole blood specimens using acceptable medical techniques to avoid hemolysis.
- Allow blood to clot and separate the serum or plasma by centrifugation.
- Test serum should be clear and non-hemolyzed. Contamination by hemolysis or lipemia should be avoided, but does not interfere with this assay.
- Specimens may be refrigerated at 2-8°C for up to five days or stored at -20°C up to six months.
- Avoid repetitive freezing and thawing of serum or plasma samples. This may result in variable loss of antibody activity.
- Testing of heat-inactivated sera is not recommended.

STORAGE AND STABILITY

- Store test kit at 2-8°C in the dark.
- Do not expose reagents to heat, sun, or strong light during storage and usage.
- Store microplate sealed and dessicated in the clip bag provided.
- Shelf life of the unopended test kit is 18 months from day of production. Unopened reagents are stable until expiration of the kit. See labels for individual batch.
- Diluted Wash Buffer and Sample Buffer are stable for at least 30 days when stored at 2-8°C. We recommend consumption on the same day.

PROCEDURAL NOTES

- Do not use kit components beyond their expiration dates.
- Do not interchange kit components from different lots and products.
- All materials must be at room temperature (20-28°C) prior to use.
- Prepare all reagents and samples. Once started, performe the test without interruption.
- Double determinations may be done. By this means pipetting errors may become obvious.
- Perform the assay steps only in the order indicated.
- Always use fresh sample dilutions.
- Pipette all reagents and samples into the bottom of the wells.
- To avoid carryover or contamination, change the pipette tip between samples and different kit controls.
- Wash microwells thoroughly and remove the last droplets of wash buffer.
- All incubation steps must be accurately timed.
- Do not re-use microplate wells.

PREPARATION OF REAGENTS

WASH

Dilute the contents of one vial of the buffered wash solution concentrate (50x) with distilled or deionised water to a final volume of 1000 ml prior to use.

DILUENT

Sample Buffer P: Prior to use dilute the contents (20 ml) of one vial of sample buffer 5x concentrate with distilled or deionised water to a final volume of 100 ml.

Preparation of samples

Dilute patient samples 1:100 before the assay: Put 990 µl of prediluted sample buffer in a polystyrene tube and add 10 µl of sample. Mix well. Note: Calibrators / Controls are ready to use and need not be diluted.

TEST PROCEDURE

Prepare enough microplate modules for all calibrators / controls and patient samples.

1. Pipette **100 µl** of calibrators, controls and prediluted patient samples into the wells.
   Incubate for **30 minutes** at room temperature (20-28 °C).
   Discard the contents of the microwells and **wash 3 times** with **300 µl** of wash solution.
2. Dispense **100 µ**l of enzyme conjugate into each well.
   Incubate for **15 minutes** at room temperature.
   Discard the contents of the microwells and **wash 3 times** with **300 µl** of wash solution.
3. Dispense **100 µ**l of TMB substrate solution into each well.
   Incubate for **15 minutes** at room temperature
4. **Add 100 µl** of stop solution to each well of the modules
   Incubate for **5 minutes** at room temperature.
   Read the optical density at 450 nm (reference 600-690nm) and calculate the results.
   The developed colour is stable for at least 30 minutes. Read during this time.

Example for a pipetting scheme:

	1	2	3	4	5	6	7	8	9	10	11	12
A	A	P1										
B	B	P2										
C	C	P3										
D	D											
E	E											
F	F											
G	C+											
H	C-											

P1, ... patient sample A-F calibrators C+, C- controls

VALIDATION

Test results are valid if the optical densities at 450 nm for calibrators / controls and the results for controls comply with the reference ranges indicated on the Certificate of Analysis enclosed in each test kit.
If these quality control criteria are not met the assay run is invalid and should be repeated.

CALCULATION OF RESULTS

For quantitative results plot the optical density of each calibrator versus the calibrator concentration to create a calibration curve. The concentration of patient samples may then be estimated from the calibration curve by interpolation.
Using data reduction software a 4-Parameter-Fit with lin-log coordinates for optical density and concentration is the data reduction method of choice.

PERFORMANCE CHARACTERISTICS

Calibration

The assay system is calibrated against the internationally recognized reference sera from CDC, Atlanta USA.

Measuring range

The calculation range of this ELISA assay is 0 - 200 U/ml

Expected values

In a normal range study with samples from healthy blood donors the following ranges have been established with this ELISA assay: Cut-off 25 U/ml

Interpretation of results

Negative:	< 15 U/ml
Borderline:	15 - 25 U/ml
Positive:	> 25 U/ml

Linearity

Patient samples containing high levels of specific antibody were serially diluted in sample buffer to demonstrate the dynamic range of the assay and the upper / lower end of linearity. Activity for each dilution was calculated from the calibration curve using a 4-Parameter-Fit with lin-log coordinates.

Sample	Dilution	Observed U/ml	Expected U/ml	O/E [%]
1	1:100	139.0	139.0	100
	1:200	67.9	69.5	98
	1:400	33.0	34.8	95
	1:800	17.2	17.4	99
2	1:100	161.6	161.6	100
	1:200	70.6	80.8	87
	1:400	39.2	40.4	97
	1:800	20.0	20.2	99

Limit of detection

Functional sensitivity was determined to be: 1 U/ml

Reproducibility

Intra-assay precision: Coefficient of variation (CV) was calculated for each of three samples from the results of 24 determinations in a single run. Results for precision-within-assay are shown in the table below.
Inter-assay precision: Coefficient of variation (CV) was calculated for each of three samples from the results of 6 determinations in 5 different runs. Results for run-to-run precision are shown in the table below.

Intra-Assay		
Sample	Mean U/ml	CV %
1	32.2	2.7
2	73.2	2.6
3	134.0	3.6

Inter-Assay		
Sample	Mean U/ml	CV %
1	33.8	6.4
2	71.3	6.2
3	133.1	1.1

Interfering substances

No interference has been observed with haemolytic (up to 1000 mg/dl) or lipemic (up to 3 g/dl triglycerides) sera or plasma, or bilirubin (up to 40 mg/dl) containing sera or plasma. Nor have any interfering effects been observed with the use of anticoagulants (Citrate, EDTA, Heparine). However for practical reasons it is recommended that grossly hemolyzed or lipemic samples should be avoided.

Study results

Study population	n	n Pos	%
Sjoegren´s syndrome	70	51	72.9
Normal human sera	100	7	7.0

		Clinical Diagnosis POS	NEG	
ORG 508	POS	51	7	
	NEG	19	93	
		70	100	170

Sensitivity: 72.9 %
Specificity: 93.0 %
Overall agreement: 84.7 %

LIMITATIONS OF THE PROCEDURE

This assay is a diagnostic aid. A definite clinical diagnosis should not be based on the results of a single test, but should be made by the physician after all clinical and laboratory findings have been evaluated concerning the entire

clinical picture of the patient. Also every decision for therapy should be taken individually.
The above pathological and normal reference ranges for antibodies in patient samples should be regarded as recommendations only. Each laboratory should establishe its own ranges according to ISO 15189 or other applicable laboratory guidelines.

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Notice to the user (European Union):
Any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the EU Member State in which the user and/or the patient is established .

Change Control
Former version: *ORG 508_IFU_EN_QM113135_2013-12-16_1.2* Reason for revision: *Introduction electronic IFU on homepage*

1. Pipet **100 µl** calibrator, control or patient sample
   → Incubate for **30 minutes** at room temperature
   → Discard the contents of the wells and wash 3 times with **300 µl** wash solution
2. Pipet **100 µl** enzyme conjugate
   → Incubate for **15 minutes** at room temperature
   → Discard the contents of the wells and wash 3 times with **300 µl** wash solution
3. Pipet **100 µl** substrate solution
   → Incubate for **15 minutes** at room temperature
4. Add **100 µl** stop solution
   → Leave untouched for **5 minutes**
   → Read at **450 nm**

CE

DiaMetra
care for quality

DCM144-2
Ed. 03/2022

Anti Cardiolipin Screen

per analisi di routine

Determinazione quantitativa degli autoanticorpi IgG o IgM contro la cardiolipina nel siero o plasma umano

IVD | **LOT** Vedere l'etichetta esterna | 2°C 8°C | Σ = 96 test | **REF DKO144**

1. SCOPO PREVISTO

Per uso diagnostico *in vitro*
Per uso professionale in laboratorio

Anti Cardiolipin Screen è un dispositivo diagnostico manuale *in vitro* destinato alla determinazione quantitativa di anticorpi di classe IgG e IgM diretti contro la cardiolipina nel siero o nel plasma umano. I risultati devono essere impiegati in associazione ad altri dati clinici e di laboratorio come ausilio nella diagnosi della sindrome da antifosfolipidi (APS).

2. RILEVANZA CLINICA

La cardiolipina è un fosfolipide caricato negativamente che si trova in genere nella membrana mitocondriale interna[1]. Gli autoanticorpi diretti contro la cardiolipina fanno parte di un gruppo noto come anticorpi antifosfolipidi che comprende gli autoanticorpi anti-β2 glicoproteina 1. La misurazione degli autoanticorpi anti-cardiolipina è considerata uno dei marcatori più importanti per sostenere la diagnosi della sindrome da antifosfolipidi (APS)[2,3].

L'APS è un disturbo autoimmune sistemico caratterizzato da una combinazione di trombosi arteriose e/o venose, complicanze della gravidanza, quali perdita fetale ricorrente, insieme a livelli elevati di anticorpi antifosfolipidi[4]. L'APS è stata descritta per la prima volta in pazienti con lupus eritematoso sistemico (LES), anche se successivamente è stato stabilito che il LES può essere indipendente da una malattia sottostante[5].

L'APS può presentarsi da sola (APS primaria o in associazione ad altre condizioni, come il LES) o come APS secondaria[6] (6). Tuttavia, è stato dimostrato che gli anticorpi anti-cardiolipina (aCL) possono essere rilevati in pazienti con LES che non sviluppano APS secondaria[7-9]. Inoltre, gli eventi tromboembolici sono la manifestazione clinica più comune dell'APS.

Gli anticorpi anti-cardiolipina possono riconoscere sia la cardiolipina sia porzioni del complesso proteina-fosfolipide β2 glicoproteina 1-cardiolipina[10,11].

Alcuni studi hanno indicato un'associazione di anticorpi IgG e IgM anti-cardiolipina[2,7,11-16], con eventi trombotici, mentre altri suggeriscono che questi siano correlati all'isotipo IgG, ma non all'IgM[6,10,17].

È stato dimostrato che gli anticorpi IgM aCL sono presenti in infezioni quali epatite C cronica, lebbra e sifilide, ma non sono direttamente coinvolti in eventi trombotici[17].

È probabile che la presenza di anticorpi antifosfolipidi, tra cui IgG e IgM aCL, costituisca il singolo fattore di rischio più riconoscibile nei casi di perdita della gravidanza ricorrente e complicanze ostetriche mediate dalla placenta tardive[4-6,11,14-15,18,19]. Laddove le pazienti possono presentare solo esiti avversi della gravidanza con eventi vascolari isolati o con manifestazioni sia ostetriche sia trombotiche[6]. È stato suggerito che gli anticorpi anti-β2-glicoproteina I – cardiolipina sono in grado di riconoscere l'antigene su tessuti placentari, inibendo la crescita e la differenziazione dei trofoblasti che possono infine causare una placentazione difettosa[20].

3. PRINCIPIO DEL METODO

Il test Anti Cardiolipin Screen permette di determinare gli autoanticorpi diretti contro il complesso cardiolipina-β2-glicoproteina attraverso due diverse curve di calibrazione e coniugati enzimatici (uno specifico per il test IgG, uno specifico per il test IgM) e una micropiastra. Il principio del metodo e la procedura di dosaggio sono gli stessi per entrambe le valutazioni. Utilizzare reagenti per IgG o reagenti per IgM a seconda dell'isotipo in esame.

Anti Cardiolipin Screen è un dosaggio immunometrico enzimatico (ELISA) a sandwich in due fasi in cui i campioni dei pazienti, i calibratori o i controlli sono incubati su piastre per microtitolazione rivestite con il complesso antigenico cardiolipina-β2 glicoproteina. Durante l'incubazione, gli anticorpi presenti nel campione di test si legano al complesso antigenico immobilizzato. Dopo l'incubazione, la separazione del legato dal libero viene eseguita con un semplice lavaggio della fase solida.

Una successiva incubazione si verifica con anti-IgM o anti-IgG umane coniugate con perossidasi di rafano (HRP), che si lega agli anticorpi immobilizzati. Viene eseguita un'ulteriore fase di lavaggio per rimuovere il coniugato in eccesso. Quindi, una soluzione di substrato cromogenico contenente TMB viene erogata nei pozzetti che reagisce con l'HRP coniugato e si sviluppa un colore blu che cambia in giallo quando viene aggiunta la soluzione di arresto (H_2SO_4). L'intensità del colore è direttamente proporzionale

alla concentrazione di IgM o IgG anti-cardiolipina (a seconda del coniugato utilizzato) nel campione.

La concentrazione di anticorpo anti-cardiolipina nel campione viene calcolata attraverso una curva di calibrazione.

4. REAGENTI, MATERIALI E STRUMENTAZIONE

4.1. Reagenti e materiali forniti nel kit

Per la determinazione di anticorpi di classe IgG

1. Calibratori di IgG anti-cardiolipina
(5 fiale, 1,2 mL ciascuno)
Tampone fosfato 0,1 M, NaN_3 < 0,1%, siero umano

CAL0	**REF DCE002/11306-0**
CAL1	**REF DCE002/11307-0**
CAL2	**REF DCE002/11308-0**
CAL3	**REF DCE002/11309-0**
CAL4	**REF DCE002/11310-0**

2. Controlli (2 fiale, 1,2 mL ciascuna, pronte all'uso)
Tampone fosfato 0,1 M, NaN_3 < 0,1%, siero umano

Controllo negativo	**REF DCE045/11301-0**
Controllo positivo	**REF DCE045/11302-0**

3. Coniugato IgG (1 fiala, 15 mL)
Coniugato anti h-IgG con perossidasi di rafano (HRP), BSA 0,1%, ProClin < 0,0015% **REF DCE002/11302-0**

Per la determinazione di anticorpi di classe IgM

1. Calibratori (5 fiale, 1,2 mL ciascuno)
Tampone fosfato 0,1 M, NaN_3 < 0,1%, siero umano

CAL0	**REF DCE002/11206-0**
CAL1	**REF DCE002/11207-0**
CAL2	**REF DCE002/11208-0**
CAL3	**REF DCE002/11209-0**
CAL4	**REF DCE002/11210-0**

2. Controlli (2 fiale, 1,2 mL ciascuna, pronte all'uso)
Tampone fosfato 0,1 M, NaN_3 < 0,1%, siero umano

Controllo negativo	**REF DCE045/11201-0**
Controllo positivo	**REF DCE045/11202-0**

3. Coniugato (1 fiala, 15 mL)
Coniugato anti h-IgM con perossidasi di rafano (HRP), BSA 0,1%, ProClin < 0,0015% **REF DCE002/11202-0**

Reagenti comuni

4. Diluente per campione (1 fiala, 100 mL)
Tampone fosfato 0,1 M, NaN_3 < 0,1%
REF DCE053-0

5. Micropiastra rivestita (1 micropiastra frangibile)
Micropiastra rivestita con complesso antigenico cardiolipina-β2-glicoproteina **REF DCE002/14403-0**

6. Substrato TMB (1 fiala, 15 mL)
H_2O_2 -TMB (0,26 g/L) *(evitare qualsiasi contatto con la pelle)*
REF DCE004-0

7. Soluzione di arresto (1 fiala, 15 mL)
Acido solforico 0,15 M *(evitare qualsiasi contatto con la pelle)*
REF DCE005-0

8. Soluzione di lavaggio conc. 10X (1 fiala, 50 mL)
Tampone fosfato 0,2 M, pH 7,4 **REF DCE054-0**

4.2. Materiali richiesti ma non forniti

Acqua distillata

4.3. Materiali e strumentazione ausiliari

Erogatore automatico
Pipette di precisione
Lettore di micropiastre (450 nm, 620-630 nm)

5. AVVERTENZE

- Questo kit è destinato all'uso *in vitro* esclusivamente da parte di professionisti. Non per uso interno o esterno in esseri umani o animali.
- Utilizzare adeguati dispositivi di protezione individuale mentre si lavora con i reagenti forniti.
- Seguire le buone prassi di laboratorio (GLP, Good Laboratory Practice) per la manipolazione di emoderivati.
- Tutto il materiale di origine umana utilizzato nella preparazione dei reagenti è stato testato e risultato negativo per gli anticorpi dell'HIV 1 e 2, HbsAg e HCV. Nessun metodo di prova, tuttavia, può offrire la completa garanzia che HIV, HBV, HCV o altri agenti infettivi siano assenti. Pertanto, i calibratori e i controlli devono essere manipolati allo stesso modo del materiale potenzialmente infettivo.
- Il materiale di origine animale utilizzato nella preparazione del kit è stato ottenuto da animali certificati come sani e la proteina bovina è stata ottenuta da Paesi non infettati dalla BSE, ma tali materiali devono essere trattati come potenzialmente infettivi.
- Alcuni reagenti contengono piccole quantità di azoturo di sodio (NaN_3) o ProClin™ 300 come conservante. Evitare il contatto con pelle o mucose.
- L'azoturo di sodio può essere tossico se ingerito o assorbito attraverso la pelle o gli occhi; inoltre, può reagire con le tubature di piombo o rame per formare azoturi metallici potenzialmente esplosivi. Se si utilizza un lavandino per rimuovere i reagenti, lavare con abbondante acqua per evitare l'accumulo di azoturi.
- Il substrato TMB contiene un irritante, che può essere dannoso se inalato, ingerito o assorbito per via cutanea. Per prevenire lesioni, evitare l'inalazione, l'ingestione o il contatto con pelle e occhi.
- La soluzione di arresto consiste in una soluzione diluita di acido solforico. L'acido solforico è velenoso e corrosivo e può essere tossico se ingerito. Per prevenire ustioni chimiche, evitare il contatto con pelle e occhi.
- Evitare l'esposizione del reagente TMB/H_2O_2 a luce solare diretta, metalli o ossidanti. Non congelare la soluzione.

6. PRECAUZIONI

- Attenersi rigorosamente alla sequenza dei passaggi di pipettaggio forniti in questo protocollo. I dati sulle prestazioni qui rappresentati sono stati ottenuti utilizzando i reagenti specifici elencati in queste istruzioni per l'uso.
- Tutti i reagenti devono essere conservati refrigerati a 2-8 °C nel contenitore originale. Tutte le eccezioni sono chiaramente indicate.
- Lasciare che tutti i componenti del kit e i campioni raggiungano la temperatura ambiente (22-28 °C) e mescolare bene prima dell'uso.
- Non scambiare i componenti di kit di lotti diversi. La data di scadenza stampata sulle etichette della confezione e delle fiale deve essere rispettata. Non utilizzare alcun componente del kit dopo la data di scadenza.

- **AVVERTENZA: il reagente coniugato è progettato per garantire la massima sensibilità per la dose e può essere contaminato da agenti esterni se non utilizzato correttamente;** pertanto, si raccomanda di utilizzare materiali di consumo monouso (puntali, flaconi, vassoi, ecc.). Per le dosi divise, prelevare l'esatta quantità di coniugato necessaria e non reintrodurre alcun prodotto di scarto nel flacone originale. Inoltre, **per le dosi erogate con l'ausilio di dispositivi automatici e semiautomatici,** prima di utilizzare il coniugato, è consigliabile pulire il sistema per la gestione dei fluidi, assicurandosi che le procedure di lavaggio, deproteinizzazione e decontaminazione siano efficaci per evitare la contaminazione del coniugato; questa procedura è altamente raccomandata quando il kit viene elaborato con analizzatori non dotati di puntali monouso.
  A tale scopo, DiaMetra fornisce un reagente di decontaminazione separato per la pulizia degli aghi.
- Se si utilizzano apparecchiature automatizzate, l'utente ha la responsabilità di assicurarsi che il kit sia stato adeguatamente testato.
- La rimozione incompleta o imprecisa del liquido dai pozzetti potrebbe influenzare la precisione del dosaggio e/o aumentare il background. Per migliorare le prestazioni del kit sui sistemi automatici, si raccomanda di aumentare il numero di lavaggi.
- È importante che il tempo di reazione in ogni pozzetto sia mantenuto costante per ottenere risultati riproducibili. Il pipettaggio dei campioni non deve andare oltre i dieci minuti per evitare deviazioni del dosaggio. Se sono necessari più di 10 minuti, seguire lo stesso ordine di erogazione. Se si utilizza più di una piastra, si raccomanda di ripetere la curva dose-risposta in ogni piastra.
- L'aggiunta della soluzione di substrato TMB avvia una reazione cinetica, che viene terminata dall'aggiunta della soluzione di arresto. Pertanto, il substrato TMB e la soluzione di arresto devono essere aggiunti nella stessa sequenza per eliminare qualsiasi deviazione temporale durante la reazione.
- Osservare le linee guida per l'esecuzione del controllo di qualità nei laboratori medici analizzando i controlli e/o i sieri in pool.
- La massima precisione è richiesta per la ricostituzione e l'erogazione dei reagenti.
- I campioni microbiologicamente contaminati, altamente lipemici o emolizzati non devono essere utilizzati nel dosaggio.
- I lettori di piastre misurano verticalmente. Non toccare il fondo dei pozzetti.

7. CONSERVAZIONE E STABILITÀ DEI REAGENTI

Conservare il kit a 2-8 °C, al buio.

- Il kit è stabile a 2-8 °C fino alla data di scadenza indicata sull'etichetta esterna del kit.
- Una volta aperto, il kit è stabile a 2-8 °C per 6 mesi.
- La soluzione di lavaggio diluita è stabile per 30 giorni a 2-8 °C.

Nota importante: aprire il sacchetto contenente la micropiastra rivestita solo quando è a temperatura ambiente e chiuderlo immediatamente dopo l'uso.

8. RACCOLTA E CONSERVAZIONE DEI CAMPIONI

Il dosaggio deve essere effettuato su campioni di siero (provette di campionamento standard o provette contenenti gel per la separazione del siero) o plasma (litio eparina, sodio eparina o EDTA di potassio).

Conservazione dei campioni	Durata
2-8 °C	96 ore
Cicli di congelamento/scongelamento	3 cicli

9. PROCEDURA

9.1. Preparazione di calibratori e controlli

I calibratori e i controlli sono pronti per l'uso.
I calibratori presentano all'incirca le seguenti concentrazioni:

	C_0	C_1	C_2	C_3	C_4
AU/mL	0	5	10	20	80

9.2. Preparazione della soluzione di lavaggio

Diluire il contenuto della fiala "Soluzione di lavaggio conc. 10X" con acqua distillata fino a un volume finale di 500 mL prima dell'uso. Per i volumi più piccoli, rispettare il rapporto di diluizione 1:10.

È possibile osservare la presenza di cristalli all'interno della soluzione di lavaggio concentrata; in tal caso, mescolare a temperatura ambiente fino alla completa dissoluzione dei cristalli. Per una maggiore precisione, diluire l'intero flacone di soluzione di lavaggio concentrata a 500 mL, avendo cura anche di trasferire completamente i cristalli sciacquando il flacone, quindi mescolare fino a quando i cristalli non si dissolvono completamente.

9.3. Preparazione dei campioni

Tutti i campioni di siero e plasma devono essere diluiti 1:100 con diluente per campione.
ad es. 10 µL di campione devono essere diluiti con 990 µL di diluente per campione.

9.4. Procedura

- **Lasciare che tutti i reagenti raggiungano la temperatura ambiente (22-28 °C) per almeno 30 minuti.** Alla fine del dosaggio, conservare immediatamente i reagenti a 2-8 °C: evitare una lunga esposizione a temperatura ambiente.
- Le strisce di micropozzetti rivestiti non utilizzate devono essere rilasciate in modo sicuro nella busta di alluminio contenente l'essiccante e conservate a 2-8 °C.
- Per evitare potenziali contaminazioni microbiche e/o chimiche, i reagenti inutilizzati non devono mai essere trasferiti nelle fiale originali.
- Poiché è necessario eseguire la determinazione in duplicato per migliorare la precisione dei risultati del test, preparare due pozzetti per ogni punto della curva di calibrazione (C_0-C_4), due per ogni controllo, due per ogni campione, uno per il bianco.

La seguente procedura è la stessa per entrambi i test degli anticorpi di classe IgG e IgM.

Reagenti	Calibratore	Campione/ Controlli	Bianco
Utilizzare reagenti per IgG o reagenti per IgM a seconda dell'isotipo in esame			
Calibratore C_0-C_4 (IgG o IgM)	**100 µL**		
Controlli (IgG o IgM)		**100 µL**	
Campione diluito		**100 µL**	
Incubare per 60 minuti a temperatura ambiente (22-28 °C). Rimuovere il contenuto da ogni pozzetto; lavare i pozzetti 3 volte con 300 µL di soluzione di lavaggio diluita. **Nota importante**: durante ogni fase di lavaggio, agitare delicatamente la piastra per 5 secondi e rimuovere la soluzione in eccesso picchiettando la piastra capovolta su un tovagliolo di carta assorbente. **Lavatore automatico**: se si utilizzano apparecchiature automatiche, lavare i pozzetti almeno 5 volte.			
Coniugato (IgG o IgM)	**100 µL**	**100 µL**	
Incubare per 60 minuti a temperatura ambiente (22-28 °C). Rimuovere il contenuto da ogni pozzetto; lavare i pozzetti 3 volte con 300 µL di soluzione di lavaggio diluita. **Lavaggio**: seguire le stesse indicazioni del punto precedente.			
Substrato TMB	**100 µL**	**100 µL**	**100 µL**
Incubare per 15 minuti, al buio, a temperatura ambiente (22-28 °C).			
Soluzione di arresto	**100 µL**	**100 µL**	**100 µL**
Agitare delicatamente la micropiastra. Leggere l'assorbanza (E) a 450 nm contro una lunghezza d'onda di riferimento di 620-630 nm o contro il bianco entro 5 minuti.			

10. CONTROLLO QUALITÀ

Le buone prassi di laboratorio (GLP) richiedono l'inclusione di campioni per il controllo della qualità in ogni serie di dosaggi al fine di verificare le prestazioni del dosaggio. I controlli devono essere trattati come campioni sconosciuti e i risultati devono essere analizzati con metodi statistici appropriati.
I controlli forniti nel kit devono essere testati come se fossero sconosciuti e hanno lo scopo di agevolare la valutazione della validità dei risultati ottenuti in ogni piastra di dosaggio.

La concentrazione media di ciascun livello di controllo è documentata nel rapporto del controllo di qualità incluso in ciascun kit. Tali livelli di concentrazione media sono determinati in base a diversi dosaggi eseguiti in duplicato in più posizioni su ciascuna piastra. Questo test è valido solo se la densità ottica a 450 nm per i controlli e per i calibratori (C_0-C_4) rientra nel rispettivo intervallo indicato sul Certificato di controllo qualità allegato a ciascun kit del test.

DiaMetra raccomanda agli utenti di conservare le annotazioni grafiche dei valori di controllo generati con ciascun dosaggio, tra cui medie mobili, DS e CV%. Queste informazioni faciliteranno l'analisi delle tendenze dei controlli per quanto riguarda le prestazioni dei lotti di controllo attuali e pregressi rispetto ai dati forniti nel controllo di qualità. Le tendenze aiuteranno a identificare i dosaggi che generano valori di controllo significativamente diversi dal rispettivo intervallo medio.

Quando si interpretano i dati dei controlli, occorre tenere conto del fatto che il prodotto è stato progettato e sviluppato come prodotto per l'utilizzo manuale. L'intervallo riportato sul certificato del controllo di qualità deve essere appropriato per i dosaggi eseguiti manualmente e rispettando rigorosamente la procedura di dosaggio descritta sopra. Gli esperti del controllo di qualità riconoscono che, a causa delle differenze di condizioni e di prassi, si avrà sempre una variabilità nei valori medi e nella precisione delle misurazioni dei controlli eseguite da laboratori diversi[21].

11. CALCOLO DEI RISULTATI

Sono disponibili vari pacchetti software di elaborazione dei dati, che possono essere utilizzati per generare la curva di calibrazione media e per calcolare le concentrazioni medie di campioni e controlli sconosciuti. È necessario un adattamento della curva logistica a 4 parametri (4PL) con coordinate log-lineari **che includa il calibratore 0.** È possibile utilizzare un adattamento uniforme della curva spline che includa il calibratore 0. Gli altri algoritmi di adattamento della curva non sono raccomandati.

In alternativa, è possibile preparare una curva di calibrazione su carta millimetrata semilogaritmica tracciando un grafico con l'assorbanza media sull'asse delle ordinate e la concentrazione dell'analita sull'asse delle ascisse. Nella curva di calibrazione deve essere incluso il calibratore 0. Leggere il valore medio dell'assorbanza di ciascun campione sconosciuto dalla curva.

12. INTERVALLO DI MISURAZIONE

12.1. Per la valutazione di anticorpi di classe IgG

L'intervallo di misurazione del test (AMR) è 2-80 AU/mL. Qualsiasi valore inferiore a 2 AU/mL deve essere indicato come "< 2 AU/mL". Qualsiasi valore superiore a 80 AU/mL deve essere indicato come "> 80 AU/mL".

12.2. Per la valutazione di anticorpi di classe IgM

L'intervallo di misurazione del test (AMR) è 2,08-80 AU/mL. Qualsiasi valore inferiore a 2,09 AU/mL deve essere indicato come "< 2,08 AU/mL". Qualsiasi valore superiore a 80 AU/mL deve essere indicato come "> 80 AU/mL".

13. METROLOGIA E TRACCIABILITÀ

13.1. Per la valutazione di anticorpi di classe IgG

I calibratori di questo kit sono tracciabili al Centres for Disease Control (CDC) Human IgG Anti-Cardiolipin Monoclonal Antibody HCAL – Catalogue [IS2717].

13.2. Per la valutazione di anticorpi di classe IgM

I calibratori di questo kit sono tracciabili al Centres for Disease Control (CDC) Human IgM Anti-Cardiolipin Monoclonal Antibody EY2C9 [IS2718].

14. INTERPRETAZIONE DEI RISULTATI

Concentrazione	Interpretazione
< 8 AU/mL	Il campione deve essere considerato negativo
8-10 AU/mL	Il campione deve essere classificato equivoco e la ripetizione dei test/campionamenti deve essere eseguita secondo le pratiche interne
> 10 AU/mL	Il campione deve essere considerato positivo

La determinazione di un intervallo di valori attesi per una popolazione "normale" di un determinato metodo dipende da diversi fattori, come la specificità e la sensibilità del metodo utilizzato e il tipo di popolazione in esame. Pertanto, ogni laboratorio deve considerare l'intervallo fornito dal produttore come un'indicazione generale e produrre il proprio intervallo di valori attesi sulla base della popolazione autoctona.

I risultati positivi devono essere verificati in relazione all'intero stato clinico del paziente e la decisione per la terapia deve essere presa in base alle condizioni di ciascun paziente. È consigliabile che ogni laboratorio stabilisca i propri intervalli normali e patologici dei valori dell'anticorpo anti-cardiolipina.

15. CARATTERISTICHE DI AZIONE

Sono mostrati i dati più rappresentativi delle prestazioni. I risultati ottenuti nei singoli laboratori possono variare.

15.1. Per la valutazione di anticorpi di classe IgG

15.1.1. Capacità di rilevamento

Il limite del bianco (LoB), il limite di rilevamento (LoD) e il limite della determinazione quantitativa (LoQ) sono stati definiti basandosi sulla procedura CLSI EP17-A, "Protocols for Determination of Limits of Detection and Limits of Quantitation" utilizzando 6 bianchi e 6 campioni a basso livello.

Sensibilità	Concentrazione
Limite del bianco (LoB)	0,59 AU/mL
Limite di rilevamento (LoD)	1,25 AU/mL
Limite della determinazione quantitativa (LoQ)	2,00 AU/mL

15.1.2. Esattezza

L'esattezza del test Anti Cardiolipin Screen per la valutazione di anticorpi di classe IgG è stata dimostrata attraverso l'esecuzione di un test di recupero utilizzando il CDC Human IgG Anti-Cardiolipin Monoclonal Antibody HCAL – Catalogue [IS2717].

15.1.3. Sensibilità e specificità diagnostica

La sensibilità e la specificità sono state determinate con CLSI EP-24 "Assessment of the Diagnostic Accuracy of Laboratory Tests Using Receiver Operating Characteristic Curves" utilizzando 50 campioni negativi e 51 positivi eseguiti su due lotti di reagenti.

		DKO144 - IgG		Totale
		Positivo	Negativo	
Stato reale	Positivo	47	4	51
	Negativo	0	57	57
Totale		47	61	108

Sensibilità diagnostica: 92%

Specificità diagnostica: 100%

15.1.4. Precisione

La precisione del test Anti Cardiolipin Screen per la determinazione degli anticorpi di classe IgG è stata determinata eseguendo un complesso studio di precisione.

Ripetibilità: un totale di 6 campioni di siero è stato analizzato in 5 repliche, una volta al giorno per 5 giorni da 3 operatori.
I dati di un lotto rappresentativo sono mostrati di seguito:

Campione	n	Conc. media (AU/mL)	Intra-test (ripetibilità)	
			DS	CV
1	75	6,95	0,38	5,5%
2	75	11,03	0,51	4,6%
3	75	20,12	0,94	4,7%
4	75	30,26	1,86	6,1%
5	75	50,11	2,58	5,1%
6	75	71,71	2,53	3,5%

Riproducibilità: un totale di 6 campioni di siero è stato analizzato in 5 repliche, una volta al giorno per 5 giorni da 3 operatori.
I risultati per i dati combinati di due lotti sono mostrati di seguito:

Campione	n	Conc. media (AU/mL)	All'interno del laboratorio (riproducibilità)	
			DS	% CV
1	150	6,98	0,49	7,0%
2	150	11,17	0,84	7,5%
3	150	20,16	1,87	9,3%
4	150	30,38	3,06	10,1%
5	150	50,76	5,02	9,9%
6	150	72,30	3,93	5,4%

15.1.5. Linearità

La linearità è stata valutata secondo le linee guida basate su CLSI EP-06, "Evaluation of the Linearity of Quantitative Measurement Procedures". Per la concentrazione di IgG anti-cardiolipina mediante il test Anti Cardiolipin Screen, la procedura di misurazione mostra linearità per l'intervallo

compreso tra 0,84 e 83,68 ng/mL entro la deviazione ammissibile di linearità (ADL) di ± 15%.

15.2. Specificità analitica

Le seguenti sostanze non interferiscono con un bias > ± 15% nel test Anti Cardiolipin Screen quando si valutano gli anticorpi di classe IgG se le concentrazioni sono inferiori alla soglia dichiarata presentata nella tabella seguente.

Reagente potenzialmente interferente	Concentrazione di soglia
Bilirubina, coniugata	15 mg/dL
Bilirubina, non coniugata	15 mg/dL
Emoglobina	200 mg/dL
Proteine totali	10 g/dL
Trigliceridi	500 mg/dL

15.2.1. Studio su siero-plasma

È stato condotto uno studio di confronto tra matrici del test Anti Cardiolipin Screen per la valutazione di anticorpi di classe IgG per valutare la differenza tra i tipi di provette (provette per la separazione del siero (SST), per plasma in litio eparina, per plasma in sodio eparina e plasma in K2 EDTA) rispetto ai campioni di controllo (siero tappo rosso, senza additivo) secondo le linee guida CLSI EP9-A3. È stato valutato un totale di 22 campioni (18 nativi, 4 additivati) per coprire l'intervallo. L'analisi di regressione lineare è stata effettuata su dati comparativi:

Tipo di campione	Pendenza [IC 95%]	Intercetta (ng/mL) [IC 95%]	Coefficiente di correlazione (r)
SST	0,96 [0,92-0,98]	0,64 [da -0,38 a 1,66]	1,00
Litio eparina	0,92 [da 0,88 a 0,96]	0,87 [da -0,24 a 1,99]	1,00
Sodio eparina	0,94 [da 0,89 a 0,98]	0,66 [da -0,75 a 2,06]	0,99
EDTA	0,95 [da 0,92 a 0,99]	0,54 [da -0,69 a 1,77]	1,00

15.3. Per la valutazione di anticorpi di classe IgM

15.3.1. Capacità di rilevamento

Il limite del bianco (LoB), il limite di rilevamento (LoD) e il limite della determinazione quantitativa (LoQ) sono stati definiti basandosi sulla procedura CLSI EP17-A, "Protocols for Determination of Limits of Detection and Limits of Quantitation" utilizzando 6 bianchi e 6 campioni a basso livello.

Sensibilità	Concentrazione
Limite del bianco (LoB)	0,76 AU/mL
Limite di rilevamento (LoD)	1,45 AU/mL
Limite della determinazione quantitativa (LoQ)	2,08 AU/mL

15.3.2. Esattezza

L'esattezza del test Anti Cardiolipin Screen per la valutazione di anticorpi di classe IgM è stata dimostrata attraverso l'esecuzione di un test di recupero utilizzando il CDC Human IgM Anti-Cardiolipin Monoclonal Antibody EY2C9 [IS2718].

15.3.3. Sensibilità e specificità diagnostica

La sensibilità e la specificità sono state determinate con CLSI EP-24 "Assessment of the Diagnostic Accuracy of Laboratory Tests Using Receiver Operating Characteristic Curves" utilizzando 73 campioni negativi e 62 positivi eseguiti su due lotti di reagenti.

		DKO144 IgM		Totale
		Positivo	Negativo	
Stato reale	**Positivo**	50	12	**62**
	Negativo	0	73	**73**
Totale		**50**	**85**	**135**

Sensibilità diagnostica: 80%

Specificità diagnostica: 100%

15.3.4. Precisione

La precisione del test Anti Cardiolipin Screen per la determinazione degli anticorpi di classe IgM è stata determinata eseguendo un complesso studio di precisione.

Ripetibilità: un totale di 6 campioni di siero è stato analizzato in 5 repliche, una volta al giorno per 5 giorni da 3 operatori.
I dati di un lotto rappresentativo sono mostrati di seguito:

Campione	n	Conc. media (AU/mL)	Intra-test (ripetibilità)	
			DS	CV
1	75	7,74	0,40	5,2%
2	75	12,46	0,51	4,1%
3	75	21,06	0,99	4,7%
4	75	32,07	1,46	4,6%
5	75	55,15	1,19	2,2%
6	75	75,45	2,33	3,1%

Riproducibilità: un totale di 6 campioni di siero è stato analizzato in 5 repliche, una volta al giorno per 5 giorni da 3 operatori.
I risultati per i dati combinati di due lotti sono mostrati di seguito:

Campione	n	Conc. media (AU/mL)	All'interno del laboratorio (riproducibilità)	
			DS	% CV
1	150	7,65	0,46	6,0%
2	150	12,33	0,74	6,0%
3	150	21,03	1,60	7,6%
4	150	31,89	1,98	6,2%
5	150	54,28	2,70	5,0%
6	150	75,46	2,63	3,5%

15.3.5. Linearità
La linearità è stata valutata secondo le linee guida basate su CLSI EP-06, "Evaluation of the Linearity of Quantitative Measurement Procedures". Per la concentrazione di IgM anti-cardiolipina mediante il test Anti Cardiolipin Screen, la procedura di misurazione mostra linearità per l'intervallo compreso tra 0,82 e 86,88 AU/mL entro la deviazione ammissibile di linearità (ADL) di ± 15%.

15.3.6. Specificità analitica
Le seguenti sostanze non interferiscono con un bias > ± 15% nel test Anti Cardiolipin Screen quando si valutano gli anticorpi di classe IgM se le concentrazioni sono inferiori alla soglia dichiarata presentata nella tabella seguente.

Reagente potenzialmente interferente	Concentrazione di soglia
Bilirubina, coniugata	15 mg/dL
Bilirubina, non coniugata	15 mg/dL
Emoglobina	200 mg/dL
Proteine totali	10 g/dL
Trigliceridi	500 mg/dL

15.3.7. Studio su siero-plasma
È stato condotto uno studio di confronto tra matrici del test Anti Cardiolipin Screen per la valutazione di anticorpi di classe IgM per valutare la differenza tra i tipi di provette (provette per la separazione del siero (SST), per plasma in litio eparina, per plasma in sodio eparina e plasma in K2 EDTA) rispetto ai campioni di controllo (siero tappo rosso, senza additivo) secondo le linee guida CLSI EP9-A3. È stato valutato un totale di 20 campioni (16 nativi, 4 additivati) per coprire l'intervallo del test. L'analisi di regressione lineare è stata effettuata su dati comparativi:

Tipo di campione	Pendenza [IC 95%]	Intercetta (ng/mL) [IC 95%]	Coefficiente di correlazione (r)
SST	1,02 [da 0,94 a 1,09]	0,19 [da -1,34 a 1,72]	0,99
Litio eparina	0,93 [da 0,82 a 1,04]	0,73 [da -1,49 a 2,95]	0,97
Sodio eparina	0,93 [da 0,84 a 1,01]	0,64 [da -1,00 a 2,28]	0,98
EDTA	0,96 [da 0,86 a 1,05]	0,65 [da -1,19 a 2,49]	0,98

16. LIMITAZIONI D'USO

- Come nel caso di qualsiasi procedura diagnostica, i risultati devono essere interpretati unitamente ai dati clinici del paziente e alle altre informazioni a disposizione del medico.
- Non sono state stabilite le caratteristiche di azione di questo dosaggio nella popolazione pediatrica.
- Gli anticorpi eterofili nel siero umano possono reagire con le immunoglobuline dei reagenti, interferendo con gli immunodosaggi *in vitro*[22]. I pazienti regolarmente esposti agli animali o a prodotti derivati da siero animale possono essere soggetti a questa interferenza, quindi si potrebbero osservare valori anomali.
- La presenza di immunocomplessi o altri aggregati di immunoglobuline nel campione del paziente può causare un aumento del livello di legame non specifico e produrre falsi positivi in questo test.

17. GESTIONE DEI RIFIUTI

I reagenti devono essere smaltiti in conformità alle normative locali.

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19. IDENTIFICATORE DELLE REVISIONI

Le aggiunte o le modifiche alle istruzioni per l'uso sono indicate dall'evidenziazione in grigio.

20. RECLAMI SUI PRODOTTI E SUPPORTO TECNICO

Per un paziente/utente/terza parte nell'Unione Europea e nei Paesi con un regime normativo simile (Regulation 2017/746/EU on IVD Medical Devices); se, durante l'uso di questo dispositivo o come risultato del suo utilizzo, si è verificato un incidente grave, segnalarlo al produttore e/o al suo rappresentante autorizzato e all'autorità normativa nazionale.
Il produttore può essere contattato tramite il relativo servizio clienti o il team di supporto tecnico. I dettagli di contatto sono disponibili di seguito e sul sito Web dell'azienda: www.diametra.com.

Ed. 03/2022 **DCM144-2**

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CE

DiaMetra
care for quality

DCM144-2
Ed. 03/2022

Anti Cardiolipin Screen

for routine analysis

Quantitative determination of IgG or IgM autoantibodies against Cardiolipin in human serum or plasma

IVD | LOT See external label | 2°C 8°C | Σ = 96 tests | REF DKO144

1. INTENDED PURPOSE

For *In Vitro* Diagnostic Use
For Laboratory Professional Use

Anti Cardiolipin Screen is a manual *in vitro* diagnostic device intended for the quantitative determination of both IgG and IgM class antibodies directed against cardiolipin in human serum or plasma. Results are to be used in conjunction with other clinical and laboratory data as an aid in the diagnosis of antiphospholipid syndrome (APS).

2. CLINICAL SIGNIFICANCE

Cardiolipin is a negatively charged phospholipid which is typically located in the inner mitochondrial membrane[1]. Autoantibodies directed against cardiolipin are part of a group known as antiphospholipid antibodies which includes anti-β2 glycoprotein 1 autoantibodies. Measurement of anti-cardiolipin autoantibodies is considered to be one of the most important markers to support diagnosis of antiphospholipid syndrome (APS)[2,3].

APS is a systemic autoimmune disorder characterised by a combination of arterial and/or venous thromboses, pregnancy complications, such as recurrent foetal loss, together with elevated levels of antiphospholipid antibodies[4]. APS was first described in patients with systemic lupus erythematosus (SLE), though it has subsequently been established that SLE may be independent of an underlying disease[5].

APS can occur alone – Primary APS, or in association with other conditions, such as SLE – Secondary APS[6] (6). However, it has been demonstrated that anti-cardiolipin antibodies (aCLs) can be detected in patients with SLE that do not develop Secondary APS[7-9]. However, thromboembolic events are the most common clinical manifestation of APS

Anti-cardiolipin antibodies can recognise both cardiolipin and portions of the phospholipid-protein complex β2 glycoprotein 1-cardiolipin[10,11].

Studies have indicated an association of anti-cardiolipin IgG and IgM antibodies[2,7,11-16], with thrombotic events whereas others suggest these to be linked with IgG isotype, but not IgM[6,10,17].

aCL IgM antibodies have been shown to occur in infections such as chronic hepatitis C, leprosy, syphilis, but they are not directly involved in thrombotic events[17].

It is likely that the presence of antiphospholipid antibodies, including aCL IgG and IgM constitutes the single most recognisable risk factor in cases of recurrent pregnancy loss and late placenta-mediated obstetric complications[4-6,11,14-15,18,19]. Where patients can present with only adverse pregnancy outcomes with isolated vascular events or with both obstetric and thrombotic manifestations[6]. It has been suggested that anti-β2-glycoprotein I – cardiolipin antibodies are able to recognise the antigen on placental tissues, inhibiting the growth and differentiation of trophoblasts which may eventually cause defective placentation[20].

3. PRINCIPLE OF THE METHOD

The Anti Cardiolipin Screen allows the determination of autoantibodies directed against the Cardiolipin-β2-glycoprotein complex through two different calibration curves and enzyme conjugates (one specific for IgG test, one specific for IgM test) and one microplate. The principle of the method and assay procedure are the same for both assessments. Use reagents for IgG or reagents for IgM depending on the isotype which is under investigation.

The Anti Cardiolipin Screen is a two-step sandwich enzyme immunometric assay (ELISA) where patient samples, calibrators or controls are incubated on microtitre plates coated with the antigenic cardiolipin-β2 glycoprotein complex. During the incubation, antibodies present in the test sample bind to the immobilised antigen complex. After the incubation, the bound/free separation is performed by a simple solid phase washing.

A subsequent incubation occurs with anti-human IgM or IgG conjugated with horseradish peroxidase (HRP), which binds to the immobilised antibodies. A further wash step is performed to remove excess conjugate. Then, a chromogenic substrate solution containing TMB is dispensed into the wells which reacts with the conjugated HRP and a blue colour develops that changes into yellow when the Stop Solution (H_2SO_4) is added. The colour intensity is directly proportional to the Anti Cardiolipin IgM or IgG concentration (depending on the conjugate used) in the sample.

Anti-cardiolipin antibody concentration in the sample is calculated through a calibration curve.

4. REAGENTS, MATERIALS AND INSTRUMENTATION

4.1. Reagents and materials supplied in the kit

For determination of IgG class antibodies

1. Anti Cardiolipin IgG Calibrators
(5 vials, 1.2 mL each)
Phosphate buffer 0.1M, NaN_3 < 0.1%, human serum

CAL0	**REF DCE002/11306-0**
CAL1	**REF DCE002/11307-0**
CAL2	**REF DCE002/11308-0**
CAL3	**REF DCE002/11309-0**
CAL4	**REF DCE002/11310-0**

2. Controls (2 vials, 1.2 mL each, ready to use)
Phosphate buffer 0.1M, NaN_3 < 0.1%, human serum

Negative Control	**REF DCE045/11301-0**
Positive Control	**REF DCE045/11302-0**

3. Conjugate IgG (1 vial, 15 mL)
Anti h-IgG conjugate with horseradish peroxidase (HRP), BSA 0.1%, ProClin < 0.0015% **REF DCE002/11302-0**

For determination of IgM class antibodies

1. Calibrators (5 vials, 1.2 mL each)
Phosphate buffer 0.1M, NaN_3 < 0.1%, human serum

CAL0	**REF DCE002/11206-0**
CAL1	**REF DCE002/11207-0**
CAL2	**REF DCE002/11208-0**
CAL3	**REF DCE002/11209-0**
CAL4	**REF DCE002/11210-0**

2. Controls (2 vials, 1.2 mL each, ready to use)
Phosphate buffer 0.1M, NaN_3 < 0.1%, human serum

Negative Control	**REF DCE045/11201-0**
Positive Control	**REF DCE045/11202-0**

3. Conjugate (1 vial, 15 mL)
Anti h-IgM conjugate with horseradish peroxidase (HRP), BSA 0.1%, ProClin < 0.0015% **REF DCE002/11202-0**

Common reagents

4. Sample Diluent (1 vial, 100 mL)
Phosphate buffer 0.1 M NaN_3 < 0.1%
REF DCE053-0

5. Coated Microplate (1 breakable microplate)
Microplate coated with antigenic Cardiolipin-β2-Glycoprotein complex **REF DCE002/14403-0**

6. TMB Substrate (1 vial, 15 mL)
H_2O_2 -TMB (0.26 g/L) *(avoid any skin contact)*
REF DCE004-0

7. Stop Solution (1 vial, 15 mL)
Sulphuric acid 0.15M *(avoid any skin contact)*
REF DCE005-0

8. 10X Conc. Wash Solution (1 vial, 50 mL)
Phosphate buffer 0.2M, pH 7.4 **REF DCE054-0**

4.2. Materials required but not provided

Distilled water

4.3. Auxiliary materials and instrumentation

Automatic dispenser
Precision Pipetting Devices
Microplate reader (450 nm, 620-630 nm)

5. WARNINGS

- This kit is intended for *in vitro* use by professional persons only. Not for internal or external use in Humans or Animals.
- Use appropriate personal protective equipment while working with the reagents provided.
- Follow Good Laboratory Practice (GLP) for handling blood products.
- All human source material used in the preparation of the reagents has been tested and found negative for antibody to HIV 1&2, HbsAg, and HCV. No test method however can offer complete assurance that HIV, HBV, HCV or other infectious agents are absent. Therefore, the Calibrators and the Controls should be handled in the same manner as potentially infectious material.
- Material of animal origin used in the preparation of the kit has been obtained from animals certified as healthy and the bovine protein has been obtained from countries not infected by BSE, but these materials should be handled as potentially infectious.
- Some reagents contain small amounts of Sodium Azide (NaN_3) or ProClin™ 300 as preservative. Avoid contact with skin or mucosa.
- Sodium Azide may be toxic if ingested or absorbed through the skin or eyes; moreover, it may react with lead or copper plumbing to form potentially explosive metal azides. If you use a sink to remove the reagents, wash through large with amounts of water to prevent azide build-up.
- The TMB Substrate contains an irritant, which harmful if inhaled, ingested or absorbed through the skin. To prevent injury, avoid inhalation, ingestion or contact with skin and eyes.
- The Stop Solution consists of a diluted sulphuric acid solution. Sulphuric acid is poisonous, corrosive and can be toxic if ingested. To prevent chemical burns, avoid contact with skin and eyes.
- Avoid the exposure of reagent TMB/H_2O_2 to direct sunlight, metals or oxidants. Do not freeze the solution.

6. PRECAUTIONS

- Please adhere strictly to the sequence of pipetting steps provided in this protocol. The performance data represented here were obtained using specific reagents listed in this Instruction For Use.
- All reagents should be stored refrigerated at 2-8°C in their original container. Any exceptions are clearly indicated.
- Allow all kit components and specimens to reach room temperature (22-28°C) and mix well prior to use.
- Do not interchange kit components from different lots. The expiry date printed on box and vials labels must be observed. Do not use any kit component beyond their expiry date.
- **WARNING: the conjugate reagent is designed to ensure maximum dose sensitivity and may be contaminated by external agents if not used properly;** therefore, it is recommended to use disposable consumables (tips, bottles, trays, etc.). For divided doses, take the exact amount of conjugate needed and do not re-introduce any waste product into the original bottle. In addition, **for doses dispensed with the aid of automatic and semi-automatic devices,** before using the conjugate, it is advisable to clean the fluid handling system, ensuring that the

procedures of washing, deproteinisation and decontamination are effective in avoiding contamination of the conjugate; this procedure is highly recommended when the kit is processed using analysers which are not equipped with disposable tips.
For this purpose, DiaMetra supplies a separate decontamination reagent for cleaning needles.

- If you use automated equipment, the user has the responsibility to make sure that the kit has been appropriately tested.
- The incomplete or inaccurate liquid removal from the wells could influence the assay precision and/or increase the background. To improve the performance of the kit on automatic systems is recommended to increase the number of washes.
- It is important that the time of reaction in each well is held constant for reproducible results. Pipetting of samples should not extend beyond ten minutes to avoid assay drift. If more than 10 minutes are needed, follow the same order of dispensation. If more than one plate is used, it is recommended to repeat the dose response curve in each plate
- Addition of the TMB Substrate solution initiates a kinetic reaction, which is terminated by the addition of the Stop Solution. Therefore, the TMB Substrate and the Stop Solution should be added in the same sequence to eliminate any time deviation during the reaction.
- Observe the guidelines for performing quality control in medical laboratories by assaying controls and/or pooled sera.
- Maximum precision is required for reconstitution and dispensation of the reagents.
- Samples microbiologically contaminated, highly lipemic or haemolysed should not be used in the assay.
- Plate readers measure vertically. Do not touch the bottom of the wells.

7. REAGENT STORAGE AND STABILITY

Store the kit at 2 – 8°C in the dark.

- The kit is stable at 2 – 8°C until the expiry date stated on the external kit label.
- Once opened, the kit is stable at 2 – 8°C for 6 months.
- The diluted wash solution is stable for 30 days at 2-8°C.

Important note: open the bag containing the Coated Microplate only when it is at room temperature and close it immediately after use.

8. SAMPLE COLLECTION AND STORAGE

The assay should be performed using serum (standard sampling tubes or tubes containing serum separating gel) or plasma (lithium heparin, sodium heparin, or potassium EDTA) samples.

Sample Storage	Duration
2 – 8 °C	96 hours
Freeze/thaw cycles	3 cycles

9. PROCEDURE

9.1. Preparation of Calibrators and Controls

The calibrators and controls are ready to use.
The calibrators and approximately the following concentrations:

	C_0	C_1	C_2	C_3	C_4
AU/mL	0	5	10	20	80

9.2. Preparation of the Wash Solution

Dilute the content of the vial “10X Conc. Wash Solution” with distilled water to a final volume of 500 mL prior to use. For smaller volumes respect the 1:10 dilution ratio.

It is possible to observe the presence of crystals within the concentrated wash solution; in this case mix at room temperature until the complete dissolution of crystals. For greater accuracy, dilute the whole bottle of concentrated wash solution to 500 mL, taking care also to transfer crystals completely by rinsing of the bottle, then mix until crystals are completely dissolved.

9.3. Preparation of Samples

All serum and plasma samples must be diluted 1:100 with sample diluent.
e.g., 10 µL of sample should be diluted with 990 µL of sample diluent.

9.4. Procedure

- **Allow all reagents to reach room temperature (22-28°C) for at least 30 minutes.** At the end of the assay, immediately store the reagents at 2-8°C: avoiding long exposure to room temperature.
- Unused coated microwell strips should be released securely in the foil pouch containing desiccant and stored at 2-8°C.
- To avoid potential microbial and/or chemical contamination, unused reagents should never be transferred into the original vials.
- As it is necessary to perform the determination in duplicate to improve accuracy of the test results, prepare two wells for each point of the calibration curve (C_0-C_4), two for each control, two for each sample, one for blank.

The following procedure is the same for both class IgG and IgM antibodies assay.

Reagents	Calibrator	Sample/ Controls	Blank
Use reagents for IgG or reagents for IgM depending on the isotype which is under investigation			
Calibrator C_0-C_4 (IgG or IgM)	**100 µL**		
Controls (IgG or IgM)		**100 µL**	
Diluted Sample		**100 µL**	
Incubate for 60 minutes at room temperature (22-28°C). Remove the content from each well; wash the wells 3 times with 300 µL diluted wash solution. **Important note**: during each washing step, gently shake the plate for 5 seconds and remove excess solution by tapping the inverted plate on an absorbent paper towel. **Automatic washer**: if you use automated equipment, wash the wells at least 5 times.			
Conjugate (IgG or IgM)	**100 µL**	**100 µL**	
Incubate for 60 minutes at room temperature (22-28°C). Remove the content from each well; wash the wells 3 times with 300 µL diluted wash solution. **Washing**: follow the same indications of the previous point.			
TMB Substrate	**100 µL**	**100 µL**	**100 µL**
Incubate for 15 minutes in the dark at room temperature (22-28°C).			
Stop Solution	**100 µL**	**100 µL**	**100 µL**
Shake the microplate gently. Read the absorbance (E) at 450 nm against a reference wavelength of 620-630 nm or against Blank within 5 minutes.			

10. QUALITY CONTROL

Good Laboratory Practice (GLP) requires the use of quality control specimens in each series of assays in order to check the performance of the assay. Controls should be treated as unknown samples, and the results analysed with appropriate statistical methods.

The kit controls provided in the kit should be tested as unknowns and are intended to assist in assessing the validity of results obtained with each assay plate.

The mean concentration of each control level is documented in the QC report included with each kit. These mean concentration levels are determined over several assays which are run in duplicate in multiple locations across each plate. This test is only valid if the optical density at 450 nm for the controls as well as for the calibrators (C_0-C_4) fall within with the respective range indicated on the Quality Control Certificate enclosed in each test kit.

DiaMetra recommends the users to maintain graphic records of the control values generated with each assay run, including the running means, SDs and %CVs. This information will facilitate the controls trending analysis relating to the performance of current and historical control lots relative to the supplied Quality Control data. The trending will assist in the identification of assays which give control values significantly different from their average range.

When interpreting control data, users should note that this product was designed and developed as a manual product. The range stated on the QC certificate should be appropriate for assays that are performed manually and with strict adherence to the Assay Procedure described above. It is recognised by Quality Control professionals, that as a result of differences in conditions and practices, there will always be variability in the mean values and precision of control measurements between different laboratories[21].

11. CALCULATION OF RESULTS

A variety of data reduction software packages are available, which may be employed to generate the mean calibration curve and to calculate the mean concentrations of unknown samples and controls. A 4-parameter logistic (4PL) curve fit with lin-log coordinates, **including Calibrator 0 is required.** A smoothed spline fit including Calibrator 0 can be used. Other curve fitting algorithms are not recommended.

Alternatively, a calibration curve may be prepared on semi-log graph paper by plotting mean absorbance on the Y-axis against concentration of analyte on the X-axis. Calibrator 0 should be included in the calibration curve. Read the mean absorbance value of each unknown sample off the curve.

12. MEASURING RANGE

12.1. For assessment of IgG class antibodies

The assay measuring range (AMR) is 2 – 80 AU/mL.
Any value that reads below 2 AU/mL should be reported as "< 2 AU/mL". Any value that reads above 80 AU/mL should be reported as "> 80 AU/mL".

12.2. For assessment of IgM class antibodies

The assay measuring range (AMR) is 2.08 – 80 AU/mL.
Any value that reads below 2.09 AU/mL should be reported as "< 2.08 AU/mL". Any value that reads above 80 AU/mL should be reported as "> 80 AU/mL".

13. METROLOGY AND TRACEABILITY

13.1. For assessment of IgG class antibodies

The calibrators of this kit are traceable to the Centres for Disease Control (CDC) Human IgG Anti-Cardiolipin Monoclonal Antibody HCAL - Catalogue [IS2717].

13.2. For assessment of IgM class antibodies

The calibrators of this kit are traceable to the Centres for Disease Control (CDC) Human IgM Anti-Cardiolipin Monoclonal Antibody EY2C9 [IS2718].

14. INTERPRETATION OF RESULTS

Concentration	Interpretation
< 8 AU/mL	The sample should be considered negative
8 – 10 AU/mL	The sample should be graded equivocal and repeat testing / sampling should be performed according to internal practices
>10 AU/mL	The sample should be considered positive

Determination of a range of expected values for a "normal" population of a given method is dependent on many factors, such as specificity and sensitivity of the method used and type of population under investigation. Therefore, each laboratory should consider the range given by the Manufacturer as a general indication and produce their own range of expected values based on the indigenous population.
Positive results should be verified concerning the entire clinical status of the patient, with the decision for therapy being taken on an individual basis. It is recommended that each laboratory establishes its own normal and pathological ranges of Anti-Cardiolipin antibody values.

15. PERFORMANCE CHARACTERISTICS

Representative performance data are shown. Results obtained at individual laboratories may vary.

15.1. For assessment of IgG class antibodies

15.1.1. Detection Capability

The limit of blank (LoB), limit of detection (LoD) and limit of quantitation (LoQ) were determined with guidance from CLSI EP17-A, "Protocols for Determination of Limits of Detection and Limits of Quantitation" using 6 blanks and 6 low level samples.

Sensitivity	Concentration
Limit of Blank (LoB)	0.59 AU/mL
Limit of Detection (LoD)	1.25 AU/mL
Limit of Quantitation (LoQ)	2.00 AU/mL

15.1.2. Trueness

Trueness of the Anti Cardiolipin Screen for assessment of IgG class antibodies has been demonstrated through performance of a recovery test using the CDC Human IgG Anti-Cardiolipin Monoclonal Antibody HCAL - Catalogue [IS2717].

15.1.3. Diagnostic Sensitivity and Specificity

The sensitivity and specificity were determined with guidance from CLSI EP-24 "Assessment of the Diagnostic Accuracy of Laboratory Tests Using Receiver Operating Characteristic Curves" using 50 negative and 51 positive samples run on two reagent lots.

		DKO144 - IgG		Total
		Positive	Negative	
True state	Positive	47	4	**51**
	Negative	0	57	**57**
Total		**47**	**61**	**108**

Diagnostic sensitivity: 92%

Diagnostic specificity: 100%

15.1.4. Precision

Precision of the Anti Cardiolipin Screen for determination of IgG class antibodies was determined by performing a complex precision study.

Repeatability: A total of 6 serum samples were assayed in 5 replicates, once a day for 5 days by 3 operators.
Data from one representative lot is shown below:

Sample	n	Mean Conc. (AU/mL)	Within run (Repeatability) SD	CV
1	75	6.95	0.38	5.5%
2	75	11.03	0.51	4.6%
3	75	20.12	0.94	4.7%
4	75	30.26	1.86	6.1%
5	75	50.11	2.58	5.1%
6	75	71.71	2.53	3.5%

Reproducibility: A total of 6 serum samples were assayed in 5 replicates, once a day for 5 days by 3 operators.
Results for the combined data from two lots is shown below:

Sample	n	Mean Conc. (AU/mL)	Within Laboratory (Reproducibility) SD	CV%
1	150	6.98	0.49	7.0%
2	150	11.17	0.84	7.5%
3	150	20.16	1.87	9.3%
4	150	30.38	3.06	10.1%
5	150	50.76	5.02	9.9%
6	150	72.30	3.93	5.4%

15.1.5. Linearity

Linearity was evaluated based on CLSI EP-06, "Evaluation of the Linearity of Quantitative Measurement Procedures". For anti-cardiolipin IgG concentration by Anti Cardiolipin Screen, the measurement procedure shows linearity for the interval from 0.84 to 83.68 ng/mL within the allowable deviation of linearity (ADL) of ±15 %.

15.2. Analytical Specificity

The following substances do not interfere with a bias of > ±15% in the Anti Cardiolipin Screen assay when assessing IgG class antibodies when the concentrations are below the stated threshold presented in the following table.

Potentially Interfering Reagent	Threshold Concentration
Bilirubin, conjugated	15 mg/dL
Bilirubin, unconjugated	15 mg/dL
Haemoglobin	200 mg/dL
Total Protein	10 g/dL
Triglyceride	500 mg/dL

15.2.1. Serum-plasma study

The Anti Cardiolipin Screen matrix comparison study for assessment of IgG class antibodies was performed to evaluate the difference across tube types (serum separator tubes (SST), lithium heparin plasma, sodium heparin plasma and K2 EDTA plasma) versus the control samples (red top serum, without additive) following CLSI EP9-A3 guidelines. A total of 22 samples (18 native, 4 spiked) to cover the range were evaluated. Linear regression analysis was performed on the comparative data:

Sample type	Slope [95% CI]	Intercept (ng/mL) [95% CI]	Correlation coefficient (r)
SST	0.96 [0.92 – 0.98]	0.64 [-0.38 to 1.66]	1.00
Lithium Heparin	0.92 [0.88 to 0.96]	0.87 [-0.24 to 1.99]	1.00
Sodium Heparin	0.94 [0.89 to 0.98]	0.66 [-0.75 to 2.06]	0.99
EDTA	0.95 [0.92 to 0.99]	0.54 [-0.69 to 1.77]	1.00

15.3. For assessment of IgM class antibodies

15.3.1. Detection Capability

The limit of blank (LoB), limit of detection (LoD) and limit of quantitation (LoQ) were determined with guidance from CLSI EP17-A, "Protocols for Determination of Limits of Detection and Limits of Quantitation" using 6 blanks and 6 low level samples.

Sensitivity	Concentration
Limit of Blank (LoB)	0.76 AU/mL
Limit of Detection (LoD)	1.45 AU/mL
Limit of Quantitation (LoQ)	2.08 AU/mL

15.3.2. Trueness

Trueness of the Anti Cardiolipin Screen for assessment of IgM class antibodies demonstrated through performance of a recovery test using the CDC Human IgM Anti-Cardiolipin Monoclonal Antibody EY2C9 [IS2718].

15.3.3. Diagnostic Sensitivity and Specificity

The sensitivity and specificity were determined with guidance from CLSI EP-24 "Assessment of the Diagnostic Accuracy of Laboratory Tests Using Receiver Operating Characteristic Curves" using 73 negative and 62 positive samples run on two reagent lots.

		DKO144 IgM		Total
		Positive	Negative	
True state	Positive	50	12	**62**
	Negative	0	73	**73**
Total		**50**	**85**	**135**

Diagnostic sensitivity: 80%

Diagnostic specificity: 100%

15.3.4. Precision

Precision of the Anti Cardiolipin Screen for determination of IgM class antibodies determined by performing a complex precision study.

Repeatability: A total of 6 serum samples were assayed in 5 replicates, once a day for 5 days by 3 operators.
Data from one representative lot is shown below:

Sample	n	Mean Conc. (AU/mL)	Within run (Repeatability) SD	CV
1	75	7.74	0.40	5.2%
2	75	12.46	0.51	4.1%
3	75	21.06	0.99	4.7%
4	75	32.07	1.46	4.6%
5	75	55.15	1.19	2.2%
6	75	75.45	2.33	3.1%

Reproducibility: A total of 6 serum samples were assayed in 5 replicates, once a day for 5 days by 3 operators.
Results for the combined data from two lots is shown below:

Sample	n	Mean Conc. (AU/mL)	Within Laboratory (Reproducibility)	
			SD	CV%
1	150	7.65	0.46	6.0%
2	150	12.33	0.74	6.0%
3	150	21.03	1.60	7.6%
4	150	31.89	1.98	6.2%
5	150	54.28	2.70	5.0%
6	150	75.46	2.63	3.5%

15.3.5. Linearity

Linearity was evaluated based on CLSI EP-06, "Evaluation of the Linearity of Quantitative Measurement Procedures". For anti-cardiolipin IgM concentration by Anti Cardiolipin Screen, the measurement procedure shows linearity for the interval from 0.82 to 86.88 AU/mL within the allowable deviation of linearity (ADL) of ±15 %.

15.3.6. Analytical Specificity

The following substances do not interfere with a bias of > ±15% in the Anti Cardiolipin Screen assay when assessing IgM class antibodies when the concentrations are below the stated threshold presented in the following table.

Potentially Interfering Reagent	Threshold Concentration
Bilirubin, conjugated	15 mg/dL
Bilirubin, unconjugated	15 mg/dL
Haemoglobin	200 mg/dL
Total Protein	10 g/dL
Triglyceride	500 mg/dL

15.3.7. Serum-plasma study

The Anti Cardiolipin Screen matrix comparison study for assessment of IgM class antibodies was performed to evaluate the difference across tube types (serum separator tubes (SST), lithium heparin plasma, sodium heparin plasma and K2 EDTA plasma) versus the control samples (red top serum, without additive) following CLSI EP9-A3 guidelines. A total of 20 samples (16 native, 4 spiked) to cover the assay range were evaluated. Linear regression analysis was performed on the comparative data:

Sample type	Slope [95% CI]	Intercept (ng/mL) [95% CI]	Correlation coefficient (r)
SST	1.02 [0.94 to 1.09]	0.19 [-1.34 to 1.72]	0.99
Lithium Heparin	0.93 [0.82 to 1.04]	0.73 [-1.49 to 2.95]	0.97
Sodium Heparin	0.93 [0.84 to 1.01]	0.64 [-1.00 to 2.28]	0.98
EDTA	0.96 [0.86 to 1.05]	0.65 [-1.19 to 2.49]	0.98

16. LIMITATIONS OF USE

- As in the case of any diagnostic procedure, results must be interpreted in conjunction with the patient's clinical presentation and other information available to the physician.
- The performance characteristics of this assay have not been established in a paediatric population.
- Heterophilic antibodies in human serum can react with reagent immunoglobulins, interfering with *in vitro* immunoassays[22]. Patients routinely exposed to animals or to animal serum products can be prone to this interference and anomalous values may be observed.
- The presence of immune complexes or other immunoglobulin aggregates in the patient sample may cause an increased level of non-specific binding and produce false positives in this assay.

17. WASTE MANAGEMENT

Reagents must be disposed of in accordance with local regulations.

18. BIBLIOGRAPHY

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19. REVISION IDENTIFIER

Additions or changes to the IFU are indicated by grey highlighting.

20. PRODUCT COMPLAINTS AND TECHNICAL SUPPORT

For a patient/user/third party in the European Union and in countries with similar regulatory regime (Regulation 2017/746/EU on IVD Medical Devices); if, during the use of this device or as a result of its use, a serious incident has occurred, please report it to the manufacturer and/or its authorised representative and to your national regulatory authority.
The manufacturer can be contacted through their customer service or technical support team. The contact details can be found below and on the company website: www.diametra.com.

Ed. 03/2022 **DCM144-2**

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CE

DiaMetra
care for quality

DCM144-2
Ed. 03/2022

Anti Cardiolipin Screen

para el análisis de rutina

Determinación cuantitativa de autoanticuerpos IgG o IgM contra la cardiolipina en suero o plasma humano

IVD | **LOT** Ver etiqueta externa | 2°C 8°C | Σ = 96 pruebas | **REF DKO144**

1. FINALIDAD PREVISTA

Para uso en diagnóstico *in vitro*
Para uso profesional de laboratorio

Anti Cardiolipin Screen es un dispositivo manual de diagnóstico *in vitro* destinado a la determinación cuantitativa de anticuerpos tanto IgG como IgM dirigidos contra la cardiolipina en suero o plasma humano. Los resultados deben utilizarse como ayuda en el diagnóstico del síndrome antifosfolípido (SAF) junto con otros datos clínicos y de laboratorio.

2. IMPORTANCIA CLÍNICA

La cardiolipina es un fosfolípido con carga negativa que suele estar localizado en la membrana mitocondrial interna[1]. Los autoanticuerpos dirigidos contra la cardiolipina forman parte de un grupo conocido como anticuerpos antifosfolípidos que incluye los autoanticuerpos anti-β2 glicoproteína 1. La medición de los autoanticuerpos anticardiolipina se considera uno de los marcadores más importantes para apoyar el diagnóstico del síndrome antifosfolípido (SAF)[2,3].

El SAF es un trastorno autoinmunitario sistémico caracterizado por una combinación de trombos arteriales y/o venosos, complicaciones del embarazo, como la pérdida gestacional recurrente, y niveles elevados de anticuerpos antifosfolípidos[4]. El SAF se describió por primera vez en pacientes con lupus eritematoso sistémico (LES), aunque posteriormente se ha establecido que el LES puede existir de forma independiente a una enfermedad subyacente[5].

El SAF puede darse por sí mismo (SAF primario) o puede estar asociado a otras condiciones médicas como el LES (SAF secundario)[6] (6). Sin embargo, se ha demostrado que pueden detectarse anticuerpos anticardiolipina (aCL) en pacientes con LES que no desarrollan SAF secundario[7-9]. No obstante, los eventos tromboembólicos son la manifestación clínica más común del SAF.

Los anticuerpos anticardiolipina pueden reconocer tanto la cardiolipina como partes del complejo de fosfolípidos y proteínas β2 glicoproteína 1-cardiolipina[10,11].

Existen estudios que indican una asociación de los anticuerpos anticardiolipina IgG e IgM[2,7,11-16] con los eventos trombóticos, mientras que otros sugieren que estos están relacionados con el isotipo IgG, pero no con el IgM[6,10,17].

Se ha demostrado que los anticuerpos IgM aCL aparecen en infecciones como la hepatitis C crónica, la lepra o la sífilis, pero no están directamente implicados en eventos trombóticos[17].

Es probable que la presencia de anticuerpos antifosfolípidos, incluidos los aCL IgG e IgM, constituya el factor de riesgo más reconocible en los casos de pérdida de recurrente del embarazo y de complicaciones obstétricas mediadas por la placenta tardía[4-6,11,14-15,18,19]. Las pacientes pueden presentar únicamente resultados adversos del embarazo con eventos vasculares aislados o con manifestaciones tanto obstétricas como trombóticas[6]. Se ha sugerido que los anticuerpos anti-β2-glicoproteína 1-cardiolipina son capaces de reconocer el antígeno en los tejidos de la placenta, inhibiendo el crecimiento y la diferenciación de los trofoblastos, lo que puede causar finalmente una placentación defectuosa[20].

3. PRINCIPIO DEL MÉTODO

Anti Cardiolipin Screen permite la determinación de autoanticuerpos dirigidos contra el complejo cardiolipina-β2-glicoproteína mediante dos curvas de calibración y conjugados enzimáticos diferentes (uno específico para la prueba IgG y otro específico para la prueba IgM) y una microplaca. El principio del método y el procedimiento de ensayo son los mismos para ambas evaluaciones. Utilice reactivos para IgG o reactivos para IgM según el isotipo que se esté investigando.

El ensayo Anti Cardiolipin Screen es un ensayo enzimático inmunométrico (ELISA) de dos pasos tipo sándwich en el que las muestras de los pacientes, los calibradores o los controles se incuban en placas de microtitulación recubiertas con el complejo antigénico cardiolipina-β2 glicoproteína. Durante la incubación, los anticuerpos presentes en la muestra de ensayo se unen al complejo de antígenos inmovilizados. Tras la incubación, la separación ligada/libre se realiza mediante un simple lavado en fase sólida.

A continuación, se realiza una incubación con IgM o IgG antihumano conjugado con peroxidasa de rábano picante (HRP), que se une a los anticuerpos inmovilizados. Se realiza otro paso de lavado para eliminar el exceso de conjugado. A continuación, se dispensa en los pocillos una solución de sustrato cromogénico que contiene TMB, que reacciona con el HRP conjugado y se desarrolla un color azul que cambia a amarillo cuando se añade la solución de detención (H_2SO_4).

La intensidad del color es directamente proporcional a la concentración de anticardiolipina IgM o IgG (dependiendo del conjugado que se utilice) de la muestra.

La concentración de anticuerpos anticardiolipina en la muestra se calcula mediante una curva de calibración.

4. REACTIVOS, MATERIALES E INSTRUMENTACIÓN

4.1. Reactivos y materiales incluidos en el kit

Para la determinación de anticuerpos de clase IgG

1. Calibradores de anticardiolipina IgG
(5 viales de 1,2 mL cada uno)
Tampón fosfato 0,1 M, NaN_3 < 0,1 %, suero humano

CAL0	**REF DCE002/11306-0**
CAL1	**REF DCE002/11307-0**
CAL2	**REF DCE002/11308-0**
CAL3	**REF DCE002/11309-0**
CAL4	**REF DCE002/11310-0**

2. Controles (2 viales de 1,2 mL cada uno, listos para usar)
Tampón fosfato 0,1 M, NaN_3 <0,1 %, suero humano

Control negativo	**REF DCE045/11301-0**
Control positivo	**REF DCE045/11302-0**

3. Conjugado IgG (1 vial, 15 mL)
IgG antihumano conjugado con peroxidasa de rábano picante (HRP), BSA 0,1 %, ProClin < 0,0015 %
REF DCE002/11302-0

Para la determinación de anticuerpos de clase IgM

1. Calibradores (5 viales de 1,2 mL cada uno)
Tampón fosfato 0,1 M, NaN_3 < 0,1 %, suero humano

CAL0	**REF DCE002/11206-0**
CAL1	**REF DCE002/11207-0**
CAL2	**REF DCE002/11208-0**
CAL3	**REF DCE002/11209-0**
CAL4	**REF DCE002/11210-0**

2. Controles (2 viales de 1,2 mL cada uno, listos para usar)
Tampón fosfato 0,1 M, NaN_3 <0,1 %, suero humano

Control negativo	**REF DCE045/11201-0**
Control positivo	**REF DCE045/11202-0**

3. Conjugado (1 vial, 15 mL)
IgM antihumano conjugado con peroxidasa de rábano picante (HRP), BSA 0,1 %, ProClin < 0,0015 %
REF DCE002/11202-0

Reactivos comunes

4. Diluyente de muestras (1 vial, 100 mL)
Tampón fosfato 0,1 M, NaN_3 < 0,1 %
REF DCE053-0

5. Microplaca recubierta (1 microplaca que se puede romper)
Microplaca recubierta con el complejo antigénico cardiolipina-β2 glicoproteína **REF DCE002/14403-0**

6. Sustrato de TMB (1 vial, 15 mL)
H_2O_2 -TMB (0,26 g/L) *(evitar el contacto con la piel)*
REF DCE004-0

7. Solución de detención (1 vial, 15 mL)
Ácido sulfúrico 0,15 M *(evitar el contacto con la piel)*
REF DCE005-0

8. Conc. 10X Solución de lavado (1 vial, 50 mL)
Tampón fosfato 0,2 M, pH 7,4 **REF DCE054-0**

4.2. Materiales necesarios pero no suministrados

Agua destilada

4.3. Materiales auxiliares e instrumentación

Dispensador automático
Dispositivos de pipetas de precisión
Lector de microplacas (450 nm, 620-630 nm)

5. ADVERTENCIAS

- Este kit está destinado al uso *in vitro* realizado exclusivamente por profesionales. No es para uso interno o externo en personas ni animales.
- Utilice el equipo de protección personal adecuado cuando trabaje con los reactivos suministrados.
- Siga las prácticas de laboratorio recomendadas (BPL) para manipular productos sanguíneos.
- Todo el material de origen humano utilizado en la preparación de los reactivos ha sido sometido a pruebas que han dado resultado negativo para los anticuerpos contra el VIH-1 y VIH-2, el HbsAg y el VHC. Sin embargo, ningún método de prueba puede ofrecer una garantía total de ausencia de VIH, VHB, VHC u otros agentes infecciosos. Por lo tanto, los calibradores y los controles deben manejarse de la misma manera que el material potencialmente infeccioso.
- El material de origen animal utilizado en la preparación del kit se ha obtenido de animales certificados como sanos y la proteína bovina se ha obtenido de países donde no hay infección de EEB, pero estos materiales deben manejarse como potencialmente infecciosos.
- Algunos reactivos contienen pequeñas cantidades de azida sódica (NaN_3) o ProClin™ 300 como conservante. Evite el contacto con la piel o las mucosas.
- La azida sódica puede ser tóxica si se ingiere o se absorbe a través de la piel o los ojos; además, puede reaccionar con las tuberías de plomo o cobre para formar azidas metálicas potencialmente explosivas. Si elimina los reactivos en un fregadero, lávelos con gran cantidad de agua para evitar la acumulación de azida.
- El sustrato de TMB contiene un irritante que es perjudicial si se inhala, se ingiere o se absorbe a través de la piel. Para evitar lesiones, evite la inhalación, la ingestión o el contacto con la piel y los ojos.
- La solución de detención consiste en una solución diluida de ácido sulfúrico. El ácido sulfúrico es venenoso, corrosivo y puede ser tóxico si se ingiere. Para evitar quemaduras químicas, evite el contacto con la piel y los ojos.
- Evite la exposición del reactivo TMB/H_2O_2 a la luz solar directa, a metales o a oxidantes. No congele la solución.

6. PRECAUCIONES

- Siga estrictamente la secuencia de pasos de pipeteado que se indica en este protocolo. Los datos de rendimiento representados en este documento se obtuvieron utilizando los reactivos específicos indicados en estas instrucciones de uso.
- Todos los reactivos deben conservarse refrigerados entre 2 y 8 °C en su envase original. Las excepciones se indican claramente.
- Deje que todos los componentes del kit y las muestras alcancen la temperatura ambiente (22-28 °C) y mezcle bien antes de usarlos.

- No intercambie componentes del kit procedentes de diferentes lotes. Debe respetarse la fecha de caducidad impresa en las etiquetas de la caja y de los viales. No utilice ningún componente del kit después de su fecha de caducidad.
- **ADVERTENCIA: el reactivo conjugado está diseñado para garantizar la máxima sensibilidad de la dosis y puede contaminarse con agentes externos si no se utiliza correctamente;** por lo tanto, se recomienda utilizar consumibles desechables (puntas, frascos, bandejas, etc.). Para dosis divididas, tome la cantidad exacta de conjugado necesaria y no vuelva a introducir ningún producto de desecho en el frasco original. Además, **para las dosis dispensadas mediante dispositivos automáticos y semiautomáticos,** antes de utilizar el conjugado, es aconsejable limpiar el sistema de manipulación de fluidos, asegurándose de que los procedimientos de lavado, desproteinización y descontaminación sean eficaces para evitar la contaminación del conjugado; este procedimiento es muy recomendable cuando el kit se procesa con analizadores que no están equipados con puntas desechables.
  Para ello, DiaMetra proporciona un reactivo de descontaminación independiente para la limpieza de las agujas.
- Si el usuario utiliza un equipo automatizado, tiene la responsabilidad de asegurarse de que el kit ha sido debidamente probado.
- La eliminación incompleta o imprecisa del líquido de los pocillos podría alterar la precisión del ensayo y/o aumentar el fondo. Para mejorar el rendimiento del kit en sistemas automáticos se recomienda aumentar el número de lavados.
- Es importante que el tiempo de reacción en cada pocillo se mantenga constante para obtener resultados reproducibles. El pipeteo de las muestras no debe prolongarse más de diez minutos para evitar errores en el ensayo. Si se necesitan más de 10 minutos, siga el mismo orden de dispensación. Si se utiliza más de una placa, se recomienda repetir la curva dosis-respuesta en cada placa.
- La adición de la solución de sustrato de TMB inicia una reacción cinética, que finaliza al añadir la solución de detención. Por lo tanto, el sustrato de TMB y la solución de detención deben añadirse en la misma secuencia para eliminar las posibles desviaciones temporales durante la reacción.
- Respete las directrices para realizar el control de calidad en los laboratorios médicos mediante el ensayo de controles y/o sueros combinados.
- Se requiere la máxima precisión en la reconstitución y dispensación de los reactivos.
- No se deben usar en el ensayo muestras contaminadas microbiológicamente, muy lipémicas o hemolizadas.
- Los lectores de placas miden en vertical. No toque el fondo de los pocillos.

7. ALMACENAMIENTO Y ESTABILIDAD DE LOS REACTIVOS

Almacene el kit a 2-8 ºC en un lugar oscuro.

- El kit es estable a 2-8 ºC hasta la fecha de caducidad indicada en su etiqueta externa.
- Una vez abierto, el kit es estable a 2-8 °C durante 6 meses.
- La solución de lavado diluida es estable durante 30 días a 2-8 °C.

Nota importante: abra la bolsa que contiene la microplaca recubierta solo cuando esté a temperatura ambiente y ciérrela inmediatamente después de su uso.

8. RECOGIDA Y ALMACENAMIENTO DE LAS MUESTRAS

El ensayo debe realizarse usando muestras de suero (tubos de muestras estándar o tubos que contienen gel de separación de suero) o plasma (heparina de litio, heparina de sodio o EDTA de potasio).

Almacenamiento de muestras	Duración
2-8 ºC	96 horas
Ciclos de congelación/descongelación	3 ciclos

9. PROCEDIMIENTO

9.1. Preparación de calibradores y controles

Los calibradores y controles están listos para usarse.
Los calibradores tienen aproximadamente las siguientes concentraciones:

	C_0	C_1	C_2	C_3	C_4
AU/mL	0	5	10	20	80

9.2. Preparación de la solución de lavado

Diluir el contenido del vial «10X Conc. Wash Solution» con agua destilada hasta un volumen final de 500 mL antes de usarlo. Para volúmenes más pequeños, respete la relación de dilución de 1:10.

Es posible que observe la presencia de cristales dentro de la solución de lavado concentrada; en este caso, mezcle a temperatura ambiente hasta la completa disolución de los cristales. Para una mayor precisión, diluya todo el frasco de solución de lavado concentrada a 500 mL, teniendo cuidado también de transferir los cristales enjuagando completamente el frasco y luego mezclando hasta que los cristales se disuelvan completamente.

9.3. Preparación de las muestras

Todas las muestras de suero y plasma deben diluirse a una concentración de 1:100 con diluyente de muestras.
Por ejemplo, 10 µL de muestra deben diluirse con 990 µL de diluyente de muestras.

9.4. Procedimiento

- **Deje que todos los reactivos alcancen la temperatura ambiente (22-28 ºC) durante al menos 30 minutos.** Al finalizar el ensayo, almacene inmediatamente los reactivos a 2-8 ºC: evite la exposición prolongada a la temperatura ambiente.
- Las tiras de micropocillos recubiertas no utilizadas deben dejarse de forma segura en el envoltorio de papel de aluminio que contiene desecante y almacenarse a 2-8 ºC.
- Para evitar que se produzca una posible contaminación microbiana y/o química, los reactivos no utilizados nunca se deberán transferir a los viales originales.

- Como es necesario realizar la determinación por duplicado para mejorar la precisión de los resultados de la prueba, prepare dos pocillos para cada punto de la curva de calibración (C_0-C_4), dos para cada control, dos para cada muestra y uno para el blanco.

El siguiente procedimiento es el mismo para el ensayo de anticuerpos de clase IgG e IgM.

Reactivos	Calibrador	Muestra/ Controles	Blanco
Utilice reactivos para IgG o reactivos para IgM según el isotipo que se esté investigando.			
Calibrador C_0-C_4 (IgG o IgM)	**100 µL**		
Controles (IgG o IgM)		**100 µL**	
Muestra diluida		**100 µL**	
Incube durante 60 minutos a temperatura ambiente (22-28 °C). Retire el contenido de cada pocillo, lave los pocillos 3 veces con 300 µL de solución de lavado diluida. **Nota importante**: en cada paso de lavado, agite ligeramente la placa durante 5 segundos y elimine el exceso de solución golpeando la placa invertida sobre un paño de papel absorbente. **Lavadora automática**: si utiliza un equipo automático, lave los pocillos al menos 5 veces.			
Conjugado (IgG o IgM)	**100 µL**	**100 µL**	
Incube durante 60 minutos a temperatura ambiente (22-28 °C). Retire el contenido de cada pocillo, lave los pocillos 3 veces con 300 µL de solución de lavado diluida. **Lavado**: siga las mismas indicaciones del punto anterior.			
Sustrato de TMB	**100 µL**	**100 µL**	**100 µL**
Incube durante 15 minutos en un lugar oscuro a temperatura ambiente (22-28 °C).			
Solución de detención	**100 µL**	**100 µL**	**100 µL**
Agite suavemente la microplaca. Compare la absorbancia (E) a 450 nm con la obtenida con una longitud de onda de referencia de 620-630 nm o con el blanco en un plazo de 5 minutos.			

10. CONTROL DE CALIDAD

Las prácticas de laboratorio recomendadas (BPL) requieren el uso de muestras de control de calidad en cada serie de ensayos para comprobar el rendimiento del ensayo. Los controles deberán tratarse como muestras desconocidas y los resultados deberán analizarse con métodos estadísticos adecuados.

Los controles incluidos en el kit deberán ser probados como desconocidos y están destinados a ayudar a evaluar la validez de los resultados obtenidos con cada placa de ensayo.

La concentración media de cada nivel de control se documenta en el informe de control de calidad que se incluye en cada kit. Los niveles de concentración media se determinan respecto de varios análisis, los cuales se realizan por duplicado en varios puntos diferentes de cada placa. Esta prueba solo es válida si la densidad óptica a 450 nm de los controles y de los calibradores (C_0-C_4) está dentro del rango respectivo indicado en el Certificado de control de calidad que se incluye con cada kit de prueba.

DiaMetra recomienda que los usuarios mantengan registros gráficos de los valores de control que se generan con cada ensayo, incluida la media de ejecución, la DE (desviación estándar) y el % CV. Esta información facilitará los ensayos de tendencia de los controles relacionados con el rendimiento de lotes de control actuales e históricos relativos a los datos de control de calidad proporcionados. La tendencia facilitará la identificación de los análisis que generan valores de control significativamente distintos de su intervalo medio.

Al interpretar los datos de control, los usuarios deberán tener en cuenta que este producto fue diseñado y desarrollado como un producto manual. El rango establecido en el certificado de control de calidad deberá ser adecuado para los ensayos que se realizan manualmente y en estricto cumplimiento del procedimiento de ensayo anteriormente descrito. Los profesionales del control de la calidad reconocen que, como resultado de las diferencias en las condiciones y en las prácticas, siempre habrá variaciones entre laboratorios en los valores medios y en la precisión de las mediciones de control[21].

11. CÁLCULO DE LOS RESULTADOS

Hay disponibles diversos paquetes de software de reducción de datos que se pueden utilizar para generar el promedio de la curva de calibración y para calcular el promedio de las concentraciones de muestras y controles desconocidos. Es necesario un ajuste de curva logístico de 4 parámetros (4PL) con coordenadas lineales y logarítmicas, **incluido el calibrador 0.** También se puede usar un ajuste de splines suavizado que incluya el calibrador 0. No se recomiendan otros algoritmos de ajuste de curva.

También se puede preparar una curva de calibración en papel semilogarítmico mediante el trazado de la absorbancia media en el eje Y frente a la concentración de analitos en el eje X. El calibrador 0 debe incluirse en la curva de calibración. Lea el valor de absorbancia medio de cada muestra desconocida que se encuentra fuera de la curva.

12. RANGO DE MEDICIÓN

12.1. Para la evaluación de anticuerpos de clase IgG

El rango de medición del ensayo (AMR) es de 2-80 AU/mL. Cualquier valor que sea inferior a 2 AU/mL debe informarse como «< 2 AU/mL». Cualquier valor que sea superior a 80 AU/mL debe informarse como «> 80 AU/mL».

12.2. Para la evaluación de anticuerpos de clase IgM
El rango de medición del ensayo (AMR) es de 2,08-80 AU/mL.
Cualquier valor que sea inferior a 2,09 AU/mL debe informarse como «< 2,08 AU/mL». Cualquier valor que sea superior a 80 AU/mL debe informarse como «> 80 AU/mL».

13. METROLOGÍA Y TRAZABILIDAD

13.1. Para la evaluación de anticuerpos de clase IgG
Los calibradores de este kit son trazables según el catálogo del Centro para el Control de Enfermedades (CDC) sobre el anticuerpo humano monoclonal anticardiolipina IgG HCAL [IS2717].

13.2. Para la evaluación de anticuerpos de clase IgM
Los calibradores de este kit son trazables según el estándar EY2C9 del Centro para el Control de Enfermedades (CDC) sobre el anticuerpo humano monoclonal anticardiolipina IgM [IS2718].

14. INTERPRETACIÓN DE LOS RESULTADOS

Concentración	Interpretación
<8 AU/mL	La muestra debe considerarse negativa.
8-10 AU/mL	La muestra debe ser calificada como equívoca y la repetición de la prueba/muestreo debe realizarse de acuerdo con las prácticas internas.
> 10 AU/mL	La muestra debe considerarse positiva.

La determinación de un rango de valores esperados para una población «normal» de un método dado depende de muchos factores, como la especificidad y la sensibilidad del método utilizado y el tipo de población en investigación. Por lo tanto, cada laboratorio debe considerar el rango dado por el fabricante como una indicación general y establecer su propio rango de valores esperados en función de la población autóctona.

Los resultados positivos deben verificarse en relación con el estado clínico general del paciente, y la decisión de la terapia se toma de forma individual. Se recomienda que cada laboratorio establezca sus propios rangos normales y patológicos de valores de anticuerpos anticardiolipina.

15. CARACTERÍSTICAS DE RENDIMIENTO

Se muestran los datos de rendimiento representativos. Los resultados obtenidos en diferentes laboratorios pueden diferir.

15.1. Para la evaluación de anticuerpos de clase IgG

15.1.1. Capacidad de detección
El límite de blanco (LoB), el límite de detección (LoD) y el límite de cuantificación (LoQ) se determinaron con orientación del documento CLSI EP17-A, “Protocols for Determination of Limits of Detection and Limits of Quantitation”, usando 6 blancos y 6 muestras de bajo nivel.

Sensibilidad	Concentración
Límite de blanco (LoB)	0,59 AU/mL
Límite de detección (LoD)	1,25 AU/mL
Límite de cuantificación (LoQ)	2,00 AU/mL

15.1.2. Veracidad
La veracidad del ensayo Anti Cardiolipin Screen para la evaluación de anticuerpos de clase IgG se ha demostrado mediante la realización de una prueba de recuperación utilizando el catálogo del CDC sobre el anticuerpo humano monoclonal anticardiolipina IgG HCAL [IS2717].

15.1.3. Sensibilidad y especificidad del diagnóstico
La sensibilidad y la especificidad se determinaron con orientación del documento CLSI EP-24 “Assessment of the Diagnostic Accuracy of Laboratory Tests Using Receiver Operating Characteristic Curves”, usando 50 muestras negativas y 51 positivas realizadas en dos lotes de reactivos.

		DKO144 - IgG		Total
		Positivo	Negativo	
Estado real	Positivo	47	4	51
	Negativo	0	57	57
Total		47	61	108

Sensibilidad del diagnóstico: 92 %

Especificidad del diagnóstico: 100 %

15.1.4. Precisión
La precisión del ensayo Anti Cardiolipin Screen para la determinación de anticuerpos de clase IgG se determinó mediante la realización de un estudio de precisión complejo.

Repetibilidad: Se analizaron un total de 6 muestras de suero en 5 réplicas, una vez al día durante 5 días por 3 operadores.
A continuación se muestran los datos de un lote representativo:

Muestra	n	Medio conc. (AU/mL)	Intraprueba (repetibilidad)	
			DE	CV
1	75	6,95	0,38	5,5 %
2	75	11,03	0,51	4,6 %
3	75	20,12	0,94	4,7 %
4	75	30,26	1,86	6,1 %
5	75	50,11	2,58	5,1 %
6	75	71,71	2,53	3,5 %

Reproducibilidad: Se analizaron un total de 6 muestras de suero en 5 réplicas, una vez al día durante 5 días por 3 operadores.
A continuación se muestran los resultados de los datos combinados de dos lotes:

Muestra	n	Medio conc. (AU/mL)	Dentro del laboratorio (reproducibilidad)	
			DE	CV %
1	150	6,98	0,49	7,0 %
2	150	11,17	0,84	7,5 %
3	150	20,16	1,87	9,3 %
4	150	30,38	3,06	10,1 %
5	150	50,76	5,02	9,9 %
6	150	72,30	3,93	5,4 %

15.1.5. Linealidad
La linealidad se evaluó en base a CLSI EP-06, “Evaluation of the Linearity of Quantitative Measurement Procedures”. Para la concentración de anticardiolipina IgG mediante el ensayo Anti Cardiolipin Screen, el procedimiento de medición muestra linealidad para el intervalo de 0,84 a 83,68 ng/mL dentro de la desviación de linealidad permitida (ADL) de ±15 %.

15.2. Especificidad analítica
Las siguientes sustancias no interfieren con un sesgo de > ±15 % en el ensayo Anti Cardiolipin Screen para la evaluación de anticuerpos de clase IgG cuando las concentraciones están por debajo del umbral indicado presentado en la siguiente tabla.

Reactivos que pueden interferir	Límite máximo de concentración
Bilirrubina, conjugada	15 mg/dL
Bilirrubina, no conjugada	15 mg/dL
Hemoglobina	200 mg/dL
Proteína total	10 g/dL
Triglicéridos	500 mg/dL

15.2.1. Estudio en suero-plasma
El estudio de comparación de la matriz de Anti Cardiolipin Screen para la evaluación de anticuerpos de clase IgG se realizó para evaluar la diferencia entre los tipos de tubos (tubos separadores de suero [SST], plasma de heparina de litio, plasma de heparina sódica y plasma K2 EDTA) frente a las muestras de control (tapón rojo para suero, sin aditivos) siguiendo las directrices de CLSI EP9-A3. Se evaluó un total de 22 muestras (18 nativas, 4 con aditivos) para cubrir el intervalo. Se realizó un análisis de regresión lineal sobre los datos comparativos:

Tipo de muestra	Pendiente [IC del 95 %]	Intersección (ng/mL) [IC del 95 %]	Coeficiente de correlación (r)
SST	0,96 [0,92 a 0,98]	0,64 [–0,38 a 1,66]	1,00
Heparina de litio	0,92 [0,88 a 0,96]	0,87 [–0,24 a 1,99]	1,00
Heparina sódica	0,94 [0,89 a 0,98]	0,66 [–0,75 a 2,06]	0,99
EDTA	0,95 [0,92 a 0,99]	0,54 [–0,69 a 1,77]	1,00

15.3. Para la evaluación de anticuerpos de clase IgM

15.3.1. Capacidad de detección
El límite de blanco (LoB), el límite de detección (LoD) y el límite de cuantificación (LoQ) se determinaron con orientación del documento CLSI EP17-A, “Protocols for Determination of Limits of Detection and Limits of Quantitation”, usando 6 blancos y 6 muestras de bajo nivel.

Sensibilidad	Concentración
Límite de blanco (LoB)	0,76 AU/mL
Límite de detección (LoD)	1,45 AU/mL
Límite de cuantificación (LoQ)	2,08 AU/mL

15.3.2. Veracidad
La veracidad del ensayo Anti Cardiolipin Screen para la evaluación de anticuerpos de clase IgM se ha demostrado mediante la realización de una prueba de recuperación utilizando el estándar EY2C9 del CDC sobre el anticuerpo humano monoclonal anticardiolipina IgM [IS2718].

15.3.3. Sensibilidad y especificidad del diagnóstico
La sensibilidad y la especificidad se determinaron con orientación del documento CLSI EP-24 “Assessment of the Diagnostic Accuracy of Laboratory Tests Using Receiver Operating Characteristic Curves”, usando 73 muestras negativas y 62 positivas realizadas en dos lotes de reactivos.

		DKO144 IgM		Total
		Positivo	Negativo	
Estado real	Positivo	50	12	62
	Negativo	0	73	73
Total		50	85	135

Sensibilidad del diagnóstico: 80 %

Especificidad del diagnóstico: 100 %

15.3.4. Precisión

La precisión del ensayo Anti Cardiolipin Screen para la determinación de anticuerpos de clase IgM se determinó mediante la realización de un estudio de precisión complejo.

Repetibilidad: Se analizaron un total de 6 muestras de suero en 5 réplicas, una vez al día durante 5 días por 3 operadores.

A continuación se muestran los datos de un lote representativo:

Muestra	n	Medio conc. (AU/mL)	Intraprueba (repetibilidad)	
			DE	CV
1	75	7,74	0,40	5,2 %
2	75	12,46	0,51	4,1 %
3	75	21,06	0,99	4,7 %
4	75	32,07	1,46	4,6 %
5	75	55,15	1,19	2,2 %
6	75	75,45	2,33	3,1 %

Reproducibilidad: Se analizaron un total de 6 muestras de suero en 5 réplicas, una vez al día durante 5 días por 3 operadores.

A continuación se muestran los resultados de los datos combinados de dos lotes:

Muestra	n	Medio conc. (AU/mL)	Dentro del laboratorio (reproducibilidad)	
			DE	CV %
1	150	7,65	0,46	6,0 %
2	150	12,33	0,74	6,0 %
3	150	21,03	1,60	7,6 %
4	150	31,89	1,98	6,2 %
5	150	54,28	2,70	5,0 %
6	150	75,46	2,63	3,5 %

15.3.5. Linealidad

La linealidad se evaluó en base a CLSI EP-06, “Evaluation of the Linearity of Quantitative Measurement Procedures”. Para la concentración de anticardiolipina IgM mediante el ensayo Anti Cardiolipin Screen, el procedimiento de medición muestra linealidad para el intervalo de 0,82 a 86,88 AU/mL dentro de la desviación de linealidad permitida (ADL) de ±15 %.

15.3.6. Especificidad analítica

Las siguientes sustancias no interfieren con un sesgo de > ±15 % en el ensayo Anti Cardiolipin Screen para la evaluación de anticuerpos de clase IgM cuando las concentraciones están por debajo del umbral indicado presentado en la siguiente tabla.

Reactivos que pueden interferir	Límite máximo de concentración
Bilirrubina, conjugada	15 mg/dL
Bilirrubina, no conjugada	15 mg/dL
Hemoglobina	200 mg/dL
Proteína total	10 g/dL
Triglicéridos	500 mg/dL

15.3.7. Estudio en suero-plasma

El estudio de comparación de la matriz de Anti Cardiolipin Screen para la evaluación de anticuerpos de clase IgM se realizó para evaluar la diferencia entre los tipos de tubos (tubos separadores de suero [SST], plasma de heparina de litio, plasma de heparina sódica y plasma K2 EDTA) frente a las muestras de control (tapón rojo para suero, sin aditivos) siguiendo las directrices de CLSI EP9-A3. Se evaluó un total de 20 muestras (16 nativas, 4 con aditivos) para cubrir el intervalo. Se realizó un análisis de regresión lineal sobre los datos comparativos:

Tipo de muestra	Pendiente [IC del 95 %]	Intersección (ng/mL) [IC del 95 %]	Coeficiente de correlación (r)
SST	1,02 [0,94 a 1,09]	0,19 [-1,34 a 1,72]	0,99
Heparina de litio	0,93 [0,82 a 1,04]	0,73 [–1,49 a 2,95]	0,97
Heparina sódica	0,93 [0,84 a 1,01]	0,64 [–1,00 a 2,28]	0,98
EDTA	0,96 [0,86 a 1,05]	0,65 [–1,19 a 2,49]	0,98

16. LÍMITES DE USO

- Como en cualquier procedimiento diagnóstico, los resultados se deberán interpretar junto con los hallazgos clínicos del paciente y otra información de la que el médico disponga.
- Las características de rendimiento de este análisis no se han establecido para una población pediátrica.
- Los anticuerpos heterofílicos en el suero humano pueden presentar reacciones con las inmunoglobulinas reactivas, que interfieren con los inmunoensayos *in vitro*[22]. Los pacientes que se exponen habitualmente a animales o a productos de suero animal pueden ser propensos a esta interferencia y puede que se observen valores anómalos.
- La presencia de inmunocomplejos u otros agregados de inmunoglobulinas en la muestra del

paciente puede causar un mayor nivel de unión no específica y dar como resultado falsos positivos en este ensayo.

17. GESTIÓN DE RESIDUOS

Los reactivos deben eliminarse de acuerdo con la normativa local.

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19. IDENTIFICADOR DE REVISIÓN

Las adiciones o cambios en las instrucciones de uso se han resaltado en gris.

20. RECLAMACIONES SOBRE PRODUCTOS Y ASISTENCIA TÉCNICA

Para un paciente/usuario/tercero en la Unión Europea y en países con un régimen regulatorio similar: Reglamento (UE) 2017/746 sobre los productos sanitarios para diagnóstico in vitro; si, durante el uso de este dispositivo o como resultado de su uso, se ha producido un incidente grave, informe del mismo al fabricante y/o a su representante autorizado y al organismo regulador nacional.

Puede contactar con el fabricante a través del servicio de atención al cliente o del equipo de asistencia técnica. Los datos de contacto se encuentran a continuación y en el sitio web de la empresa: www.diametra.com.

Ed. 03/2022 **DCM144-2**

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DiaMetra	Packaging Information Sheet	Mod. PIS000-1

IVD	DE ES FR GB IT PT	In vitro Diagnostikum Producto sanitario para diagnóstico In vitro Dispositif medical de diagnostic in vitro In vitro Diagnostic Medical Device Dispositivo medico-diagnostico in vitro Dispositivos medicos de diagnostico in vitro		DE ES FR GB IT PT	Hergestellt von Elaborado por Fabriqué par Manufacturer Produttore Produzido por
	DE ES FR GB IT PT	Achtung, Begleitdokumente Precaución, consulte los documentos adjuntos Attention, veuillez consulter les documents d'accompagnement Caution, consult accompanying documents Attenzione, consultare la documentazione allegata Atenção,consultar os documentos de acompanhamento	 yyyy-mm	DE ES FR GB IT PT	Herstellungs datum Fecha de fabricacion Date de fabrication Date of manufacture Data di produzione Data de produção
 yyyy-mm-dd	DE ES FR GB IT PT	Verwendbar bis Establa hasta (usar antes de último día del mes) Utiliser avant (dernier jour du mois indiqué) Use by (last day of the month) Utilizzare prima del (ultimo giorno del mese) Utilizar (antes ultimo dia do mês)		DE ES FR GB IT PT	Biogefährdung Riesco biológico Risque biologique Biological risk Rischio biologico Risco biológico
	DE ES FR GB IT PT	Gebrauchsanweisung beachten Consultar las instrucciones Consulter le mode d'emploi Consult instructions for use Consultare le istruzioni per l'uso Consultar instruções para uso	LOT	DE ES FR GB IT PT	Chargenbezeichnung Codigo de lote Numero de lot Batch code Codice del lotto Codigo do lote
 Σ = xx	DE ES FR GB IT PT	Ausreichend für "n" Tests Contenido suficiente para "n" tests Contenu suffisant pour "n" tests Contains sufficient for "n" tests Contenuto sufficiente per "n" saggi Contém o suficiente para "n" testes	CONT	DE ES FR GB IT PT	Inhalt Contenido del estuche Contenu du coffret Contents of kit Contenuto del kit Conteúdo do kit
Max  Min	DE ES FR GB IT PT	Temperaturbereich Límitaciôn de temperatura Limites de température de conservation Temperature limitation Limiti di temperatura Temperaturas limites de conservação	REF	DE ES FR GB IT PT	Bestellnummer Nûmero de catálogo Référéncès du catalogue Catalogue number Numero di Catalogo Número do catálogo
	DE ES FR GB IT PT	Vor direkter sonneneinstrahlung schützen Mantener alejado de la luz solar Tenir à l'écart de la lumière du soleil Keep away from sunlight Tenere lontano dalla luce solare Mantenha longe da luz solar			

SUGGERIMENTI PER LA RISOLUZIONE DEI PROBLEMI/TROUBLESHOOTING

ERRORE CAUSE POSSIBILI/ SUGGERIMENTI

Nessuna reazione colorimetrica del saggio

- mancata dispensazione del coniugato
- contaminazione del coniugato e/o del Substrato
- errori nell'esecuzione del saggio (es. Dispensazione accidentale dei reagenti in sequenza errata o provenienti da flaconi sbagliati, etc.)

Reazione troppo blanda (OD troppo basse)

- coniugato non idoneo (es. non proveniente dal kit originale)
- tempo di incubazione troppo breve, temperatura di incubazione troppa bassa

Reazione troppo intensa (OD troppo alte)

- coniugato non idoneo (es. non proveniente dal kit originale)
- tempo di incubazione troppo lungo, temperatura di incubazione troppa alta
- qualità scadente dell'acqua usata per la soluzione di lavaggio (basso grado di deionizzazione,)
- lavaggi insufficienti (coniugato non completamente rimosso)

Valori inspiegabilmente fuori scala

- contaminazione di pipette, puntali o contenitori- lavaggi insufficienti (coniugato non completamente rimosso)

CV% intrasaggio elevato

- reagenti e/o strip non portate a temperatura ambiente prima dell'uso
- il lavatore per micropiastre non lava correttamente (suggerimento: pulire la testa del lavatore)

CV% intersaggio elevato

- condizioni di incubazione non costanti (tempo o temperatura)
- controlli e campioni non dispensati allo stesso tempo (con gli stessi intervalli) (controllare la sequenza di dispensazione)
- variabilità intrinseca degli operatori

ERROR POSSIBLE CAUSES / SUGGESTIONS

No colorimetric reaction

- no conjugate pipetted reaction after addition
- contamination of conjugates and/or of substrate
- errors in performing the assay procedure (e.g. accidental pipetting of reagents in a wrong sequence or from the wrong vial, etc.)

Too low reaction (too low ODs)

- incorrect conjugate (e.g. not from original kit)
- incubation time too short, incubation temperature too low

Too high reaction (too high ODs)

- incorrect conjugate (e.g. not from original kit)
- incubation time too long, incubation temperature too high
- water quality for wash buffer insufficient (low grade of deionization)
- insufficient washing (conjugates not properly removed)

Unexplainable outliers

- contamination of pipettes, tips or containers

insufficient washing (conjugates not properly removed) too high within-run

- reagents and/or strips not pre-warmed to CV% Room Temperature prior to use
- plate washer is not washing correctly (suggestion: clean washer head)

too high between-run - incubation conditions not constant (time, CV % temperature)

- controls and samples not dispensed at the same time (with the same intervals) (check pipetting order)
- person-related variation

ERROR / POSIBLES CAUSAS / SUGERENCIAS

No se produce ninguna reacción colorimétrica del ensayo

- no se ha dispensado el conjugado
- contaminación del conjugado y/o del substrato
- errores en la ejecución del ensayo (p. ej., dispensación accidental de los reactivos en orden incorrecto o procedentes de frascos equivocados, etc.)

Reacción escasa (DO demasiado bajas)

- conjugado no idóneo (p. ej., no procedente del kit original)
- tiempo de incubación demasiado corto, temperatura de incubación demasiado baja

Reacción demasiado intensa (DO demasiado altas)

- conjugado no idóneo (p. ej., no procedente del kit original)
- tiempo de incubación demasiado largo, temperatura de incubación demasiado alta
- calidad escasa del agua usada para la solución de lavado (bajo grado de desionización)
- lavados insuficientes (el conjugado no se ha retirado completamente)

Valores inexplicablemente fuera de escala

- contaminación de pipetas, puntas o contenedores- lavados insuficientes (el conjugado no se ha retirado completamente)

CV% intraensayo elevado

- los reactivos y/o tiras no se encontraban a temperatura ambiente antes del uso
- el lavador de microplacas no funciona correctamente (sugerencia: limpiar el cabezal del lavador)

CV% interensayo elevado

- condiciones de incubación no constantes (tiempo o temperatura)
- controles y muestras no dispensados al mismo tiempo (con los mismos intervalos) (controlar la secuencia de dispensación)
- variación en función de los operadores

ERREUR CAUSES POSSIBLES / SUGGESTIONS

Aucune réaction colorimétrique de l'essai

- non distribution du conjugué
- contamination du conjugué et/ou du substrat
- erreurs dans l'exécution du dosage (par ex., distribution accidentelle des réactifs dans le mauvais ordre ou en provenance des mauvais flacons, etc.)

Réaction trop faible (DO trop basse)

- conjugué non approprié (par ex., ne provenant pas du coffret original)
- temps d'incubation trop court, température d'incubation trop basse

Réaction trop intense (DO trop élevée)

- conjugué non approprié (par ex., ne provenant pas du coffret original)
- temps d'incubation trop long, température d'incubation trop élevée
- mauvaise qualité de l'eau utilisée pour la solution de lavage (bas degré de déionisation)
- lavages insuffisants (conjugué non entièrement éliminé)

Valeurs inexplicablement hors plage

- contamination des pipettes, embouts ou récipients - lavages insuffisants (conjugué non entièrement éliminé)

CV% intra-essai élevé

- les réactifs et/ou les bandes n'ont pas atteint la température ambiante avant usage
- le laveur de microplaques ne lave pas correctement (suggestion : nettoyer la tête du laveur)

CV% inter-essai élevé

- conditions d'incubation non constantes (temps ou température)
- contrôles et échantillons non distribués en même temps (avec les mêmes intervalles) (contrôler l'ordre de distribution)
- variabilité intrinsèque des opérateurs

CE

DiaMetra
care for quality

DCM114-7
Ed. 09/2018

Anti Phospholipid Screen

per analisi di routine

Determinazione quantitativa degli autoanticorpi anti fosfolipidi in siero o plasma umano

IVD | **LOT** Vedere etichetta esterna | 2°C 8°C | Σ = 96 test | **REF DKO114**

DESTINAZIONE D'USO

Il kit Anti Phospholipid Screen è un test immunoenzimatico indiretto in fase solida per la misurazione quantitativa degli auto-anticorpi di classe IgG e IgM diretti contro fosfolipidi anionici del siero mediati dalla β2-glicoproteina (Cardiolipina, Fosfatidil serina, Fosfatidil inositolo, Acido fosfatidico, Fosfatidil colina, Lisofosfatidil colina e Fosfatidil etanol-ammina) su siero o plasma umano. Il test si intende per uso diagnostico in vitro come supporto nella diagnosi di aumentato rischio di trombosi in pazienti con Lupus Eritematosus Sistemico (LES) o disordini simili.
Il kit Anti Phospholipid Screen è destinato al solo uso di laboratorio.

1. SIGNIFICATO CLINICO

Il primo studio sugli anticorpi anti-fosfolipidi iniziò nel 1906 quando Wasserman introdusse un test sierologico per la sifilide. Nel 1942 fu scoperto che la componente attiva era un fosfolipide indicato con il nome di Cardiolipina. Negli anni '50 emerse che un elevato numero di persone risultava essere positivo al test per la sifilide senza però presentare alcuna evidenza della malattia. All' inizio si etichettò il fenomeno come una serie di falsi positivi al test per la sifilide, in seguito emerse che in questo gruppo di pazienti vi era una elevata prevalenza di disordini autoimmuni tra cui il Lupus Eritematosus Sistemico (LES) e la Sindrome di Sjögrens.
Il termine Lupus anticoagulante (LA), utilizzato per la prima volta nel 1972, deriva da osservazioni sperimentali nelle quali si osservò un aumento del rischio di trombosi paradossalmente alla presenza di alcuni fattori anticoagulanti; il termine LA non è del tutto corretto poiché la patologia si presenta più frequentemente in pazienti senza lupus ed è associato a trombosi piuttosto che a sanguinamento anormale.
Negli anni più recenti è stato investigato il ruolo di un cofattore , la proteina β2-glicoproteina I (apolipoproteina H) detto anche β2GPI, e le sue interazioni con i fosfolipidi anionici contenuti nel siero/plasma umano. Questo cofattore è una β-globulina con peso molecolare 50 kDa che si trova nel plasma alla concentrazione di circa 200 µg/mL. Si è scoperto che β2GPI è coinvolto nella regolazione della coagulazione del sangue, inibendo la via intrinseca. β2GPI in vivo è associato con sostanze cariche negativamente, quali ad esempio fosfolipidi anionici, eparina e lipoproteine. La regione che lega i fosfolipidi è situata nel suo quinto dominio.
Con l'acronimo "aPL" (anticorpi anti-fosfolipidi) si intendono impropriamente anticorpi diretti contro fosfolipidi carichi negativamente quali la Cardiolipina (CL), Fosfatidil serina (PS) Fosfatidil inositolo (PI) e Acido fosfatidico (PA); secondo una accezione più corretta del termine, vanno intesi come anticorpi anti-fosfolipidi quegli anticorpi diretti contro il complesso tra β2GPI e i fosfolipidi anionici in grado di legarsi al dominio quinto della β2GPI.Tra questi, la Cardiolipina è il fosfolipide usato più comunemente come antigene per dosare gli aPL con metodo ELISA. Anticorpi diretti contro il complesso tra β2GPI e fosfolipidi carichi negativamente, quali Fosfatidil serina (PS) Fosfatidil inositolo (PI) e Acido fosfatidico (PA) vengono misurati nel laboratorio diagnostico.
Alcuni ricercatori hanno suggerito che l'utilizzo della PS al posto della Cardiolipina nei dosaggi ELISA permetterebbe una diagnosi più precisa. Tuttavia, questi anticorpi anti fosfolipidi sono usati meno comunemente e il loro utilizzo aggiuntivo può aumentare la sensibilità clinica nei campioni di pazienti con sospetta Sindrome Anti-fosfolipidica (APS), ma non possono sostituire la determinazione degli autoanticorpi anti Cardiolipina.

2. PRINCIPIO DEL METODO

Il dosaggio Anti Phospholipid Screen si basa sul legame degli anticorpi presenti nel siero e diretti contro i complessi antigenici formati da fosfolipidi anionici (Cardiolipina, Fosfatidil serina, Fosfatidil inositolo, Acido fosfatidico, Fosfatidil colina, Lisofosfatidil colina e Fosfatidil etanol-ammina) e la β2-Glicoproteina; questi complessi sono adsorbiti sulla micropiastra. Gli anticorpi di tipo IgG e IgM diretti verso questi antigeni e presenti nei calibratori, nei controlli, e nei campioni di siero o plasma prediluiti dei pazienti, si legano quindi ai rispettivi antigeni.
Dopo 60 minuti di incubazione la micropiastra viene lavata con Wash Solution per la rimozione delle componenti del siero che non hanno reagito.
Una soluzione di immunoglobuline anti-human IgG (Conjugate IgG, reattivo 4) o anti-human IgM (Conjugate IgM, reattivo 5) riconosce gli anticorpi di classe IgG o IgM (rispettivamente) legati agli antigeni immobilizzati.
Dopo 30 minuti di incubazione, l'eccesso di coniugato enzimatico che non si è legato specificamente, viene rimosso tramite lavaggio con apposito tampone (Wash Solution).
Si aggiunge poi una soluzione substrato cromogenica (TMB Substrate) contenente Tetrametilbenzidina. Dopo 15 minuti di incubazione si blocca lo sviluppo del colore mediante aggiunta della Stop Solution. Il colore della soluzione diventa giallo, e l'intensità di colore sviluppata è direttamente proporzionale alla concentrazione di anticorpi IgG o IgM presenti nel campione originale.
La concentrazione di anticorpi IgG o IgM presenti nel campione è calcolata sulla base di una curva di calibrazione.

3. REATTIVI, MATERIALI E STRUMENTAZIONE

3.1. Reattivi e materiali forniti nel kit

1. Calibrators (5 flaconi, 1,2 mL ciascuno)
Tampone fosfato 0,1M, NaN_3 < 0,1%, siero umano

CAL0	**REF DCE002/11406-0**
CAL1	**REF DCE002/11407-0**
CAL2	**REF DCE002/11408-0**
CAL3	**REF DCE002/11409-0**
CAL4	**REF DCE002/11410-0**

2. Controlli (2 flaconi, 1,2 mL ciascuno, pronti all'uso)
Tampone fosfato 0,1M, NaN_3 < 0,1%, siero umano

Positive Control	**REF DCE045/11402-0**
Negative Control	**REF DCE045/11401-0**

3. Sample Diluent (1 flacone, 100 mL)
Tampone fosfato 0,1M, NaN_3 < 0,1%
REF DCE053-0

4. Conjugate IgG (1 flacone, 15 mL)
Anti h-IgG coniugato con perossidasi di rafano (HRP), BSA 0,1%, Proclin < 0,0015% **REF DCE002/11402-G**

5. Conjugate IgM (1 flacone, 15 mL)
Anti h-IgM coniugate con perossidasi di rafano (HRP), BSA 0,1%, Proclin < 0,0015% **REF DCE002/11402-M**

6. Coated Microplate (1 micropiastra breakable)
Complessi antigenici di fosfolipidi e β2-Glicoproteina adsorbiti su micropiastra **REF DCE002/11403-0**

7. TMB Substrate (1 flacone, 15 mL)
H_2O_2-TMB (0,26 g/L) *(evitare il contatto con la pelle)*
REF DCE004-0

8. Stop Solution (1 flacone, 15 mL)
Acido solforico 0,15M *(evitare il contatto con la pelle)*
REF DCE005-0

9. 10X Conc. Wash Solution (1 flacone, 50 mL)
Tampone fosfato 0,2M, pH 7.4 **REF DCE054-0**

3.2. Reattivi necessari non forniti nel kit

Acqua distillata.

3.3. Materiale e strumentazione ausiliare

Dispensatori automatici.
Lettore per micropiastre (450 nm, 620-630 nm).

Note

Conservare tutti i reattivi a 2÷8°C, al riparo dalla luce.
Aprire la busta del Reattivo 6 (Coated Microplate) solo dopo averla riportata a temperatura ambiente e chiuderla subito dopo il prelievo delle strip da utilizzare; una volta aperta è stabile fino alla data di scadenza del kit.

4. AVVERTENZE

- Questo test kit è per uso in vitro, da eseguire da parte di personale esperto. Non per uso interno o esterno su esseri Umani o Animali.
- Usare i previsti dispositivi di protezione individuale mentre si lavora con i reagenti forniti.
- Seguire le Buone Pratiche di Laboratorio (GLP) per la manipolazione di prodotti derivati da sangue.
- Materiali di origine animale usati per la preparazione di questo kit sono stati ottenuti da animali sani e le proteine bovine sono state ottenute da paesi non affetti da BSE, ma comunque questi materiali dovrebbero essere usati come potenzialmente contagiosi.
- Tutti i reattivi di origine umana usati nella preparazione dei reagenti sono stati testati e sono risultati negativi per la presenza di anticorpi anti-HIV 1&2, per HbsAg e per anticorpi anti-HCV. Tuttavia nessun test offre la certezza completa dell'assenza di HIV, HBV, HCV o di altri agenti infettivi. Pertanto, i Calibratori ed i Controlli devono essere maneggiati come materiali potenzialmente infettivi.
- Alcuni reagenti contengono piccole quantità di Sodio Azide (NaN_3) o di Proclin 300^R come conservante. Evitare il contatto con la pelle e le mucose.
- La Sodio Azide può essere tossica se ingerita o assorbita attraverso la cute o gli occhi; inoltre, può reagire con le tubature di piombo o rame formando azidi metalliche potenzialmente esplosive. Se si usa un lavandino per eliminare i reagenti, lasciar scorrere grandi quantità di acqua per prevenire la formazione di azidi.
- Il TMB Substrato contiene un irritante, che può essere dannoso se inalato, ingerito o assorbito attraverso la cute. Per prevenire lesioni, evitare l'inalazione, l'ingestione o il contatto con la cute e con gli occhi.
- La Stop Solution è costituita da una soluzione di acido solforico diluito. L'acido solforico è velenoso e corrosivo e può essere tossico se ingerito. Per prevenire possibili ustioni chimiche, evitare il contatto con la cute e con gli occhi.
- Evitare l'esposizione del reagente TMB/H_2O_2 a luce solare diretta, metalli o ossidanti. Non congelare la soluzione.

5. PREACUZIONI

- Si prega di attenersi rigorosamente alla sequenza dei passaggi indicata in questo protocollo. I risultati presentati qui sono stati ottenuti usando specifici reagenti elencati in queste Istruzioni per l'Uso.
- Tutti i reattivi devono essere conservati a temperatura controllata di 2-8°C nei loro contenitori originali. Eventuali eccezioni sono chiaramente indicate. I reagenti sono stabili fino alla data di scadenza se conservati e trattati seguendo le istruzioni fornite.
- Prima dell'uso lasciare tutti i componenti dei kit e i campioni a temperatura ambiente (22-28°C) e mescolare accuratamente.
- Non scambiare componenti dei kit di lotti diversi. Devono essere osservate le date di scadenza riportate sulle etichette della scatola e di tutte le fiale. Non utilizzare componenti oltre la data di scadenza.
- **ATTENZIONE**: **il reagente Coniugato è stato studiato per garantire la massima sensibilità di dosaggio, e pertanto, se non opportunamente usato, può essere contaminato da agenti esterni**; si raccomanda pertanto di utilizzare consumabili (puntali, flaconi, vaschette ecc.) usa e getta. Per dosaggi frazionati, prelevare l'esatta quantità di coniugato necessaria ed evitare di re-introdurre l'eventuale scarto nel flacone originale. Inoltre, **per dosaggi effettuati con l'ausilio di strumentazione automatica e semi-automatica**, si consiglia, prima di utilizzare il coniugato, di effettuare uno step di pulizia della fluidica, assicurandosi che le procedure di lavaggio, deproteinizzazione e decontaminazione siano efficaci nell'evitare la contaminazione del coniugato; questa procedura è fortemente raccomandata quando il kit è processato con analizzatori non dotati di puntali monouso.
  A tale scopo Dia.Metra rende disponibile separatamente un reattivo decontaminante per il lavaggio degli aghi.
- Qualora si utilizzi strumentazione automatica, è responsabilità dell'utilizzatore assicurarsi che il kit sia stato opportunamente validato.
- Un lavaggio incompleto o non accurato dei pozzetti può causare una scarsa precisione e/o un'elevato background. Per migliorare le prestazioni del kit su strumentazione automatica, si consiglia di aumentare il numero di lavaggi.

- Per la riproducibilità dei risultati, è importante che il tempo di reazione di ogni pozzetto sia lo stesso. Per evitare il time shifting durante la dispensazione degli reagenti, il tempo di dispensazione dei pozzetti non dovrebbe estendersi oltre i 10 minuti. Se si protrae oltre, si raccomanda di seguire lo stesso ordine di dispensazione. Se si utilizza più di una piastra, si raccomanda di ripetere la curva di calibrazione in ogni piastra.
- L'aggiunta del TMB Substrato dà inizio ad una reazione cinetica, la quale termina con l'aggiunta della Stop Solution. L'aggiunta del TMB Substrato e della Stop Solution deve avvenire nella stessa sequenza per evitare tempi di reazione differenti.
- Osservare le linee guida per l'esecuzione del controllo di qualità nei laboratori clinici testando controlli e/o pool di sieri.
- Osservare la massima precisione nella ricostituzione e dispensazione dei reagenti.
- Non usare campioni microbiologicamente contaminati, altamente lipemici o emolizzati.
- I lettori di micropiastre leggono l'assorbanza verticalmente. Non toccare il fondo dei pozzetti.

6. PROCEDIMENTO

6.1. Preparazione dei Calibratori (C_0…C_4)

I Calibratori sono pronti all'uso e sono misti, pertanto contengono sia gli anticorpi IgG che IgM. I Calibratori hanno le seguenti concentrazioni:

	C_0	C_1	C_2	C_3	C_4
AU/mL	0	5	10	20	80

Una volta aperti sono stabili 6 mesi a 2-8°C.

6.2. Preparazione del campione

Per l'esecuzione del test si possono utilizzare campioni di siero o di plasma umano. I campioni da utilizzare devono essere limpidi. Si consiglia di evitare contaminazioni dovute a iperlipemia, anche se queste non interferiscono con l'analisi. I campioni possono essere conservati refrigerati a 2-8 °C fino a 5 giorni, oppure conservati a -20°C fino a 6 mesi. Si consiglia di evitare congelamenti e scongelamenti ripetuti dei campioni di siero che potrebbero determinare una perdita variabile dell'attività degli autoanticorpi. Non è raccomandata l'analisi di campioni inattivati al calore.

Tutti i campioni di siero e plasma devono essere prediluiti 1:100 con sample diluent; per esempio 10 µL di siero vengono diluiti con 990 µL di sample diluent.

I Controlli sono pronti all'uso.

6.3. Preparazione della Wash Solution

Prima dell'uso, diluire il contenuto di ogni flacone di soluzione di lavaggio tamponata concentrata (10X) con acqua distillata fino al volume di 500 mL. Per preparare volumi minori rispettare il rapporto di diluizione di 1:10. La soluzione di lavaggio diluita è stabile a 2-8°C per almeno 30 giorni.

Nella wash solution concentrata è possibile osservare la presenza di cristalli, in tal caso agitare a temperatura ambiente fino a completa dissoluzione dei cristalli, per una maggiore precisione diluire tutto il flacone della soluzione di lavaggio concentrata a 500 mL avendo cura di trasferire anche i cristalli, poi agitare fino a completa dissoluzione.

6.4. Procedimento

- **Portare tutti i reagenti a temperatura ambiente (22-28°C) per almeno 30 minuti.** Al termine del dosaggio riporre immediatamente tutti i reagenti a 2-8°C: evitare lunghi periodi a temperatura ambiente.
- Le strisce di pozzetti non utilizzate devono essere rimesse immediatamente nella busta richiudibile contenente il materiale essicante e conservate a 2-8°C.
- Per evitare potenziali contaminazioni microbiche e/o chimiche non rimettere i reagenti inutilizzati nei flaconi originali.
- Al fine di aumentare l'accuratezza dei risultati del test è necessario operare in doppio, allestendo due pozzetti per ogni punto della curva di calibrazione (C_0-C_4), due per ogni Controllo, due per ogni Campione ed uno per il Bianco.

Reagenti	Calibratore	Campione /Controlli	Bianco
Calibratore C_0-C_4	**100 µL**		
Controlli		**100 µL**	
Campione diluito		**100 µL**	
Incubare 60 minuti a temperatura ambiente (22-28°C). Allontanare la miscela di reazione, lavare i pozzetti 3 volte con 300 µL di wash solution diluita. **Nota importante**: ad ogni step di lavaggio, agitare delicatamente la piastra per 5 secondi e successivamente rimuovere l'eccesso di soluzione di lavaggio sbattendo delicatamente la micropiastra capovolta su fogli di carta assorbente. **Lavaggi automatici**: se si utilizza strumentazione automatica effettuare almeno 5 lavaggi.			
Conjugate (IgG or IgM)	**100 µL**	**100 µL**	
Incubare 30 minuti a temperatura ambiente (22-28°C). Allontanare la miscela di reazione, lavare i pozzetti 3 volte con 300 µL di wash solution diluita. **Lavaggi**: seguire le stesse indicazioni del punto precedente.			
TMB Substrate	**100 µL**	**100 µL**	**100 µL**
Incubare 15 minuti a temperatura ambiente al riparo dalla luce (22-28°C).			
Stop Solution	**100 µL**	**100 µL**	**100 µL**
Agitare delicatamente la micropiastra. Leggere l'assorbanza (E) a 450 nm contro una lunghezza d'onda di riferimento di 620-630 nm oppure contro il Bianco entro 5 minuti.			

7. CONTROLLO DI QUALITA'

- Il Controllo Positivo per anti-fosfolipidi deve essere incluso ogni volta che si esegue il test per assicurare che tutti i reagenti ed il test funzionino in modo corretto.
- Poichè il Controllo è prediluito, esso non rappresenta un controllo procedurale per le tecniche di diluizione utilizzate per i campioni.
- Ulteriori sieri di controllo possono essere preparati raccogliendo un pool di sieri umani, aliquotandolo e conservandolo a < -20°C.
- Perchè i risultati del test siano considerati validi, tutti i seguenti criteri devono essere soddisfatti. Se anche uno solo non rientra nei valori specificati, i risultati non dovrebbero essere considerati validi ed il test dovrebbe essere ripetuto:

- Il Controllo Positivo serve per controllare un'eventuale malfunzionamento dei reagenti e non assicura la precisione in corrispondenza del valore soglia del test.
- Il test è valido solo se la densità ottica a 450 nm del Controllo Positivo come pure quelle dei calibratori (C_0-C_4) coincidono con gli intervalli corrispondenti indicati nel Certificato di Controllo di Qualità incluso nel kit.

8. CALCOLO DEI RISULTATI

Per il kit Anti Phospholipid Screen il metodo di scelta per il trattamento dei risultati è una elaborazione 4 parametri con assi lin-log per densità ottica e concentrazione rispettivamente. Inoltre si possono utilizzare un'approssimazione spline e coordinate log-log. Tuttavia si raccomanda di utilizzare una curva lin-log.
Innanzitutto occorre calcolare la media delle densità ottiche relative ai calibratori. Utilizzare un foglio di carta lin-log e tracciare le densità ottiche medie di ogni calibratore verso la rispettiva concentrazione. Disegnare la curva che approssima nel modo migliore tutti i punti di calibrazione. I punti dei calibratori possono anche essere collegati con segmenti di linea retta. La concentrazione dei campioni incogniti può essere determinata per interpolazione dalla curva di calibrazione.

9. VALORI DI RIFERIMENTO

In uno studio sui valori normali eseguito con campioni di siero provenienti da donatori sani sono stati determinati i seguenti intervalli di normalità con il test Anti Phospholipid Screen:

	IgG (GPL AU/mL)	IgM (MPL AU/mL)
Normale	< 10	< 10
Elevato	≥ 10	≥ 10

È importante tenere presente che la determinazione di un range di valori attesi in un dato metodo per una popolazione "normale" è dipendente da molteplici fattori, quali la specificità e sensibilità del metodo in uso, e la popolazione in esame. Perciò ogni laboratorio dovrebbe considerare i range indicati dal Fabbricante come un'indicazione generale e produrre range di valori attesi propri basati sulla popolazione indigena dove il laboratorio risiede.
I risultati positivi dovrebbero essere verificati relativamente allo stato clinico del paziente. Inoltre, ogni decisione relativa alla terapia dovrebbe essere presa individualmente. Si raccomanda che ogni laboratorio stabilisca i sui propri intervalli normale e patologico di anticorpi anti fosfolipidi serici.

10. LIMITAZIONI DEL TEST

La presenza nel campione di complessi immuni o di altri aggregati di immunoglobuline può determinare delle reazioni aspecifiche con conseguenti risultati falsi positivi.

11. PARAMETRI CARATTERISTICI

11.1. Precisione e riproducibilità

La precisione e la riproducibilità sono state valutate testando due campioni in due esperimenti diversi con due lotti di kit differenti.
Le operazioni di dispensazione e lavaggio sono state eseguite da un operatore manualmente.
I risultati in termini di deviazione standard e coefficiente di variazione sono riportati di seguito:

	IgG			
Campione	1		2	
	SD	CV%	SD	CV%
Intra-assay	1.03	5.9	1.31	7.4
Inter-assay	0.26	9.2	5.25	11.7
	IgM			
Campione	1		2	
	SD	CV%	SD	CV%
Intra-assay	0.61	7.6	1.97	5.9
Inter-assay	0.15	7.1	2.98	6.6

11.2. Sensibilità:

La sensibilità clinica del saggio Anti Phospholipid Screen IgG è 92,3%.
La sensibilità clinica del saggio Anti Phospholipid Screen IgM è 68,8%.

11.3. Specificità:

La specificità clinica del saggio Anti Phospholipid Screen IgG è 84,6%.
La specificità clinica del saggio Anti Phospholipid Screen IgM è > 99,9%.

11.4. Limite di rilevabilità:

La minor concentrazione che può essere distinta dal Calibratore zero è di circa 0,03 AU/mL per IgG e 0,16 AU/mL per IgM.

12. DISPOSIZIONI PER LO SMALTIMENTO

I reagenti devono essere smaltiti in accordo con le leggi locali.

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DiaMetra
care for quality

DCM114-7
Ed. 09/2018

Anti Phospholipid Screen

for routine analysis

Quantitative determination of auto-antibodies against phospholipids in human serum or plasma

IVD

LOT
See external label

2°C 8°C

Σ = 96 test

REF DKO114

INTENDED USE

Anti Phospholipid Screen is an indirect solid phase immunoassay kit for the quantitative measurement of IgG and IgM class auto-antibodies directed against β2-glycoprotein mediated anionic phospholipids in human serum or plasma, including Cardiolipin, Phosphatidyl serine, Phosphatidyl inositol, Phosphatidic acid, Phosphatidyl choline, Lyso-phosphatidyl choline, Phosphatidyl ethanolamine. The assay is intended for in vitro diagnostic use only as an aid in the diagnosis of increased risk of thrombosis in patients with Systemic Lupus Eritematosus (SLE) or similar disorders.
Anti Phospholipid Screen kit is intended for laboratory use only.

1. CLINICAL SIGNIFICANCE

The first study on the anti-phospholipid antibodies began in 1906 when Wasserman introduced a serological test for syphilis. In 1942 it was found the active component that is a phospholipid indicated by the name of Cardiolipin. In the 50's it was observed that a large number of people appeared to be positive for syphilis tests but did not show any evidence of disease. At the beginning the phenomenon was classified as a series of false positive syphilis test, then a more accurate analysis revealed, for this group of patients, a high prevalence of autoimmune disorders including Systemic Lupus Eritematosus (SLE) and Sjögrens syndrome.
The term lupus anticoagulant (LA), used for the first time in 1972, derives from experimental observations in which it was observed an increased risk of thrombosis, paradoxically, with the presence of some anticoagulants factors; the term LA is not totally correct, in fact the disease is present more frequently in patients without lupus and it is associated with thrombosis rather than to abnormal bleeding.
Some years later the role of a cofactor has been investigated, the β2-glycoprotein I (apolipoprotein H) also said β2GPI, and its interactions with anionic phospholipids in human serum / plasma. This cofactor is a β-globulin with a molecular weight of 50 kDa that has the concentration of 200 g / mL in plasma. The β2GPI is involved in the regulation of blood coagulation, inhibiting the intrinsic way. β2GPI in vivo is associated with negatively charged substances such as anionic phospholipids, heparin and lipoproteins. The region that binds phospholipids is in its fifth domain.
The acronym "aPL" (anti-phospholipid antibodies) indicates improperly antibodies directed against phospholipids negatively charged like Cardiolipin (CL), Phosphatidyl serine (PS) Phosphatidyl inositol (PI) and phosphatidic acid (PA); more correctly the term anti-phospholipid antibodies indicate those antibodies directed against the complex between β2GPI and anionic phospholipids that can bind to the fifth domain of β2GPI. Among these, the Cardiolipin is the most commonly used phospholipid as an antigen for determining the aPL with ELISA method. Diagnostic laboratories measure the antibodies directed against the complex between β2GPI and negatively charged phospholipids, as Phosphatidyl serine (PS) Phosphatidyl inositol (PI) and phosphatidic acid (PA). Some researchers suggest the use of PS instead of Cardiolipin in ELISA assays, for a more precise diagnosis. However, these antibodies against phospholipids are less commonly used, even if their use may increase the clinical sensitivity of patients samples with suspected Anti-phospholipid Syndrome (APS), but it can' t replace the determination of autoantibodies anti-Cardiolipin.

2. PRINCIPLE

Anti Phospholipid Screen test is based on the binding of antibodies in human serum directed against the antigenic complex between anionic phospholipids (Cardiolipin, Phosphatidyl serine, Phosphatidyl inositol, Phosphatidic acid, Phosphatidyl choline, Lyso-phosphatidyl choline, Phosphatidyl ethanolamine) and β2-Glycoprotein; these complexes are coated on the microplate. Any antibody of IgG class or IgM class in calibrators, controls or prediluted patient samples binds to its respective antigen.
After 60 minutes incubation, the microplate is washed with wash buffer for removing non-reactive serum components. An anti-human IgG conjugate solution (Conjugate IgG, reactive 4) or an anti-human IgM conjugate solution (Conjugate IgM, reactive 5) recognize IgG class or IgM class antibodies, respectively, bound to the immobilized antigens.
After a 30 minutes incubation any excess enzyme conjugate which is not specifically bound is washed away with the wash buffer.
A chromogenic substrate solution containing TMB is then dispensed into the wells. After a 15 minutes incubation the color development is stopped by adding the stop solution. The solutions color change into yellow. The amount of color is directly proportional to the concentration of IgG or IgM antibodies present in the original sample.
The concentration of IgG or IgM antibodies in the original sample is calculated through a calibration curve.

3. REAGENTS, MATERIALS AND INSTRUMENTATION

3.1. Reagents and materials supplied in the kit

1. Calibrators (5 vials, 1,2 mL each)
Phosphate buffer 0,1M, NaN_3 < 0,1%, human serum

CAL0	**REF DCE002/11406-0**
CAL1	**REF DCE002/11407-0**
CAL2	**REF DCE002/11408-0**
CAL3	**REF DCE002/11409-0**
CAL4	**REF DCE002/11410-0**

2. Controls (2 vials, 1,2 mL each, ready to use)
Phosphate buffer 0,1M, NaN_3 < 0,1%, human serum

Positive Control	**REF DCE045/11402-0**
Negative Control	**REF DCE045/11401-0**

3. Sample Diluent (1 vial, 100 mL)
Tampone fosfato 0,1 M NaN_3 < 0,1%
REF DCE053-0

4. Conjugate IgG (1 vial, 15 mL)
Anti h-IgG conjugate with horseradish peroxidise (HRP), BSA 0,1%, Proclin < 0,0015% **REF DCE002/11402-G**

5. Conjugate IgM (1 vial, 15 mL)
Anti h-IgM conjugate with horseradish peroxidise (HRP), BSA 0,1%, Proclin < 0,0015% **REF DCE002/11402-M**

6. Coated Microplate (1 breakable microplate)
Antigenic phospholipid and β2-Glicoprotein complexes coated on the microplate **REF DCE002/11403-0**

7. TMB Substrate (1 vial, 15 mL)
H_2O_2 -TMB (0,26 g/L) *(avoid any skin contact)*
REF DCE004-0

8. Stop Solution (1 vial, 15 mL)
Sulphuric acid 0,15M *(avoid any skin contact)*
REF DCE005-0

9. 10X Conc. Wash Solution (1 vial, 50 mL)
Phosphate buffer 0,2M pH 7.4 **REF DCE054-0**

3.2. Reagents necessary not supplied

Distilled water.

3.3. Auxiliary materials and instrumentation

Automatic dispenser.
Microplate reader (450 nm, 620-630 nm).

Notes

Store all reagents between 2÷8°C in the dark.
Open the bag of reagent 6 (Coated Microplate) only when it is at room temperature and close it immediately after use; once opened, it is stable until expiry date of the kit.

4. WARNINGS

- This kit is intended for in vitro use by professional persons only. Not for internal or external use in Humans or Animals.
- Use appropriate personal protective equipment while working with the reagents provided.
- Follow Good Laboratory Practice (GLP) for handling blood products.
- Material of animal origin used in the preparation of the kit has been obtained from animals certified as healthy and the bovine protein has been obtained from countries not infected by BSE, but these materials should be handled as potentially infectious.
- All human source material used in the preparation of the reagents has been tested and found negative for antibody to HIV 1&2, HbsAg, and HCV. No test method however can offer complete assurance that HIV, HBV, HCV or other infectious agents are absent. Therefore, Calibrators and Controls should be handled in the same manner as potentially infectious material.
- Some reagents contain small amounts of Sodium Azide (NaN_3) or Proclin 300^R as preservatives. Avoid the contact with skin or mucosa.
- Sodium Azide may be toxic if ingested or absorbed through the skin or eyes; moreover it may react with lead or copper plumbing to form potentially explosive metal azides. If you use a sink to remove the reagents, allow scroll through large amounts of water to prevent azide build-up.
- The TMB Substrate contains an irritant, which may be harmful if inhaled, ingested or absorbed through the skin. To prevent injury, avoid inhalation, ingestion or contact with skin and eyes.
- The Stop Solution consists of a diluted sulphuric acid solution. Sulphuric acid is poisonous and corrosive and can be toxic if ingested. To prevent chemical burns, avoid contact with skin and eyes.
- Avoid the exposure of reagent TMB/H_2O_2 to directed sunlight, metals or oxidants. Do not freeze the solution.

5. PRECAUTIONS

- Please adhere strictly to the sequence of pipetting steps provided in this protocol. The performance data represented here were obtained using specific reagents listed in this Instruction For Use.
- All reagents should be stored refrigerated at 2-8°C in their original container. Any exceptions are clearly indicated. The reagents are stable until the expiry date when stored and handled as indicated.
- Allow all kit components and specimens to reach room temperature (22-28°C) and mix well prior to use.
- Do not interchange kit components from different lots. The expiry date printed on box and vials labels must be observed. Do not use any kit component beyond their expiry date.
- **WARNING: the conjugate reagent is designed to ensure maximum dose sensitivity and may be contaminated by external agents if not used properly;** therefore, it is recommended to use disposable consumables (tips, bottles, trays, etc.). For divided doses, take the exact amount of conjugate needed and do not re-introduce any waste product into the original bottle. In addition, **for doses dispensed with the aid of automatic and semi-automatic devices,** before using the conjugate, it is advisable to clean the fluid handling system, ensuring that the procedures of washing, deproteinization and decontamination are effective in avoiding contamination of the conjugate; this procedure is highly recommended when the kit is processed using analyzers which are not equipped with disposable tips.
  For this purpose, Dia.Metra supplies a separate decontamination reagent for cleaning needles.
- If you use automated equipment, the user has the responsibility to make sure that the kit has been appropriately tested.
- The incomplete or inaccurate liquid removal from the wells could influence the assay precision and/or increase the background. To improve the performance of the kit on automatic systems is recommended to increase the number of washes.
- It is important that the time of reaction in each well is held constant for reproducible results. Pipetting of samples should not extend beyond ten minutes to avoid assay drift. If more than 10 minutes are needed, follow the same order of dispensation. If more than one plate is used, it is recommended to repeat the dose response curve in each plate
- Addition of the TMB Substrate solution initiates a kinetic reaction, which is terminated by the addition of the Stop

Solution. Therefore, the TMB Substrate and the Stop Solution should be added in the same sequence to eliminate any time deviation during the reaction.
- Observe the guidelines for performing quality control in medical laboratories by assaying controls and/or pooled sera.
- Maximum precision is required for reconstitution and dispensation of the reagents.
- Samples microbiologically contaminated, highly lipemeic or haemolysed should not be used in the assay.
- Plate readers measure vertically. Do not touch the bottom of the wells.

6. PROCEDURE

6.1. Preparation of Calibrators (C_0…C_4)

The Calibrators are ready to use and are mixed, so they have both IgG and IgM antibodies. The Calibrators have the following concentrations:

	C_0	C_1	C_2	C_3	C_4
AU/mL	0	5	10	20	80

Once opened, the Calibrators are stable 6 months at 2-8°C.

6.2. Sample Preparation

Either human serum or plasma samples can be used for the test execution. Test samples should be clear. Contamination by lipemia should be avoided, although it does not interfere with this assay. Specimens may be refrigerated at 2-8°C for up to five days or stored at -20°C up to six months. Avoid repetitive freezing and thawing of serum samples. This may result in variable loss of autoantibody activity. Testing of heat-inactivated sera is not recommended.

All serum and plasma samples have to be diluted 1:100 with sample diluent; for example 10 L of sample may be diluted with 990 L of sample diluent.

The Controls are ready to use.

6.3. Wash Solution Preparation

Dilute the contents of each vial of the buffered wash solution concentrate (10X) with distilled water to a final volume of 500 mL prior to use. For smaller volumes respect the 1:10 dilution ratio. The diluted wash solution is stable for 30 days at 2-8°C.

In concentrated wash solution it is possible to observe the presence of crystals. In this case mix at room temperature until complete dissolution of crystals is observed. For greater accuracy dilute the whole bottle of concentrated wash solution to 500 mL taking care also to transfer the crystals completely, then mix until the crystals are completely dissolved.

6.4. Procedure

- **Allow all reagents to reach room temperature (22-28°C) for at least 30 minutes.** At the end of the assay store immediately the reagents at 2-8°C: avoid long exposure to room temperature.
- Unused coated microwell strips should be released securely in the foil pouch containing desiccant and stored at 2-8°C.
- To avoid potential microbial and/or chemical contamination, unused reagents should never be transferred into the original vials.
- As it is necessary to perform the determination in duplicate in order to improve accuracy of the test results, prepare two wells for each point of the calibration curve (C_0-C_4), two for each Control, two for each sample, one for Blank.

Reagents	Calibrator	Sample/ Controls	Blank
Calibrator C_0-C_4	**100 µL**		
Controls		**100 µL**	
Diluted Sample		**100 µL**	
Incubate 60 minutes at room temperature (22-28°C). Remove the content from each well, wash the wells 3 times with 300 µL of diluted wash solution. **Important note**: during each washing step, gently shake the plate for 5 seconds and remove excess solution by tapping the inverted plate on an absorbent paper towel. **Automatic washer**: if you use automated equipment, wash the wells at least 5 times.			
Conjugate (IgG or IgM)	**100 µL**	**100 µL**	
Incubate 30 minutes at room temperature (22-28°C). Remove the content from each well, wash the wells 3 times with 300 µL of diluted wash solution. **Washing**: follow the same indications of the previous point.			
TMB Substrate	**100 µL**	**100 µL**	**100 µL**
Incubate 15 minutes in the dark at room temperature (22-28°C).			
Stop Solution	**100 µL**	**100 µL**	**100 µL**
Shake the microplate gently. Read the absorbance (E) at 450 nm against a reference wavelength of 620-630 nm or against Blank within 5 minutes.			

7. QUALITY CONTROL

- The anti-phopholipids Positive Control should be run with every batch of samples to ensure that all reagents and procedures perform properly.
- Because Positive Control is prediluted, it do not control for procedural methods associated with dilution of specimens.
- Additional suitable control sera may be prepared by aliquoting pooled human serum specimens and storing at < -20°C.
- In order for the test results to be considered valid, all of the criteria listed below must be met. If any of these are not met, the test should be considered invalid and the assay repeated:

- The Positive Control are intended to monitor for substantial reagent failure and they will not ensure precision at the assay cut-off.
- This test is only valid if the optical density at 450 nm for Positive Control as well as for the Calibrator (C_0-C_4) complies with the respective range indicated on the Quality Control Certificate enclosed to each test kit.

8. RESULTS

For Anti Phospholipid Screen kit a 4-Parameter-Fit with lin-log coordinates for optical density and concentration is the data reduction method of choice. Smoothed-Spline Approximation and log-log coordinates are also suitable. However we recommend using a Lin-Log Plot.

First calculate the averaged optical densities for each calibrator well. Use lin-log graph paper and plot the averaged optical density of each calibrator versus the concentration. Draw the best fitting curve approximating the path of all calibrator points. The calibrator points may

also be connected with straight line segments. The concentration of unknowns may then be estimated from the calibration curve by interpolation.

9. REFERENCE VALUES

In a normal range study with serum samples from healthy blood donors the following ranges have been established with the Anti Phospholipid Screen test:

	IgG (GPL AU/mL)	IgM (MPL AU/mL)
Normal	< 10	< 10
Elevated	≥ 10	≥ 10

Please pay attention to the fact that the determination of a range of expected values for a “normal” population in a given method is dependent on many factors, such as specificity and sensitivity of the method used and type of population under investigation. Therefore each laboratory should consider the range given by the Manufacurer as a general indication and produce their own range of expected values based on the indigenous population where the laboratory works.
Positive results should be verified concerning the entire clinical status of the patient. Also every decision for therapy should be taken individually. It is recommended that each laboratory establishes its own normal and pathological ranges of seric Ab-Anti-Phospholipid.

10. LIMITATIONS OF PROCEDURE

The presence of immune complexes or other immunoglobulin aggregates in the patient sample may cause an increased level of non-specific binding and produce false positives in this assay

11. PERFORMANCE AND CHARACTERISTICS

11.1. Precision and reproducibility

Precision and reproducibility are evaluated by eight reply of two positive samples by two different runs with two different lots. Dispensing and washing operations were performed manually by an operator.
The results in terms of standard deviation and coefficient of variation were below:

	IgG			
Sample	1		2	
	SD	CV%	SD	CV%
Intra-assay	1.03	5.9	1.31	7.4
Inter-assay	0.26	9.2	5.25	11.7
	IgM			
Sample	1		2	
	SD	CV%	SD	CV%
Intra-assay	0.61	7.6	1.97	5.9
Inter-assay	0.15	7.1	2.98	6.6

11.2. Sensitivity

The clinicl sensitivity of Anti Phoshpolipids Screen IgG assay is 92,3%.
The clinicl sensitivity of Anti Phoshpolipids Screen IgM assay is 68,8%.

11.3. Specificity

The clinical specificity of Anti Phoshpolipids Screen IgG assay is 84,6%.
The clinical specificity of Anti Phoshpolipids Screen IgM assay is > 99,9%.

11.4. Detection Limit

The lowest concentration that can be distinguished from Calibrator zero is 0.03 AU/mL for IgG and 0.16 AU/mL for IgM.

12. WASTE MANAGEMENT

Reagents must be disposed off in accordance with local regulations..

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Anti Phospholipid Screen

para análisis de rutina

Determinación cuantitativa de autoanticuerpos anti-fosfolípidos en suero o plasma

IVD | **LOT** Ver etiqueta externa | 2°C 8°C | Σ = 96 ensayos | **REF DKO114**

USO PREVISTO

El kit de análisis anti-fosfolípidos es un ensayo inmunoenzimático en fase sólida para la determinación cuantitativa en suero o plasma humano de autoanticuerpos de tipo IgG e IgM contra los fosfolípidos aniónicos mediados por la β2 glicoproteína, incluyendo cardiolipina, fosfatidil serina, fosfatidil inositolo, ácido fosfatídico, fosfatidil colina, lisofosfatidil colina y fosfatidil etanolamina.
El ensayo está diseñado para uso diagnóstico *in vitro*, como coadyuvante en el diagnóstico de mayor riesgo de trombosis en pacientes con lupus eritematoso sistémico (LES) o trastornos similares.
El kit Anti Phospholipid Screen está destinado al uso en laboratorio exclusivamente.

1. SIGNIFICADO CLÍNICO

El primer estudio sobre anticuerpos anti-fosfolípidos data de 1906, cuando Wasserman introdujo un análisis serológico para la sífilis. En 1942 se descubrió que el componente activo era un fosfolípido indicado con el nombre de cardiolipina. En los años 50 se observó que un gran número de personas era positiva al análisis de la sífilis aunque no presentaba ninguno de los síntomas de la enfermedad. Al principio, el fenómeno fue catalogado como una serie de resultados falsamente positivos al análisis de sífilis; más tarde se advirtió que en este grupo de pacientes había una gran preponderancia de trastornos autoinmunes, entre ellos el lupus eritematoso sistémico (LES) y el síndrome de Sjögrens.
El término *lupus anticoagulante* (LA), utilizado por primera vez en 1972, deriva de observaciones experimentales en las que se advirtió un aumento del riesgo de trombosis, paradójicamente en presencia de algunos factores anticoagulantes; el término LA no es del todo incorrecto, porque la patología es más frecuente en pacientes sin lupus y va asociada a trombosis antes que a sangrado anormal.
En años más recientes, se investigó el papel de un cofactor, la proteína β2-glicoproteina I (apolipoproteína H) también llamada β2GPI, y su interacción con los fosfolípidos aniónicos contenidos en el suero o plasma humanos. Este cofactor es una β-globulina con peso molecular 50 kDa que se encuentra en el plasma en concentración aproximada de 200 μg/mL. Se descubrió que β2GPI interviene en la regulación de la coagulación de la sangre, inhibiendo la vía intrínseca. *In vivo,* la β2GPI está asociada a sustancias de carga negativa, por ejemplo fosfolípidos aniónicos, heparina y lipoproteínas. La región que liga los fosfolípidos está ubicada en su quinto dominio.
Con el acrónimo "aPL" (anticuerpos anti-fosfolípidos) se identifican impropiamente anticuerpos contra fosfolípidos de carga negativa como la cardiolipina (CL), la fosfatidil serina (PS), el fosfatil inositolo (PI) y el ácido fosfatídico (PA); según una acepción más correcta del término, se entiende por anticuerpos anti-fosfolípidos aquellos anticuerpos dirigidos contra el complejo formado por la β2GPI y los fosfolípidos aniónicos capaces de ligarse al dominio quinto de la β2GPI. De éstos, la cardiolipina es el fosfolípido utilizado más frecuentemente como antígeno para dosificar los aPL con el método ELISA. En el laboratorio clínico se miden los anticuerpos contra el complejo de β2GPI y fosfolípidos de carga negativa, tales como fosfatidil serina (PS), fosfatidil inositol (PI) y ácido fosfatídico (PA).
Algunos investigadores sugieren que el empleo de la PS en lugar de la cardiolipina en los ensayos ELISA permitiría un diagnóstico más exacto. Sin embargo, estos anticuerpos anti-fosfolípidos se utilizan con menos frecuencia aunque su empleo puede aumentar la sensibilidad clínica en las muestras de pacientes en que se sospecha la síndrome anti-fosfolipídica, pero no pueden reemplazar la determinación de los autoanticuerpos anti-cardiolipina.

2. PRINCIPIO DEL MÉTODO

El ensayo de detección de anti-fosfolípidos se basa en el ligado de los anticuerpos presentes en el suero contra los complejos antigénicos formados por fosfolípidos aniónicos (cardiolipina, fosfatidil serina, fosfatidil inositolo, ácido fosfatídico, fosfatidil colina, lisofosfatidil colina y fosfatidil etanolamina) y la β2 glicoproteína; estos complejos están inmovilizados en la microplaca. Los anticuerpos de tipos IgG y IgM dirigidos contra estos antígenos, presentes en los calibradores, controles y muestras de suero o plasma diluidas de pacientes, se ligan a sus respectivos antígenos. Tras 60 minutos de incubación, se lava la microplaca con solución de lavado para eliminar los componentes del suero inactivos. Una solución de inmunoglobulinas anti-humanas IgG (IgG conjugado, reactivo 4) o anti-humano IgM (IgM Conjugado, reactivo 5) reconoce la IgG o IgM (respectivamente) unido a los antígenos inmovilizados.
Tras 30 minutos de incubación, el exceso de conjugado enzimático no ligado específicamente se elimina mediante lavado con solución de lavado. A continuación se añade una solución sustrato cromogénica que contiene tetrametilbenzidina (sustrato TMB). Se incuba 15 minutos y se para el desarrollo del color añadiendo la solución de parada. El color de la solución se vuelve amarillo y la intensidad de color desarrollada es directamente proporcional a la concentración de anticuerpos IgG o IgM de la muestra original.

3. REACTIVOS, MATERIALES E INSTRUMENTACIÓN

3.1. Reactivos y materiales suministrados en el kit

1. Calibradores (5 frascos, 1,2 mL cada uno)
Tampón fosfato 0,1 M, NaN_3 < 0,1%, suero humano

CAL0	**REF DCE002/11406-0**
CAL1	**REF DCE002/11407-0**
CAL2	**REF DCE002/11408-0**
CAL3	**REF DCE002/11409-0**
CAL4	**REF DCE002/11410-0**

2. Controles (2 frascos, 1,2 mL cada uno)
Tampón fosfato 0,1 M, NaN_3 < 0,1%, suero humano

Control negativo	**REF DCE045/11401-0**
Control positivo	**REF DCE045/11402-0**

3. Diluyente de muestra (1 frasco, 100 mL)
Tampón fosfato 0,1 M, NaN_3 < 0,1%
REF DCE053-0

4. Conjugado IgG (1 frasco, 15 mL)
Anti h-IgG conjugado con peroxidasa de rabano (HRP), BSA 0,1%, Proclin < 0,0015% **REF DCE002/11402-G**

5. Conjugado IgM (1 frasco, 15 mL)
Anti h-IgM conjugado con peroxidasa de rabano (HRP), BSA 0,1%, Proclin < 0,0015% **REF DCE002/11402-M**

6. Microplaca recubierta
(1 microplaca rompible con el complejo antigénico de fosfolípidos y β2 glicoproteína adsorbidos)
REF DCE002/11403-0

7. Substrato TMB (1 frasco, 15 mL)
H_2O_2-TMB (0,26 g/L) *(evitar el contacto con la piel)*
REF DCE004-0

8. Solución de parada (1 frasco, 15 mL)
0,15M ácido sulfúrico *(evitar el contacto con la piel)*
REF DCE005-0

9. Solución de lavado conc. 10X (1 frasco, 50 mL)
Tampón fosfato 0,2M pH 7.4 **REF DCE054-0**

3.2. Reactivos necesarios no suministrados en el kit

Agua destilada.

3.3. Material e instrumentación auxiliares

Dispensadores automáticos.
Lector de microplacas (450 nm, 620-630 nm).

Notas

Conservar todos los reactivos a 2÷8°C, protegidos de la luz. Abrir la bolsa del reactivo 6 (microplaca recubierta) solo cuando se encuentre a temperatura ambiente y cerrarla inmediatamente después de extraer las tiras que se vayan a utilizar; una vez abierta, permanece estable hasta la fecha de caducidad del kit.

4. ADVERTENCIAS

- Este kit de ensayo está previsto para usarse in vitro y por personal experto. No es para uso interno o externo en humanos o animales.
- Usar los equipos de protección individual previstos al trabajar con los reactivos suministrados.
- Siga las Buenas Prácticas de Laboratorio (GLP) en el manejo de las muestras sanguíneas y sus derivados.
- Todos los reactivos de origen humano usados en la preparación de los Calibradores y de los controles se han comprobado y han resultado negativos para la presencia de anticuerpos anti-VIH 1 y 2, para HbsAg y para anticuerpos anti-VHC. Sin embargo, ningún ensayo ofrece seguridad absoluta de la ausencia de VIH, VHB, VHC o de otros agentes infecciosos. Por lo tanto, los Calibradores y lo control positivo deben manipularse como material potencialmente infeccioso.
- Materiales de origen animal utilizadas para la elaboración de este kit se obtuvieron a partir de animales sanos y de las proteínas de bovino se obtuvieron de los países no afectados por la EEB, pero estos materiales se debe utilizar como potencialmente infecciosos.
- Algunos reactivos contienen pequeñas cantidades de Azida de Sodio (NaN_3) o Proclin 300^R como conservante. Evite el contacto con la piel y las mucosas.
- La Azida de Sodio, usada como conservante, puede ser tóxica si se ingiere o se absorbe a través de la piel o de los ojos; además, puede reaccionar con las tuberías de plomo o cobre formando azidas metálicas potencialmente explosivas. Dejar que corra gran cantidad de agua, si se usa un lavabo para eliminar los reactivos, para prevenir la formación de azidas.
- El cromógeno TMB contiene un irritante que puede ser dañino si se inhala, se ingiere o se absorbe a través de la piel. Para prevenir lesiones, evitar la inhalación, la ingestión o el contacto con la piel y con los ojos.
- La solución de parada está formada por una solución de ácido sulfúrico diluido. El ácido sulfúrico es venenoso y corrosivo, y puede ser tóxico si se ingiere. Para prevenir posibles quemaduras químicas, evitar el contacto con la piel y con los ojos.
- Evite la exposición de los reactivos TMB/H_2O_2 a la luz solar directa, metales u oxidantes. No congelar la solución.

5. PRECAUCIONES

- Respetar rigurosamente la secuencia de los pasos indicados en este protocolo. Los resultados aquí presentados se han obtenido utilizando los reactivos específicos que figuran en estas instrucciones de uso.
- Todos los reactivos deben conservarse a una temperatura controlada de 2-8°C en sus recipientes originales. Todas las excepciones están claramente marcados. Los reactivos son estables hasta la fecha de caducidad cuando se almacenan y manipulan de acuerdo con las instrucciones proporcionadas.
- Antes del uso, esperar hasta que todos los componentes del kit y las muestras se encuentren a temperatura ambiente (22-28°C) y mezclar cuidadosamente.
- No mezclar componentes de kits de lotes distintos. Se debe observar la fecha de caducidad indicada en la etiqueta de la caja y de todas las ampollas. No usar componentes después de la fecha de caducidad.
- **ATENCIÓN**: **se ha estudiado el reactivo conjugado para garantizar la máxima sensibilidad en la determinación y, por lo tanto, si no se usa adecuadamente, podría contaminarse por agentes externos**; se recomienda utilizar consumibles (puntas, frascos, bandejas, etc.) desechables. Para determinaciones fraccionadas, tomar la cantidad necesaria exacta de conjugando y evitar volver a introducir los posibles restos en el frasco original. Además, **para determinaciones realizadas con la ayuda de instrumentación automática y semiautomática**, se recomienda, antes de usar el conjugado, realizar una fase de limpieza de la fluídica, asegurándose de que los procedimientos de lavado, desproteinización y descontaminación resulten eficaces para evitar la contaminación del conjugado; este procedimiento se recomienda especialmente cuando el kit se procesa con analizadores que no están dotados de puntas monouso. Para tal fin, Dia.Metra pone a su disposición por separado un reactivo descontaminante para el lavado de las agujas.

- Si utiliza un equipo automático, es responsabilidad del usuario asegurar que el equipo ha sido debidamente validada.
- Un lavado incompleto o impreciso y la aspiración insuficiente del líquido de los pocillos ELISA pueden causar una precisión pobre y/o un elevado fondo. Para mejorar el rendimiento del kit en los sistemas automatizados, se recomienda aumentar el número de lavados.
- Para la reproducibilidad de los resultados, es importante que el tiempo de reacción sea igual para cada pocillo. El tiempo de dispensación de los pocillos no debe superar los 10 minutos; si se prolongara más allá de los 10 minutos, respétese el orden de dispensación. si utiliza más de una placa, se recomienda repetir la curva de calibración en cada plato.
- Al añadir el substrato TMB inicia una reacción cinética que termina al agregar la solución de parada. Tanto el sustrato como la solución de parada deben agregarse en la misma secuencia para evitar diferentes tiempos de reacción.
- Observar las directrices para la ejecución del control de calidad en los laboratorios clínicos al comprobar controles y/o pool de sueros.
- Observar la máxima precisión en la reconstitución y dispensación de los reactivos.
- No use muestras con contaminación microbiana, altamente lipémicas o hemolizadas.
- Los lectores de microplacas leen las DO verticalmente, por tanto no debe tocarse el fondo de los pocillos.

6. PROCEDIMIENTO

6.1. Preparación de los Calibradores (C_0…C_4)

Los Calibradores están listos para usar y son mixtos, es decir, contienen tanto los anticuerpos IgG como IgM. Los Calibradores están listos para usarse y tienen las siguientes concentraciones:

	C_0	C_1	C_2	C_3	C_4
AU/mL	0	5	10	20	80

Una vez abiertos, los Calibratores permanecen estables 6 meses conservados a 2-8°C.

6.2. Preparación de la muestra

Para realizar el ensayo se pueden usar muestras de suero o plasma humano. Las muestras que se van a usar deben estar limpias. Se recomienda evitar la contaminación por hiperlipemia, aunque esta no interfiera con el análisis. Las muestras pueden conservarse refrigeradas a 2-8°C durante 5 días, o a -20°C hasta 6 meses. Se recomienda no congelar y descongelar repetidamente las muestras de suero o plasma, puesto que esto podría provocar una pérdida variable de la actividad de los autoanticuerpos. No se recomienda el análisis de muestras inactivadas por calor.

Todas las muestras de suero o plasma deben prediluirse 1:100 con diluyente de muestras. por ejemplo 10 µL de suero o plasma pueden diluirse con 990 µL de diluyente de muestras.

Los controles están listos para usar.

6.3. Preparación de la solución de lavado

Antes del uso, diluir el contenido de cada frasco de solución de lavado tamponada concentrada (10x) con agua destilada hasta un volumen de 500 mL. Para preparar volúmenes menores, respetar la relación de dilución de 1:10. La solución de lavado diluida se mantiene estable a 2-8°C durante al menos 30 días. En la solución de lavado concentrada es posible observar la presencia de cristales. En ese caso, agitar a temperatura ambiente hasta que los cristales se disuelvan por completo. Para una mayor precisión, diluir todo el frasco de la solución de lavado concentrada a 500 mL, teniendo cuidado para transferir también los cristales y, a continuación, agitar hasta que se disuelvan por completo.

6.4. Procedimiento

- **Esperar hasta que todos los reactivos se encuentren a temperatura ambiente (22-28°C) durante al menos 30 minutos.** Al final del ensayo inmediatamente poner todos los reactivos a 2-8°C para evitar largos periodos a temperatura ambiente.
- Las tiras de pocillos no utilizados se deben guardar de inmediato en la bolsa desechable que contiene desecantes y almacenarse a 2-8°C.
- Para evitar la contaminación microbiana y/o química no regrese porciones de reactivos no usados en los viales originales.
- Para aumentar la precisión de los resultados de la prueba es necesario trabajar en duplicado: preparar dos pocillos para cada punto de la curva de calibración (C_0-C_4), dos para cada control, dos para cada muestra, uno para el blanco.

Reactivos	Calibrador	Muestra/ Controles	Blanco
Calibrador C_0-C_4	**100 µL**		
Controles		**100 µL**	
Muestra diluida		**100 µL**	
Incubar 60 minutos a temperatura ambiente (22-28°C). Retirar la mezcla de reacción y lavar los pocillos tres veces con 300 µL de solución de lavado diluida. **Nota importante**: agite suavemente la placa durante 5 segundos en cada paso del lavado. Después del último lavado asegúrese haber eliminado completamente la solución de lavado de los pozos, invierta la placa y golpéela repetidas veces contra una servilleta de papel absorbente. **Lavados automático**: si está utilizando una lavadora automática, lavar los pocillos al menos 5 veces.			
Conjugado (IgG o IgM)	**100 µL**	**100 µL**	
Incubar 30 minutos a temperatura ambiente (22-28 °C). Retirar la mezcla de reacción y lavar los pocillos tres veces con 300 µL de solución de lavado diluida. **Lavados**: siga las mismas instrucciones del punto anterior.			
Substrato TMB	**100 µL**	**100 µL**	**100 µL**
Incubar 15 minutos a temperatura ambiente (22-28°C), en la oscuridad.			
Solución de parada	**100 µL**	**100 µL**	**100 µL**
Agitar la microplaca con cuidado. Leer la absorbancia (E) a 450 nm frente una segunda lectura de referencia a 620-630 nm o frente al blanco entre 5 minutos.			

7. CONTROL DE CALIDAD

- Los controles positivo y negativo deben incluirse cada vez que se realice el ensayo para asegurar que todos los reactivos y el ensayo funcionen de forma correcta.
- Puesto que los controles están prediluidos, no representan un control de procedimiento para las técnicas de dilución usadas para las muestras.
- Se pueden preparar sueros de control adicionales recogiendo un pool de sueros humanos, dividiéndolo en alícuotas y conservándolo a < -20 °C.
- Para que los resultados del ensayo se consideren válidos, se deben cumplir todos los criterios siguientes. Aunque solo uno no se encuentre dentro de los valores especificados, los resultados no deberán considerarse válidos y el ensayo deberá repetirse:
  - La absorbancia del control positivo debe ser mayor que la del control negativo.
  - El control negativo y el positivo sirven para controlar un eventual malfuncionamiento de los reactivos y no aseguran la precisión en correspondencia con el valor límite del ensayo.
  - El ensayo es válido solo si la densidad óptica a 450 nm del control negativo y del control positivo, así como las de los calibradores (C_0-C_4), coinciden con los intervalos correspondientes indicados en el Certificado de control de calidad incluido en el kit.

8. CÁLCULO DE LOS RESULTADOS

Para Anti Phospholipid Screen, el método de elección para el tratamiento de los resultados es un procesamiento de 4 parámetros con ejes lin-log para la densidad óptica y la concentración respectivamente. Además, se pueden usar una aproximación spline y coordenadas log-log. Sin embargo, se recomienda usar una curva Lin-Log.
En primer lugar, calcular la media de las densidades ópticas relativas a los calibradores. Usar una hoja de papel lin-log y trazar las densidades ópticas medias de cada calibrador frente a la respectiva concentración. Dibujar la curva que mejor se aproxime a todos los puntos de calibración. Los puntos de los calibradores también pueden unirse con segmentos de línea recta. La concentración de las muestras desconocidas puede determinarse por interpolación de la curva de calibración.

9. VALORES DE REFERENCIA

En un estudio sobre los valores normales realizado con muestras de suero procedentes de donantes sanos se han determinado los siguientes intervalos de normalidad con el ensayo Anti-Phospholipid Screen:

	IgG (GPL AU/mL)	**IgM (MPL AU/mL)**
Normal	< 10	< 10
Alto	≥ 10	≥ 10

Es importante señalar que la determinación de un rango de valores esperados en un método dado para una población "normal" depende de muchos factores, tales como la especificidad y sensibilidad del método en uso, y la población en estudio. Por lo tanto, cada laboratorio debe considerar el intervalo especificado por el fabricante como una guía general y producir su propio rango de valores calculados en base al estadístico obtenido por el laboratorio, donde reside la población local.
Los resultados positivos deben verificarse con relación al estado clínico del paciente. Además, cada decisión relativa a la terapia debe tomarse individualmente. Se recomienda que cada laboratorio establezca sus propios intervalos normal y patológico de anticuerpos anti-fosfolipidos sérica.

10. LIMITACIONES DEL ENSAYO

La presencia en la muestra de complejos inmunes o de otros agregados de inmunoglobulinas puede dar lugar a reacciones no específicas con resultados falsos positivos.

11. PARÁMETROS CARACTERÍSTICOS

11.1. Precisión y reproducibilidad

La precisión y la reproducibilidad se evaluaron analizando ocho duplicados de dos muestras positivas en dos ensayos diferentes con dos lotes de kits diferentes.
La dispensación y el lavado las efectuó manualmente un operador.
Los resultados de desviación Calibración y coeficiente de variación se indican a continuación:

	IgG			
Muestra	1		2	
	SD	CV%	SD	CV%
Intra-ensayo	1.03	5.9	1.31	7.4
Entre-ensayos	0.26	9.2	5.25	11.7
	IgM			
Muestra	1		2	
	SD	CV%	SD	CV%
Intra-ensayo	0.61	7.6	1.97	5.9
Entre-ensayos	0.15	7.1	2.98	6.6

11.2. Sensibilidad

La sensibilidad clínica del ensayo anti-fosfolípidos IgG es de 92,3%.
La sensibilidad clínica del ensayo anti-fosfolípidos IgM es un 68,8%.

11.3. Especificidad

La especificidad clínica del ensayo anti-fosfolípidos IgG es de 84,6%.
La especificidad clínica del ensayo anti-fosfolípidos IgM es un 100%.

11.4. Límite de detección:

La concentración mínima que puede distinguirse del Calibración cero es de aproximadamente 0,03 AU/mL para IgG y 0,16 AU/mL para IgM.

12. DISPOSICIONES PARA LA ELIMINACIÓN

Los reactivos deben eliminarse de acuerdo con las leyes locales.

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DiaMetra	Packaging Information Sheet	Mod. PIS000-1

IVD	DE ES FR GB IT PT	In vitro Diagnostikum Producto sanitario para diagnóstico In vitro Dispositif medical de diagnostic in vitro In vitro Diagnostic Medical Device Dispositivo medico-diagnostico in vitro Dispositivos medicos de diagnostico in vitro		DE ES FR GB IT PT	Hergestellt von Elaborado por Fabriqué par Manufacturer Produttore Produzido por
	DE ES FR GB IT PT	Achtung, Begleitdokumente Precaución, consulte los documentos adjuntos Attention, veuillez consulter les documents d'accompagnement Caution, consult accompanying documents Attenzione, consultare la documentazione allegata Atenção,consultar os documentos de acompanhamento	 yyyy-mm	DE ES FR GB IT PT	Herstellungs datum Fecha de fabricacion Date de fabrication Date of manufacture Data di produzione Data de produção
 yyyy-mm-dd	DE ES FR GB IT PT	Verwendbar bis Establa hasta (usar antes de último día del mes) Utiliser avant (dernier jour du mois indiqué) Use by (last day of the month) Utilizzare prima del (ultimo giorno del mese) Utilizar (antes ultimo dia do mês)		DE ES FR GB IT PT	Biogefährdung Riesco biológico Risque biologique Biological risk Rischio biologico Risco biológico
	DE ES FR GB IT PT	Gebrauchsanweisung beachten Consultar las instrucciones Consulter le mode d'emploi Consult instructions for use Consultare le istruzioni per l'uso Consultar instruções para uso	LOT	DE ES FR GB IT PT	Chargenbezeichnung Codigo de lote Numero de lot Batch code Codice del lotto Codigo do lote
 Σ = xx	DE ES FR GB IT PT	Ausreichend für "n" Tests Contenido suficiente para "n" tests Contenu suffisant pour "n" tests Contains sufficient for "n" tests Contenuto sufficiente per "n" saggi Contém o suficiente para "n" testes	CONT	DE ES FR GB IT PT	Inhalt Contenido del estuche Contenu du coffret Contents of kit Contenuto del kit Conteúdo do kit
Max  Min	DE ES FR GB IT PT	Temperaturbereich Límitaciôn de temperatura Limites de température de conservation Temperature limitation Limiti di temperatura Temperaturas limites de conservação	REF	DE ES FR GB IT PT	Bestellnummer Nûmero de catálogo Référéncès du catalogue Catalogue number Numero di Catalogo Número do catálogo
	DE ES FR GB IT PT	Vor direkter sonneneinstrahlung schützen Mantener alejado de la luz solar Tenir à l'écart de la lumière du soleil Keep away from sunlight Tenere lontano dalla luce solare Mantenha longe da luz solar			

SUGGERIMENTI PER LA RISOLUZIONE DEI PROBLEMI/TROUBLESHOOTING

ERRORE CAUSE POSSIBILI/ SUGGERIMENTI

Nessuna reazione colorimetrica del saggio

- mancata dispensazione del coniugato
- contaminazione del coniugato e/o del Substrato
- errori nell'esecuzione del saggio (es. Dispensazione accidentale dei reagenti in sequenza errata o provenienti da flaconi sbagliati, etc.)

Reazione troppo blanda (OD troppo basse)

- coniugato non idoneo (es. non proveniente dal kit originale)
- tempo di incubazione troppo breve, temperatura di incubazione troppa bassa

Reazione troppo intensa (OD troppo alte)

- coniugato non idoneo (es. non proveniente dal kit originale)
- tempo di incubazione troppo lungo, temperatura di incubazione troppa alta
- qualità scadente dell'acqua usata per la soluzione di lavaggio (basso grado di deionizzazione,)
- lavaggi insufficienti (coniugato non completamente rimosso)

Valori inspiegabilmente fuori scala

- contaminazione di pipette, puntali o contenitori- lavaggi insufficienti (coniugato non completamente rimosso)

CV% intrasaggio elevato

- reagenti e/o strip non portate a temperatura ambiente prima dell'uso
- il lavatore per micropiastre non lava correttamente (suggerimento: pulire la testa del lavatore)

CV% intersaggio elevato

- condizioni di incubazione non costanti (tempo o temperatura)
- controlli e campioni non dispensati allo stesso tempo (con gli stessi intervalli) (controllare la sequenza di dispensazione)
- variabilità intrinseca degli operatori

ERROR POSSIBLE CAUSES / SUGGESTIONS

No colorimetric reaction

- no conjugate pipetted reaction after addition
- contamination of conjugates and/or of substrate
- errors in performing the assay procedure (e.g. accidental pipetting of reagents in a wrong sequence or from the wrong vial, etc.)

Too low reaction (too low ODs)

- incorrect conjugate (e.g. not from original kit)
- incubation time too short, incubation temperature too low

Too high reaction (too high ODs)

- incorrect conjugate (e.g. not from original kit)
- incubation time too long, incubation temperature too high
- water quality for wash buffer insufficient (low grade of deionization)
- insufficient washing (conjugates not properly removed)

Unexplainable outliers

- contamination of pipettes, tips or containers

insufficient washing (conjugates not properly removed) too high within-run

- reagents and/or strips not pre-warmed to CV% Room Temperature prior to use
- plate washer is not washing correctly (suggestion: clean washer head)

too high between-run - incubation conditions not constant (time, CV % temperature)

- controls and samples not dispensed at the same time (with the same intervals) (check pipetting order)
- person-related variation

ERROR / POSIBLES CAUSAS / SUGERENCIAS

No se produce ninguna reacción colorimétrica del ensayo

- no se ha dispensado el conjugado
- contaminación del conjugado y/o del substrato
- errores en la ejecución del ensayo (p. ej., dispensación accidental de los reactivos en orden incorrecto o procedentes de frascos equivocados, etc.)

Reacción escasa (DO demasiado bajas)

- conjugado no idóneo (p. ej., no procedente del kit original)
- tiempo de incubación demasiado corto, temperatura de incubación demasiado baja

Reacción demasiado intensa (DO demasiado altas)

- conjugado no idóneo (p. ej., no procedente del kit original)
- tiempo de incubación demasiado largo, temperatura de incubación demasiado alta
- calidad escasa del agua usada para la solución de lavado (bajo grado de desionización)
- lavados insuficientes (el conjugado no se ha retirado completamente)

Valores inexplicablemente fuera de escala

- contaminación de pipetas, puntas o contenedores- lavados insuficientes (el conjugado no se ha retirado completamente)

CV% intraensayo elevado

- los reactivos y/o tiras no se encontraban a temperatura ambiente antes del uso
- el lavador de microplacas no funciona correctamente (sugerencia: limpiar el cabezal del lavador)

CV% interensayo elevado

- condiciones de incubación no constantes (tiempo o temperatura)
- controles y muestras no dispensados al mismo tiempo (con los mismos intervalos) (controlar la secuencia de dispensación)
- variación en función de los operadores

ERREUR CAUSES POSSIBLES / SUGGESTIONS

Aucune réaction colorimétrique de l'essai

- non distribution du conjugué
- contamination du conjugué et/ou du substrat
- erreurs dans l'exécution du dosage (par ex., distribution accidentelle des réactifs dans le mauvais ordre ou en provenance des mauvais flacons, etc.)

Réaction trop faible (DO trop basse)

- conjugué non approprié (par ex., ne provenant pas du coffret original)
- temps d'incubation trop court, température d'incubation trop basse

Réaction trop intense (DO trop élevée)

- conjugué non approprié (par ex., ne provenant pas du coffret original)
- temps d'incubation trop long, température d'incubation trop élevée
- mauvaise qualité de l'eau utilisée pour la solution de lavage (bas degré de déionisation)
- lavages insuffisants (conjugué non entièrement éliminé)

Valeurs inexplicablement hors plage

- contamination des pipettes, embouts ou récipients - lavages insuffisants (conjugué non entièrement éliminé)

CV% intra-essai élevé

- les réactifs et/ou les bandes n'ont pas atteint la température ambiante avant usage
- le laveur de microplaques ne lave pas correctement (suggestion : nettoyer la tête du laveur)

CV% inter-essai élevé

- conditions d'incubation non constantes (temps ou température)
- contrôles et échantillons non distribués en même temps (avec les mêmes intervalles) (contrôler l'ordre de distribution)
- variabilité intrinsèque des opérateurs

CE

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DCM092-5
Ed. 09/2018

Anti MPO (p-ANCA)

per analisi di routine

Determinazione quantitativa degli anticorpi IgG contro la mieloperossidasi (MPO) nel siero o plasma umano

IVD | **LOT** Vedere etichetta esterna | 2°C 8°C | Σ = 96 test | **REF DKO092**

DESTINAZIONE D'USO
L'Anti MPO (p-ANCA) è un test immunoenzmatico in fase solida (ELISA) indiretto per la determinazione quantitativa deli autoanticorpi di classe IgG diretti contro la mieloperossidasi (MPO) nel siero o plasma umano. Il test è da intendersi per uso diagnostico in vitro come supporto nella diagnosi di alcune vasculiti autoimmuni quali la gronulomatosi di Wegener.
Il kit Anti MPO (p-ANCA) è destinato al solo uso di laboratorio.

1. SIGNIFICATO CLINICO

Gli anticorpi anti citoplasma-neutrofilico (ANCA) rappresentano un gruppo di autoanticorpi diretti contro le componenti citoplasmatiche dei granulociti neutrofili e dei monociti. I metodi classici per la determinazione degli ANCA sono i test di immunofluorescenza (IF) Con queste tecniche di immunofluorescenza indiretta si riconoscono 2 modelli, il tipo citoplasmatico (c-ANCA) e il tipo perinucleare (p-ANCA).
L'antigene principale per il c-ANCA è la proteinasi 3 (PR3) presente nei granuli primari. Gli anticorpi anti p-ANCA presenti nei sieri positivi sono principalmente diretti contro la mieloperossidasi (MPO). Anticorpi diretti verso altri antigeni quali ad esempio la lattoferrina, l'elastasi, la catepsina-G e anche il lisozima spesso danno origine a positività per p-ANCA. Questo rende difficile l'esatta interpretazione e classificazione dei profili di immunofluorescenza. Perciò ogni risposta positiva all'IF per ANCA dovrebbere essere differenziata mediante tecnica ELISA utilizzando antigeni purificati.
PR3-ANCA e MPO-ANCA sono marcatori sierologici affidabili nella diagnosi di vasculite. PR3-ANCA è l'autoantigene classico nella granulomatosi di Wegener con una specificità clinica maggiore del 95%. La presenza di c-ANCA è stata documentata in diverse malattie. Gli anticorpi anti–MPO sono altamente specifici per glomerulonefriti idiopatiche e associate a vasculiti e per la poliarteite nodosa, la sindrome di Churg-Strauss e la poliangite senza coinvolgimento renale.
I metodi ELISA specifici per PR3 e MPO possono fornire un importante risultato di conferma oltre all'interoretazione di risultati di difficile lettura per l'immunofluorescenza, ad esempio nei casi in cui questa rileva positività contemporanea per più anticorpi o elevato background di fluorescenza

2. PRINCIPIO DEL METODO

Il test Anti MPO (p-ANCA) si basa sul legame degli anticorpi presenti nel campione con la mieloperossidasi da neutrofili umani legata sulle micropiastre. Nella prima fase gli anticorpi presenti nei calibratori, nei controlli e nei campioni prediluiti dei pazienti si legano sulla superficie interna dei pozzetti. Dopo 60 minuti di incubazione, la micropiastra viene lavata con un tampone di lavaggio per rimuovere le componenti del siero che non hanno reagito.
Nella seconda fase una soluzione di immunoglobuline anti-human IgG coniugate con perossidasi di rafano (HRP) riconosce gli anticorpi di classe IgG legati agli antigeni immobilizzati. Dopo 30 minuti di incubazione, l'eccesso di coniugato enzimatico che non si è legato specificamente viene rimosso mediante un lavaggio con tampone.
Successivamente si aggiunge ai pozzetti una soluzione substrato cromogenica contenente TMB. Dopo 15 minuti di incubazione si blocca lo sviluppo del colore mediante aggiunta della soluzione di stop. Il colore della soluzione diventa giallo. La quantità di colore sviluppato è direttamente proporzionale alla concentrazione di anticorpi IgG presenti nel campione originale.
La concentrazione di anticorpi IgG è calcolata sulla base di una curva di calibrazione.

3. REAGENTI, MATERIALI E STRUMENTAZIONE

3.1. Reattivi e materiali forniti nel kit

1. Calibrators (5 flaconi, 1,2 mL ciascuno)
Tampone fosfato 0,1M, NaN_3 < 0,1%, siero umano

CAL0	**REF DCE002/09206-0**
CAL1	**REF DCE002/09207-0**
CAL2	**REF DCE002/09208-0**
CAL3	**REF DCE002/09209-0**
CAL4	**REF DCE002/09210-0**

2. Controlli (2 flaconi, 1,2 mL ciascuno, pronti all'uso)
Tampone fosfato 0,1M, NaN_3 < 0,1%, siero umano

Controllo Negativo	**REF DCE045/09201-0**
Controllo Positivo	**REF DCE045/09202-0**

3. Sample Diluent (1 flacone, 100 mL)
Tampone fosfato 0,1M, NaN_3 < 0,1%
REF DCE053-0

4. Conjugate (1 flacone, 15 mL)
Anti h-IgG coniugato con perossidasi di rafano (HRP), BSA 0,1%, Proclin < 0,0015% **REF DCE002/09202-0**

5. Coated Microplate (1 micropiastra breakable)
Micropiastra con mieloperossidasi da neutrofili umani adorbita **REF DCE002/09203-0**

6. TMB Substrate (1 flacone, 15 mL)
H_2O_2 -TMB (0,26 g/L) *(evitare il contatto con la pelle)*
REF DCE004-0

7. Stop Solution (1 flacone, 15 mL)
Acido solforico 0,15M *(evitare il contatto con la pelle)*
REF DCE005-0

8. 10X Conc. Wash Solution (1 flacone, 50 mL)
Tampone fosfato 0,2M, pH 7.4 **REF DCE054-0**

3.2. Reattivi necessari non forniti nel kit
Acqua distillata.

3.3. Materiale e strumentazione ausiliare
Dispensatori automatici.
Lettore per micropiastre (450 nm, 620-630 nm).

Note
Conservare tutti i reattivi a 2÷8°C, al riparo dalla luce.
Aprire la busta del Reattivo 5 (Coated Microplate) solo dopo averla riportata a temperatura ambiente e chiuderla subito dopo il prelievo delle strip da utilizzare; una volta aperta è stabile fino alla data di scadenza del kit.

4. AVVERTENZE
- Questo test kit è per uso in vitro, da eseguire da parte di personale esperto. Non per uso interno o esterno su esseri Umani o Animali.
- Usare i previsti dispositivi di protezione individuale mentre si lavora con i reagenti forniti.
- Seguire le Buone Pratiche di Laboratorio (GLP) per la manipolazione di prodotti derivati da sangue.
- Tutti i reattivi di origine umana usati nella preparazione dei reagenti sono stati testati e sono risultati negativi per la presenza di anticorpi anti-HIV 1&2, per HbsAg e per anticorpi anti-HCV. Tuttavia nessun test offre la certezza completa dell'assenza di HIV, HBV, HCV o di altri agenti infettivi. Pertanto, i Calibratori ed i Controlli devono essere maneggiati come materiali potenzialmente infettivi.
- Materiali di origine animale usati per la preparazione di questo kit sono stati ottenuti da animali sani e le proteine bovine sono state ottenute da paesi non affetti da BSE, ma comunque questi materiali dovrebbero essere usati come potenzialmente contagiosi.
- Alcuni reagenti contengono piccole quantità di Sodio Azide (NaN_3) o di Proclin 300^R come conservante. Evitare il contatto con la pelle e le mucose.
- La Sodio Azide può essere tossica se ingerita o assorbita attraverso la cute o gli occhi; inoltre, può reagire con le tubature di piombo o rame formando azidi metalliche potenzialmente esplosive. Se si usa un lavandino per eliminare i reagenti, lasciar scorrere grandi quantità di acqua per prevenire la formazione di azidi.
- Il TMB Substrato contiene un irritante, che può essere dannoso se inalato, ingerito o assorbito attraverso la cute. Per prevenire lesioni, evitare l'inalazione, l'ingestione o il contatto con la cute e con gli occhi.
- La Stop Solution è costituita da una soluzione di acido solforico diluito. L'acido solforico è velenoso e corrosivo e può essere tossico se ingerito. Per prevenire possibili ustioni chimiche, evitare il contatto con la cute e con gli occhi.
- Evitare l'esposizione del reagente TMB/H_2O_2 a luce solare diretta, metalli o ossidanti. Non congelare la soluzione.

5. PRECAUZIONI
- Si prega di attenersi rigorosamente alla sequenza dei passaggi indicata in questo protocollo. I risultati presentati qui sono stati ottenuti usando specifici reagenti elencati in queste Istruzioni per l'Uso.
- Tutti i reattivi devono essere conservati a temperatura controllata di 2-8°C nei loro contenitori originali. Eventuali eccezioni sono chiaramente indicate. I reagenti sono stabili fino alla data di scadenza se conservati e trattati seguendo le istruzioni fornite.
- Prima dell'uso lasciare tutti i componenti dei kit e i campioni a temperatura ambiente (22-28°C) e mescolare accuratamente.
- Non scambiare componenti dei kit di lotti diversi. Devono essere osservate le date di scadenza riportate sulle etichette della scatola e di tutte le fiale. Non utilizzare componenti oltre la data di scadenza.
- **ATTENZIONE**: **il reagente Coniugato è stato studiato per garantire la massima sensibilità di dosaggio, e pertanto, se non opportunamente usato, può essere contaminato da agenti esterni**; si raccomanda pertanto di utilizzare consumabili (puntali, flaconi, vaschette ecc.) usa e getta. Per dosaggi frazionati, prelevare l'esatta quantità di coniugato necessaria ed evitare di re-introdurre l'eventuale scarto nel flacone originale. Inoltre, **per dosaggi effettuati con l'ausilio di strumentazione automatica e semi-automatica**, si consiglia, prima di utilizzare il coniugato, di effettuare uno step di pulizia della fluidica, assicurandosi che le procedure di lavaggio, deproteinizzazione e decontaminazione siano efficaci nell'evitare la contaminazione del coniugato; questa procedura è fortemente raccomandata quando il kit è processato con analizzatori non dotati di puntali monouso.
  A tale scopo Dia.Metra rende disponibile separatamente un reattivo decontaminante per il lavaggio degli aghi.
- Qualora si utilizzi strumentazione automatica, è responsabilità dell'utilizzatore assicurarsi che il kit sia stato opportunamente validato.
- Un lavaggio incompleto o non accurato dei pozzetti può causare una scarsa precisione e/o un'elevato background. Per migliorare le prestazioni del kit su strumentazione automatica, si consiglia di aumentare il numero di lavaggi.
- Per la riproducibilità dei risultati, è importante che il tempo di reazione di ogni pozzetto sia lo stesso. Per evitare il time shifting durante la dispensazione degli reagenti, il tempo di dispensazione dei pozzetti non dovrebbe estendersi oltre i 10 minuti. Se si protrae oltre, si raccomanda di seguire lo stesso ordine di dispensazione. Se si utilizza più di una piastra, si raccomanda di ripetere la curva di calibrazione in ogni piastra.
- L'addizione del TMB Substrato dà inizio ad una reazione cinetica, la quale termina con l'addizione della Stop Solution. L'addizione del TMB Substrato e della Stop Solution deve avvenire nella stessa sequenza per evitare tempi di reazione differenti.
- Osservare le linee guida per l'esecuzione del controllo di qualità nei laboratori clinici testando controlli e/o pool di sieri.
- Osservare la massima precisione nella ricostituzione e dispensazione dei reagenti.
- Non usare campioni microbiologicamente contaminati, altamente lipemici o emolizzati.
- I lettori di micropiastre leggono l'assorbanza verticalmente. Non toccare il fondo dei pozzetti.

6. PROCEDIMENTO

6.1. Preparazione dei Calibratori (C_0…C_4)
Dal momento che non sono disponibili preparati di riferimento internazionale per gli anticorpi anti MPO, il sistema di analisi è calibrato in unità relative arbitrarie.
I Calibratori sono pronti all'uso ed hanno le seguenti concentrazioni:

	C_0	C_1	C_2	C_3	C_4
AU/mL	0	10	20	40	160

Una volta aperti sono stabili per 6 mesi a 2÷8°C.

6.2. Preparazione del campione

Per l'esecuzione del test si possono utilizzare campioni di siero o plasma umano. I campioni da utilizzare devono essere limpidi. Si consiglia di evitare contaminazioni dovute a iperlipemia, anche se queste non interferiscono con l'analisi. I campioni possono essere conservati refrigerati a 2-8°C fino a 5 giorni, oppure conservati a -20°C fino a 6 mesi. Si consiglia di evitare congelamenti e scongelamenti ripetuti dei campioni che potrebbero determinare una perdita variabile dell'attività degli autoanticorpi. Non è raccomandata l'analisi di campioni inattivati al calore.

Tutti i campioni di siero o plasma devono essere prediluiti 1:100 con sample diluent; per esempio 10 µL di campione possono essere diluiti con 990 µL di sample diluent.

I Controli sono pronti all'uso.

6.3. Preparazione della Wash Solution

Prima dell'uso, diluire il contenuto di ogni fiala di "10X Conc. Wash Solution" con acqua distillata fino al volume di 500 mL. Per preparare volumi minori rispettare il rapporto di diluizione di 1:10. La soluzione di lavaggio diluita è stabile a 2-8°C per almeno 30 giorni.

Nella wash solution concentrata è possibile osservare la presenza di cristalli; in tal caso agitare a temperatura ambiente fino a completa dissoluzione dei cristalli; per una maggiore precisione diluire tutto il flacone della soluzione di lavaggio concentrata a 500 mL, avendo cura di trasferire anche i cristalli, poi agitare fino a completa dissoluzione dei cristalli.

6.4. Procedimento

- **Portare tutti i reagenti a temperatura ambiente (22-28°C) per almeno 30 minuti.** Al termine del dosaggio riporre immediatamente tutti i reagenti a 2-8°C: evitare lunghi periodi a temperatura ambiente.
- Le strisce di pozzetti non utilizzate devono essere rimesse immediatamente nella busta richiudibile contenente il materiale essicante e conservate a 2-8°C.
- Per evitare potenziali contaminazioni microbiche e/o chimiche non rimettere i reagenti inutilizzati nei flaconi originali.
- Al fine di aumentare l'accuratezza dei risultati del test è necessario operare in doppio, allestendo due pozzetti per ogni punto della curva di calibrazione (C_0-C_4), due per ogni Controllo, due per ogni Campione ed uno per il Bianco.

Reagenti	Calibrator	Campione /Controlli	Bianco
Calibrator C_0-C_4	**100 µL**		
Controlli		**100 µL**	
Campione diluito		**100 µL**	
Incubare 60 minuti a temperatura ambiente (22-28°C). Allontanare la miscela di reazione, lavare i pozzetti 3 volte con 300 µL di wash solution diluita. **Nota importante**: ad ogni step di lavaggio, agitare delicatamente la piastra per 5 secondi e successivamente rimuovere l'eccesso di soluzione di lavaggio sbattendo delicatamente la micropiastra capovolta su fogli di carta assorbente. **Lavaggi automatici**: se si utilizza strumentazione automatica effettuare almeno 5 lavaggi.			
Coniugato	**100 µL**	**100 µL**	
Incubare 30 minuti a temperatura ambiente (22-28°C). Allontanare la miscela di reazione, lavare i pozzetti 3 volte con 300 µL di wash solution diluita. **Lavaggi**: seguire le stesse indicazioni del punto precedente.			
TMB Substrato	**100 µL**	**100 µL**	**100 µL**
Incubare 15 minuti a temperatura ambiente al riparo dalla luce (22-28°C).			
Stop Solution	**100 µL**	**100 µL**	**100 µL**
Agitare delicatamente la micropiastra. Leggere l'assorbanza (E) a 450 nm contro una lunghezza d'onda di riferimento di 620-630 nm oppure contro il Bianco entro 5 minuti.			

7. CONTROLLO DI QUALITA'

- Il Controllo Positivo per il test Anti MPO (p-ANCA) e il Controllo Negativo devono essere inclusi ogni volta che si esegue il test per assicurare che tutti i reagenti ed il test funzionino in modo corretto.
- Poichè i Controlli sono prediluiti, essi non rappresentano un controllo procedurale per le tecniche di diluizione utilizzate per i campioni.
- Ulteriori sieri di controllo possono essere preparati raccogliendo un pool dei sieri umani, aliquotandolo e conservandolo a < -20°C.
- Perchè i risultati del test siano considerati validi, tutti i seguenti criteri devono essere soddisfatti. Se anche uno solo non rientra nei valori specificati, i risultati non dovrebbero essere considerati validi ed il test dovrebbe essere ripetuto:
  - L'assorbanza del Controllo Positivo deve essere maggiore di quella del Controllo Negativo.
  - Il Controllo Negativo e quello Positivo servono per controllare un'eventuale malfunzionamento dei reagenti e non assicurano la precisione in corrispondenza del valore soglia del test.
  - Il test è valido solo se la densità ottica a 450 nm del Controllo Positivo (1) e del Controllo Negativo (2) come pure quelle dei calibratori (C_0-C_5) coincidono con gli intervalli corrispondenti indicati nel Certificato di Controllo di Qualità incluso nel kit.

8. CALCOLO DEI RISULTATI

Per il kit Anti MPO (p-ANCA) il metodo di scelta per il trattamento dei risultati è una elaborazione 4 parametri con assi lin-log per densità ottica e concentrazione rispettivamente. Inoltre si possono utilizzare un'approssimazione spline e coordinate log-log. Tuttavia si raccomanda di utilizzare una curva Lin-Log.

Innanzitutto occorre calcolare la media delle densità ottiche relative ai calibratori. Utilizzare un foglio di carta lin-log e tracciare le densità ottiche medie di ogni calibratore verso la rispettiva concentrazione. Disegnare la curva che approssima nel modo migliore tutti i punti di calibrazione. I punti dei calibratori possono anche essere collegati con segmenti di linea retta. La concentrazione dei campioni incogniti può essere determinata per interpolazione dalla curva di calibrazione.

9. VALORI DI RIFERIMENTO

In uno studio sui valori normali eseguito con campioni di siero provenienti da donatori sani sono stati determinati i seguenti intervalli di normalità con il test Anti MPO(p-ANCA):

	Anti MPO (p-ANCA)
Cut-off	20 AU/mL

È importante tenere presente che la determinazione di un range di valori attesi in un dato metodo per una popolazione "normale" è dipendente da molteplici fattori, quali la specificità e sensibilità del metodo in uso, e la popolazione in esame. Perciò ogni laboratorio dovrebbe considerare i range indicati dal Fabbricante come un'indicazione generale e produrre range di valori attesi propri basati sulla popolazione indigena dove il laboratorio risiede.
I risultati positivi dovrebbero essere verificati relativamente allo stato clinico del paziente. Inoltre, ogni decisione relativa alla terapia dovrebbe essere presa individualmente. Si raccomanda che ogni laboratorio stabilisca i sui propri intervalli normale e patologico di Anti-MPO serica.

10. LIMITAZIONI DEL TEST

La presenza nel campione di complessi immuni o di altri aggregati di immunoglobuline può determinare delle reazioni aspecifiche con conseguenti risultati falsi positivi.

11. PARAMETRI CARATTERISTICI

11.1. Specificità

Test di correlazione contro un analogo kit commerciale di riferimento, effettuati su 32 sieri (di cui 8 positivi e 24 negativi) hanno mostrato una specificità del 100%.

11.2. Sensibilità:

Test di correlazione contro un analogo kit commerciale di riferimento, effettuati su 32 sieri (di cui 8 positivi e 24 negativi) hanno mostrato una sensibilità del 100%.

11.3. Limite di rilevabilità:

La minor concentrazione di anti MPO che può essere distinta dal Calibratore zero è di circa 0,73 AU/mL con limite di confidenza del 98%.

11.4. Precisione e riproducibilità

11.4.1 Intra-Assay

La variabilità all'interno dello stesso kit è stata determinata replicando la misura di tre diversi sieri con valori situati dentro il range di lavoro della curva di calibrazione. La variabilità intra-assay è ≤ 6,1%.

11.4.2 Inter-Assay

La variabilità tra kit differenti è stata determinata replicando la misura di due differenti sieri di controllo con kit appartenenti a lotti diversi e/o con diverse combinazioni di lotti di reagenti. La variabilità inter-assay è ≤ 12,3%.

12. DISPOSIZIONI PER LO SMALTIMENTO

I reagenti devono essere smaltiti in accordo con le leggi locali.

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DCM092-5
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Anti MPO (p-ANCA)

for routine analysis

Quantitative determination of IgG class antibodies against myeloperoxidase (MPO) in human serum or plasma

IVD | **LOT** See external label | 2°C 8°C | Σ = 96 tests | **REF DKO092**

INTENDED USE

Anti MPO (p-ANCA) kit is an indirect solid phase enzyme immunoassay (ELISA) for the quantitative measurement of IgG class autoantibodies against myeloperoxidase (MPO) in human serum or plasma. The assay is intended for in vitro diagnostic use only as an aid in the diagnosis of certain autoimmune vasculitites such as Wegener´s granulomatosis.
Anti MPO (p-ANCA) kit is intended for laboratory use only.

1. CLINICAL SIGNIFICANCE

Anti-neutrophilic-cytoplasm antibodies (ANCA) represent a group of autoantibodies directed towards the cytoplasmatic components of the neutrophilic granulocytes and monocytes. The classical methods for the determination of ANCA are the immunofluorescent methods.With these indirect immunofluorescence techniques two main patterns are recognized, a cytoplasmatic (c-ANCA) and a perinuclear (p-ANCA) type.
The main antigen for the c-ANCA is the proteinase 3 (PR3), which is a serine proteinase of the present in primary granules.Antibodies of p-ANCA positive sera are mainly directed to myeloperoxidase (MPO). Antibodies to other antigens e.g. lactoferrin, elastase, cathepsin-G and also lysozyme often result in a similar p-ANCA pattern. Beside different untypical variants of p- ANCA IF patterns granulocyte specific antinuclear antibodies (GS-ANA) is indistinguishable from p-ANCA. This makes a clear interpretation and classification of the IF patterns difficult.
Therefore every positive IF-ANCA findings esp. p-ANCA should be differenciated by ELISA techniques using purified antigens. A survey of documented clinical indications of specific ANCA is given in the table below. PR3- ANCA and MPO-ANCA are reliable serologic markers in the diagnostics of vasculitides.
PR3- ANCA is the classical autoantigen in Wegener´s granulomatosis with a clinical specificity of more than 95%. c-ANCA is documented to be present in different diseases. Anti-MPO antibodies are highly specific for idiopathic and vasculitis associated crescentic glomerulonephritis and also for classic polyarteritis nodosa, Churg-Strauss syndrome and the polyangitis overlap syndrome without renal involvement.7-10 With respect to sensitivity, either MPO or PR-3 antibodies were found in 77 to 100% of patients with idiopathic and vasculitis associated crescentic glomerulonephritis. In WG, anti-MPO antibodies were detected only occasionally and generally in patients negative for PR-3 antibodies.
The MPO and PR-3 specific ELISA methods can provide an important confirmatory result for two of the more important of the identified antigens. ELISA is also useful for interpreting "difficult" samples by IFA such as those which exhibit several antibodies simultaneously or those with high background fluorescence.

2. PRINCIPLE

Anti MPO (p-ANCA) test is based on the binding of the antibodies in the sample to the human neutrophil myeloperoxidase coated on the microplates. In the first step the antibodies in calibrators, controls or prediluted patient samples bind to the inner surface of the wells. After a 60 minutes incubation the microplate is washed with a wash buffer to remove the non-reactive serum components. In the second step an anti-human-IgG horseradish peroxidase conjugated solution recognizes the IgG class antibodies bound to the immobilized antigens. After a 30 minutes incubation any excess of enzyme conjugate which is not specifically bound is washed away with the wash buffer. Then a chromogenic substrate solution containing TMB is dispensed into the wells. After 15 minutes of incubation the color development is stopped by adding the stop solution. The solutions color change into yellow. The amount of color is directly proportional to the concentration of IgG antibodies present in the original sample. The concentration of IgG antibodies in the sample is calculated through a calibration curve.

3. REAGENTS, MATERIALS AND INSTUMENTATION

3.1. Reagents and materials supplied in the kit

1. Calibrators (5 vials, 1.2 mL each)
Phosphate buffer 0,1M, NaN_3 < 0,1%, human serum

CAL0	**REF DCE002/09206-0**
CAL1	**REF DCE002/09207-0**
CAL2	**REF DCE002/09208-0**
CAL3	**REF DCE002/09209-0**
CAL4	**REF DCE002/09210-0**

2. Controls (2 vials, 1.2 mL each, ready for use)
Phosphate buffer 0,1M, NaN_3 < 0,1%, human serum

Negative control	**REF DCE045/09201-0**
Positive control	**REF DCE045/09202-0**

3. Sample Diluent (1 vial, 100 mL)
Phosphate buffer 0,1M, NaN_3 < 0,1%
REF DCE053-0

4. Conjugate (1 vial, 15 mL)
Anti h-IgG conjugated with horseradish peroxidase (HRP), BSA 0,1%, Proclin < 0,0015% **REF DCE002/09202-0**

5. Coated Microplate (1 breakable microplate)
Microplate coated with human neutrophil myeloperoxidase
REF DCE002/09203-0

6. TMB Substrate (1 vial, 15 mL)
H_2O_2-TMB 0.26 g/L *(avoid any skin contact)*
REF DCE004-0

7. Stop Solution (1 vial, 15 mL)
Sulphuric acid 0.15M *(avoid any skin contact)*
REF DCE005-0

8. 10X Conc. Wash Solution (1 vial, 50 mL)
Phosphate buffer 0,2M, pH 7.4 **REF DCE054-0**

3.2. Reagents necessary not supplied
Distilled water.

3.3. Auxiliary materials and instrumentation
Automatic dispenser.
Microplate reader (450 nm, 620-630 nm).

Notes
Store all reagents between 2÷8°C in the dark.
Open the bag of reagent 5 (Coated Microplate) only when it is at room temperature and close it immediately after use; once opened, it is stable until expiry date of the kit.

4. WARNINGS
- This kit is intended for in vitro use by professional persons only. Not for internal or external use in Humans or Animals.
- Use appropriate personal protective equipment while working with the reagents provided.
- Follow Good Laboratory Practice (GLP) for handling blood products.
- All human source material used in the preparation of the reagents has been tested and found negative for antibody to HIV 1&2, HbsAg, and HCV. No test method however can offer complete assurance that HIV, HBV, HCV or other infectious agents are absent. Therefore, Calibrators and Controls should be handled in the same manner as potentially infectious material.
- Material of animal origin used in the preparation of the kit has been obtained from animals certified as healthy and the bovine protein has been obtained from countries not infected by BSE, but these materials should be handled as potentially infectious.
- Some reagents contain small amounts of Sodium Azide (NaN_3) or Proclin 300^R as preservatives. Avoid the contact with skin or mucosa.
- Sodium Azide may be toxic if ingested or absorbed through the skin or eyes; moreover it may react with lead or copper plumbing to form potentially explosive metal azides. If you use a sink to remove the reagents, allow scroll through large amounts of water to prevent azide build-up.
- The TMB Substrate contains an irritant, which may be harmful if inhaled, ingested or absorbed through the skin. To prevent injury, avoid inhalation, ingestion or contact with skin and eyes.
- The Stop Solution consists of a diluted sulphuric acid solution. Sulphuric acid is poisonous and corrosive and can be toxic if ingested. To prevent chemical burns, avoid contact with skin and eyes.
- Avoid the exposure of reagent TMB/H_2O_2 to directed sunlight, metals or oxidants. Do not freeze the solution.

5. PRECAUTIONS
- Please adhere strictly to the sequence of pipetting steps provided in this protocol. The performance data represented here were obtained using specific reagents listed in this Instruction For Use.
- All reagents should be stored refrigerated at 2-8°C in their original container. Any exceptions are clearly indicated. The reagents are stable until the expiry date when stored and handled as indicated.
- Allow all kit components and specimens to reach room temperature (22-28°C) and mix well prior to use.
- Do not interchange kit components from different lots. The expiry date printed on box and vials labels must be observed. Do not use any kit component beyond their expiry date.
- **WARNING: the conjugate reagent is designed to ensure maximum dose sensitivity and may be contaminated by external agents if not used properly;** therefore, it is recommended to use disposable consumables (tips, bottles, trays, etc.). For divided doses, take the exact amount of conjugate needed and do not re-introduce any waste product into the original bottle. In addition, **for doses dispensed with the aid of automatic and semi-automatic devices,** before using the conjugate, it is advisable to clean the fluid handling system, ensuring that the procedures of washing, deproteinization and decontamination are effective in avoiding contamination of the conjugate; this procedure is highly recommended when the kit is processed using analyzers which are not equipped with disposable tips.
  For this purpose, Dia.Metra supplies a separate decontamination reagent for cleaning needles.
- If you use automated equipment, the user has the responsibility to make sure that the kit has been appropriately tested.
- The incomplete or inaccurate liquid removal from the wells could influence the assay precision and/or increase the background. To improve the performance of the kit on automatic systems is recommended to increase the number of washes.
- It is important that the time of reaction in each well is held constant for reproducible results. Pipetting of samples should not extend beyond ten minutes to avoid assay drift. If more than 10 minutes are needed, follow the same order of dispensation. If more than one plate is used, it is recommended to repeat the dose response curve in each plate
- Addition of the TMB Substrate solution initiates a kinetic reaction, which is terminated by the addition of the Stop Solution. Therefore, the TMB Substrate and the Stop Solution should be added in the same sequence to eliminate any time deviation during the reaction.
- Observe the guidelines for performing quality control in medical laboratories by assaying controls and/or pooled sera.
- Maximum precision is required for reconstitution and dispensation of the reagents.
- Samples microbiologically contaminated, highly lipemeic or haemolysed should not be used in the assay.
- Plate readers measure vertically. Do not touch the bottom of the wells.

6. PROCEDURE

6.1. Preparation of the Calibrators (C_0…C_4)
Since no international reference preparation for Anti-myeloperoxidase antibodies is available, the assay system is calibrated in relative arbitrary units. The Calibrators are to use and have the following concentration:

	C_0	C_1	C_2	C_3	C_4
AU/mL	0	10	20	40	160

Once opened, the Calibrators are stable 6 months at 2-8°C.

6.2. Preparation of the Sample
Either human serum or plasma samples can be used for the test execution. Test samples should be clear. Contamination by lipemia is best avoided, but does not interfere with this assay.
Specimens may be refrigerated at 2-8°C for up to five days or stored at -20°C up to six months. Avoid repetitive freezing and thawing of serum samples. This may result in variable loss of autoantibody activity.

Testing of heat-inactivated sample is not recommended.
All serum and plasma samples must be prediluted 1:100 with sample diluents; for example 10 L of sample should be diluted with 990 L of sample diluent.
The Controls are ready to use.

6.3. Preparation of the Wash Solution

Dilute the content of each vial of the "10X Conc. Wash Solution" with distilled water to a final volume of 500 mL prior to use. For smaller volumes respect the 1:10 dilution ratio. The diluted wash solution is stable for 30 days at 2-8°C.
In concentrated wash solution is possible to observe the presence of crystals; in this case mix at room temperature until the complete dissolution of crystals; for greater accuracy, dilute the whole bottle of concentrated wash solution to 500 mL, taking care to transfer completely the crystals, then mix until crystals are completely dissolved.

6.4. Procedure

- **Allow all reagents to reach room temperature (22-28°C) for at least 30 minutes.** At the end of the assay store immediately the reagents at 2-8°C: avoid long exposure to room temperature.
- Unused coated microwell strips should be released securely in the foil pouch containing desiccant and stored at 2-8°C.
- To avoid potential microbial and/or chemical contamination, unused reagents should never be transferred into the original vials.
- As it is necessary to perform the determination in duplicate in order to improve accuracy of the test results, prepare two wells for each point of the calibration curve (C_0-C_4), two for each Control, two for each sample, one for Blank.

Reagent	Calibrator	Sample/ Controls	Blank
Calibrator C_0-C_4	**100 µL**		
Controls		**100 µL**	
Diluted Sample		**100 µL**	
Incubate 60 minutes at room temperature (22-28°C). Remove the contents from each well, wash the wells 3 times with 300 µL of diluted wash solution. **Important note**: during each washing step, gently shake the plate for 5 seconds and remove excess solution by tapping the inverted plate on an absorbent paper towel. **Automatic washer**: if you use automated equipment, wash the wells at least 5 times.			
Conjugate	**100 µL**	**100 µL**	
Incubate 30 minutes at room temperature (22-28°C). Remove the contents from each well, wash the wells 3 times with 300 µL of diluted wash solution. **Washing**: follow the same indications of the previous point.			
TMB Substrate	**100 µL**	**100 µL**	**100 µL**
Incubate 15 minutes in the dark at room temperature (22-28°C).			
Stop Solution	**100 µL**	**100 µL**	**100 µL**
Shake the microplate gently. Read the absorbance (E) at 450 nm against a reference wavelength of 620-630 nm or against Blank within 5 minutes.			

7. QUALITY CONTROL

- The MPO IgG Positive and Negative Control should be run with every batch of samples to ensure that all reagents and procedures perform properly.
- Because Positive and the Negative Control are prediluted, they do not control for procedural methods associated with dilution of specimens.
- Additional suitable control sera may be prepared by aliquoting pooled human serum specimens and storing at < -20°C.
- In order for the test results to be considered valid, all of the criteria listed below must be met. If any of these are not met, the test should be considered invalid and the assay repeated:
- The absorbance of the prediluted MPO IgG Positive must be greater than the absorbance of the prediluted Negative Control.
- The Negative and Positive Control are intended to monitor for substantial reagent failure and they will not ensure precision at the assay cutoff.
- This test is only valid if the optical density at 450 nm for Positive Control (1) and Negative Control (2) as well as for the Calibrator S0-S5 complies with the respective range indicated on the Quality Control Certificate enclosed to each test kit: If any of these criteria is not met, the results are invalid and the test should be repeated.

8. RESULTS

For Anti MPO (p-ANCA) test a 4-Parameter-Fit with lin-log coordinates for optical density and concentration is the data reduction method of choice. Smoothed-Spline Approximation and log-log coordinates are also suitable.
Recommended Lin-Log Plot
First calculate the averaged optical densities for each calibrator well. Use lin-log graph paper and plot the averaged optical density of each calibrator versus the concentration. Draw the best fitting curve approximating the path of all calibrator points. The calibrator points may also be connected with straight line segments. The concentration of unknowns may then be estimated from the calibration curve by interpolation.

9. REFERENCE VALUES

In a normal range study with serum samples from healthy blood donors the following ranges have been established with the Anti MPO (p-ANCA) tests:

	Anti MPO (p-ANCA)
Cut-off	20 AU/mL

Please pay attention to the fact that the determination of a range of expected values for a “normal” population in a given method is dependent on many factors, such as specificity and sensitivity of the method used and type of population under investigation. Therefore each laboratory should consider the range given by the Manufacurer as a general indication and produce their own range of expected values based on the indigenous population where the laboratory works.
Positive results should be verified concerning the entire clinical status of the patient. Also every decision for therapy should be taken individually. It is recommended that each

laboratory establishes its own normal and pathological ranges of serum Anti-MPO.

10. LIMITATIONS OF PROCEDURE

The presence of immune complexes or other immunoglobulin aggregates in the patient sample may cause an increased level of non-specific binding and produce false positives in this assay.

11. PERFORMANCE AND CHARACTERISTICS

11.1. Specificity

Comparison test against a commercial reference kit, performed on 32 sera (8 of them positive sera and 24 negative sera) showed a 100% specificity.

11.2. Sensitivity

Comparison test against a commercial reference kit, performed on 32 sera (8 of them positive sera and 24 negative sera) showed a 100% sensitivity.

11.3. Detection Limit

The lowest concentration of anti MPO antibodies that can be distinguished from the Calibrator 0 is about 0.73 AU/mL with a confidence limit of 98%.

11.4. Precision and reproducibility

11.4.1. Intra-Assay

Within run variation was determined by replicate the measurements of three different sera with values in the range of the calibration curve. The within assay variability is ≤ 6.1%.

11.4.2. Inter-Assay

Between run variation was determined by replicate the measurements of two different control sera with different lots of kits and/or different mix of lots of reagents. The between assay variability is ≤ 12.3%.

12. WASTE MANAGEMENT

Reagents must be disposed off in accordance with local regulations.

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DiaMetra
care for quality

DCM092-5
Ed. 09/2018

Anti MPO (p-ANCA)

para análisis de rutina

Determinación cuantitativa de los anticuerpos IgG contra la mieloperoxidasa en suero o plasma humano.

IVD | **LOT** Ver etiqueta externa | 2°C 8°C | Σ = 96 ensayos | **REF DKO092**

USO PREVISTO
Anti MPO (p-ANCA) es un ensayo inmunoenzimático indirecto en fase sólida (ELISA) para la determinación cuantitativa de los autoanticuerpos de clase IgG directos contra la mieloperoxidasa (MPO) en suero o plasma humano. El ensayo está destinado al uso diagnóstico in vitro como apoyo en el diagnóstico de algunas vasculitis autoinmunes, como la granulomatosis de Wegener.
El kit Anti MPO (p-ANCA) está destinado al uso en laboratorio exclusivamente.

1. SIGNIFICADO CLÍNICO

Los anticuerpos anti citoplasma-neutrofílico (ANCA) representan un grupo de autoanticuerpos directos contra los componentes citoplasmáticos de los granulocitos neutrófilos y de los monocitos. Los métodos clásicos para la determinación de los ANCA son los ensayos de inmunofluorescencia (IF). Con estas técnicas de inmunofluorescencia indirecta se reconocen 2 modelos, el tipo citoplasmático (c-ANCA) y el tipo perinuclear (p-ANCA). El antígeno principal para el c-ANCA es la proteinasa 3 (PR3) presente en los gránulos primarios. Los anticuerpos anti p-ANCA presentes en los sueros positivos son principalmente directos contra la mieloperoxidasa (MPO). Anticuerpos directos contra otros antígenos, como por ejemplo lactoferrina, elastasa, catepsina G y también lisozima, a menudo dan lugar a positividad para p-ANCA. Esto dificulta la interpretación y clasificación exactas de los perfiles de inmunofluorescencia. Por lo tanto, cada respuesta positiva a IF para ANCA debe diferenciarse mediante la técnica ELISA usando antígenos purificados.
PR3-ANCA y MPO-ANCA son marcadores serológicos fiables en el diagnóstico de vasculitis. PR3-ANCA es el autoantígeno clásico en la granulomatosis de Wegener con una especificidad clínica superior al 95%. La presencia de c-ANCA se ha documentado en distintas enfermedades. Los anticuerpos anti-MPO son altamente específicos para glomerulonefritis idiopática y asociada con vasculitis, y para la poliarteritis nodosa, el síndrome de Churg-Strauss y la poliangeítis sin afectación renal.
Los métodos ELISA específicos para PR3 y MPO pueden proporcionar un resultado importante de confirmación además de la interpretación de resultados de difícil lectura para la inmunofluorescencia, por ejemplo, en casos en los que esta detecta positividad simultánea para varios anticuerpos o elevado fondo de fluorescencia.

2. PRINCIPIO DEL MÉTODO

El ensayo anti-MPO-IgG se basa en la unión de los anticuerpos presentes en la muestra con la mieloperoxidasa de neutrófilos humanos unida a las microplacas. Los anticuerpos presentes en los calibradores, en los controles y en las muestras prediluidas de los pacientes se unen a la superficie interna de los pocillos. Tras 60 minutos de incubación, la microplaca se lava con un tampón de lavado para retirar los componentes del suero que no han reaccionado.
Una solución de inmunoglobulinas anti-IgG humana conjugadas con peroxidasa reconoce los anticuerpos de clase IgG unidos a los antígenos inmovilizados. Tras 30 minutos de incubación, el exceso de conjugado enzimático que no se ha unido específicamente se retira mediante un lavado con el tampón.
Se añade a los pocillos una solución de substrato cromogénico que contiene TMB. Tras 15 minutos de incubación se bloquea el desarrollo del color mediante la adición de la solución de parada. El color de la solución se vuelve amarillo. La cantidad del color desarrollado es directamente proporcional a la concentración de anticuerpos IgA presentes en la muestra original.

3. REACTIVOS, MATERIALES E INSTRUMENTACIÓN

3.1. Reactivos y materiales suministrados en el kit

1. Calibradores (CAL) de anti-MPO (5 frascos, 1,2 mL cada uno)

Tampón fosfato 0,1 M, NaN_3 < 0,1%, suero humano

CAL0	**REF DCE002/09206-0**
CAL1	**REF DCE002/09207-0**
CAL2	**REF DCE002/09208-0**
CAL3	**REF DCE002/09209-0**
CAL4	**REF DCE002/09210-0**

2. Controles (2 frascos, 1,2 mL cada uno, listo para usar)

Tampón fosfato 0,1 M, NaN_3 < 0,1%, suero humano

Control negativo	**REF DCE045/09201-0**
Control positivo	**REF DCE045/09202-0**

3. Diluyente de muestra (1 frasco, 100 mL)

Tampón fosfato 0,1 M, NaN_3 < 0,1%

REF DCE053-0

4. Conjugado (1 frasco, 1 mL)

Anti h-IgG conjugado con peroxidasa de rabano (HRP), BSA 0,1%, Proclin < 0,0015% **REF DCE002/09202-0**

5. Microplaca recubierta (1 microplaca rompible)

Microplaca con mieloperoxidasa de neutrófilos humanos absorbida **REF DCE002/09203-0**

6. Substrato TMB (1 frasco, 15 mL)

H_2O_2 -TMB (0,26 g/L) *(evítese el contacto con la piel)*

REF DCE004-0

7. Solución de parada (1 frasco, 15 mL)

0,15M ácido sulfúrico *(evítese el contacto con la piel)*

REF DCE005-0

8. Solución de lavado conc. 10X (1 frasco, 50 mL)
Tampón fosfato 0,2M pH 7.4 **REF DCE054-0**

3.2. Reactivos necesarios no suministrados en el kit
Agua destilada.
3.3. Material e instrumentación auxiliares
Dispensadores automáticos.
Lector de microplacas (450 nm, 620-630 nm).

Notas
Conservar los reactivos a oscuras, a temperatura entre 2 y 8 ºC.
Atemperar a temperatura ambiente la bolsa del reactivo 5 (microplaca recubierta) antes de abrirla; cerrarla de inmediato después de sacar las tiras que se han de utilizar; una vez abierta, permanece estable hasta la fecha de caducidad del kit.

4. ADVERTENCIAS

- Este kit de ensayo está previsto para usarse in vitro y por personal experto. No es para uso interno o externo en humanos o animales.
- Usar los equipos de protección individual previstos al trabajar con los reactivos suministrados.
- Siga las Buenas Prácticas de Laboratorio (GLP) en el manejo de las muestras sanguíneas y sus derivados.
- Todos los reactivos de origen humano usados en la preparación de los Calibradores y de los controles se han comprobado y han resultado negativos para la presencia de anticuerpos anti-VIH, para HbsAg y para anticuerpos anti-VHC. Sin embargo, ningún ensayo ofrece seguridad absoluta de la ausencia de VIH, VHB, VHC o de otros agentes infecciosos. Por lo tanto, los Calibradores y los controles positivo y negativo deben manipularse como material potencialmente infeccioso.
- Materiales de origen animal utilizadas para la elaboración de este kit se obtuvieron a partir de animales sanos y de las proteínas de bovino se obtuvieron de los países no afectados por la EEB, pero estos materiales se debe utilizar como potencialmente infecciosos.
- Algunos reactivos contienen pequeñas cantidades de Azida de Sodio (NaN_3) o Proclin 300^R como conservante. Evite el contacto con la piel y las mucosas.
- La Azida de Sodio, usada como conservante, puede ser tóxica si se ingiere o se absorbe a través de la piel o de los ojos; además, puede reaccionar con las tuberías de plomo o cobre formando azidas metálicas potencialmente explosivas. Dejar que corra gran cantidad de agua, si se usa un lavabo para eliminar los reactivos, para prevenir la formación de azidas.
- El cromógeno TMB contiene un irritante que puede ser dañino si se inhala, se ingiere o se absorbe a través de la piel. Para prevenir lesiones, evitar la inhalación, la ingestión o el contacto con la piel y con los ojos.
- La solución de parada está formada por una solución de ácido sulfúrico diluido. El ácido sulfúrico es venenoso y corrosivo, y puede ser tóxico si se ingiere. Para prevenir posibles quemaduras químicas, evitar el contacto con la piel y con los ojos.
- Evite la exposición de los reactivos TMB/H_2O_2 a la luz solar directa, metales u oxidantes. No congelar la solución.

5. PRECAUCIONES

- Respetar rigurosamente la secuencia de los pasos indicados en este protocolo. Los resultados aquí presentados se han obtenido utilizando los reactivos específicos que figuran en estas instrucciones de uso.
- Todos los reactivos deben conservarse a una temperatura controlada de 2-8 °C en sus recipientes originales. Todas las excepciones están claramente marcados. Los reactivos son estables hasta la fecha de caducidad cuando se almacenan y manipulan de acuerdo con las instrucciones proporcionadas.
- Antes del uso, esperar hasta que todos los componentes del kit y las muestras se encuentren a temperatura ambiente (22-28°C) y mezclar cuidadosamente.
- No mezclar componentes de kits de lotes distintos. Se debe observar la fecha de caducidad indicada en la etiqueta de la caja y de todas las ampollas. No usar componentes después de la fecha de caducidad.
- **ATENCIÓN**: **se ha estudiado el reactivo conjugado para garantizar la máxima sensibilidad en la determinación y, por lo tanto, si no se usa adecuadamente, podría contaminarse por agentes externos**; se recomienda utilizar consumibles (puntas, frascos, bandejas, etc.) desechables. Para determinaciones fraccionadas, tomar la cantidad necesaria exacta de conjugando y evitar volver a introducir los posibles restos en el frasco original. Además, **para determinaciones realizadas con la ayuda de instrumentación automática y semiautomática**, se recomienda, antes de usar el conjugado, realizar una fase de limpieza de la fluídica, asegurándose de que los procedimientos de lavado, desproteinización y descontaminación resulten eficaces para evitar la contaminación del conjugado; este procedimiento se recomienda especialmente cuando el kit se procesa con analizadores que no están dotados de puntas monouso. Para tal fin, Dia.Metra pone a su disposición por separado un reactivo descontaminante para el lavado de las agujas.
- Si utiliza un equipo automático, es responsabilidad del usuario asegurar que el equipo ha sido debidamente validada.
- Un lavado incompleto o impreciso y la aspiración insuficiente del líquido de los pocillos ELISA pueden causar una precisión pobre y/o un elevado fondo. Para mejorar el rendimiento del kit en los sistemas automatizados, se recomienda aumentar el número de lavados.
- Para la reproducibilidad de los resultados, es importante que el tiempo de reacción sea igual para cada pocillo. El tiempo de dispensación de los pocillos no debe superar los 10 minutos; si se prolongara más allá de los 10 minutos, respétese el orden de dispensación. si utiliza más de una placa, se recomienda repetir la curva de calibración en cada plato.
- Al añadir el substrato TMB inicia una reacción cinética que termina al agregar la solución de parada. Tanto el sustrato como la solución de parada deben agregarse en la misma secuencia para evitar diferentes tiempos de reacción.
- Observar las directrices para la ejecución del control de calidad en los laboratorios clínicos al comprobar controles y/o pool de sueros.
- Observar la máxima precisión en la reconstitución y dispensación de los reactivos.
- No use muestras con contaminación microbiana, altamente lipémicas o hemolizadas.
- Los lectores de microplacas leen las DO verticalmente, por tanto no debe tocarse el fondo de los pocillos.

6. PROCEDIMIENTO

6.1. Preparación de los Calibradores (C_0….C_4)

Puesto que no hay disponibles preparados de referencia internacional para los anticuerpos anti-MPO, el sistema de análisis se calibra en unidades relativas arbitrarias.
Los Calibradores son listos para usar y tienen las siguientes concentraciones:

	C_0	C_1	C_2	C_3	C_4
AU/mL	0	10	20	40	160

Una vez abiertos, los Calibratores permanecen estables 6 meses conservados a 2-8°C.

6.2. Preparación de la muestra

Para realizar el ensayo se pueden usar muestras de suero o plasma humano. Las muestras que se van a usar deben estar limpias. Se recomienda evitar la contaminación por hiperlipemia, aunque esta no interfiera con el análisis. Las muestras pueden conservarse refrigeradas a 2-8°C durante 5 días, o a -20°C hasta 6 meses. Se recomienda no congelar y descongelar repetidamente las muestras de suero o plasma, puesto que esto podría provocar una pérdida variable de la actividad de los autoanticuerpos. No se recomienda el análisis de muestras inactivadas por calor.
Todas las muestras de suero o plasma deben prediluirse 1:100 con diluyente de muestras. por ejemplo, 10 µL de muestra pueden diluirse con 990 µL de diluyente de muestras.
Los Controles son listo para usar.

6.3. Preparación de la solución de lavado

Diluir el contenido de cada frasco de solución de lavado concentrada (10x) con agua destilada hasta un volumen final de 500 mL. Mantener refrigerada: estable a 2-8 °C durante al menos 30 días tras la preparación o hasta la fecha de caducidad indicada en la etiqueta.
En la solución de lavado concentrada es posible observar la presencia de cristales. En ese caso, agitar a temperatura ambiente hasta que los cristales se disuelvan por completo. Para una mayor precisión, diluir todo el frasco de la solución de lavado concentrada a 500 mL, teniendo cuidado para transferir también los cristales y, a continuación, agitar hasta que se disuelvan por completo.

6.4. Procedimiento

- **Esperar hasta que todos los reactivos se encuentren a temperatura ambiente (22-28°C) durante al menos 30 minutos.** Al final del ensayo inmediatamente poner todos los reactivos a 2-8°C para evitar largos periodos a temperatura ambiente.
- Las tiras de pocillos no utilizados se deben guardar de inmediato en la bolsa desechable que contiene desecantes y almacenarse a 2-8°C.
- Para evitar la contaminación microbiana y/o química no regrese porciones de reactivos no usados en los viales originales.
- Para aumentar la precisión de los resultados de la prueba es necesario trabajar en duplicado: preparar dos pocillos para cada punto de la curva de calibración (C_0-C_4), dos para cada control, dos para cada muestra, uno para el blanco.

Reactivos	Calibrador	Muestra/ controles	Blanco
Calibrador C_0-C_4	**100 µL**		
Controles		**100 µL**	
Muestra diluida		**100 µL**	
Incubar 60 minutos a temperatura ambiente (22-28°C). Retirar la mezcla de reacción y lavar los pocillos 3 veces con 300 µL de solución de lavado diluida. **Nota importante**: agite suavemente la placa durante 5 segundos en cada paso del lavado. Después del último lavado asegúrese haber eliminado completamente la solución de lavado de los pozos, invierta la placa y golpéela repetidas veces contra una servilleta de papel absorbente. **Lavados automático**: si está utilizando una lavadora automática, lavar los pocillos al menos 5 veces.			
Conjugado	**100 µL**	**100 µL**	
Incubar 30 minutos a temperatura ambiente (22-28 °C). Retirar la mezcla de reacción y lavar los pocillos 3 veces con 300 µL de solución de lavado diluida. **Lavados**: siga las mismas instrucciones del punto anterior.			
Substrato TMB	**100 µL**	**100 µL**	**100 µL**
Incubar 15 minutos a temperatura ambiente (22-28 °C), en la oscuridad.			
Solución de parada	**100 µL**	**100 µL**	**100 µL**
Agitar la microplaca con cuidado. Leer la absorbancia (E) a 450 nm frente una segunda lectura de referencia a 620-630 nm o frente al blanco dentro de los 5 minutos.			

7. CONTROL DE CALIDAD

- Los controles positivo para IgG anti-MPO y negativo deben incluirse cada vez que se realice el ensayo para asegurar que todos los reactivos y el ensayo funcionen de forma correcta.
- Puesto que los controles están prediluidos, no representan un control de procedimiento para las técnicas de dilución usadas para las muestras.
- Se pueden preparar sueros de control adicionales recogiendo un pool de sueros humanos, dividiéndolo en alícuotas y conservándolo a < -20 °C.
- Para que los resultados del ensayo se consideren válidos, se deben cumplir todos los criterios siguientes. Aunque solo uno no se encuentre dentro de los valores especificados, los resultados no deberán considerarse válidos y el ensayo deberá repetirse:
  - La absorbancia del control positivo debe ser mayor que la del control negativo.
  - El control negativo y el positivo sirven para controlar un eventual malfuncionamiento de los reactivos y no aseguran la precisión en correspondencia con el valor límite del ensayo.
  - El ensayo es válido solo si la densidad óptica a 450 nm del control positivo (1) y del control negativo (2), así como las de los calibradores (S_0-S_5), coinciden con los intervalos correspondientes indicados en el Certificado de control de calidad incluido en el kit.

8. CÁLCULO DE LOS RESULTADOS

Para Anti-MPO IgG, el método de elección para el tratamiento de los resultados es un procesamiento de 4 parámetros con ejes lin-log para la densidad óptica y la concentración respectivamente. Además, se pueden usar una aproximación spline y coordenadas log-log. Sin embargo, se recomienda usar una curva Lin-Log.
En primer lugar, calcular la media de las densidades ópticas relativas a los calibradores. Usar una hoja de papel lin-log y trazar las densidades ópticas medias de cada calibrador frente a la respectiva concentración. Dibujar la curva que mejor se aproxime a todos los puntos de calibración. Los puntos de los calibradores también pueden unirse con segmentos de línea recta. La concentración de las muestras desconocidas puede determinarse por interpolación de la curva de calibración.

9. VALORES DE REFERENCIA

En un estudio sobre los valores normales realizado con muestras de suero procedentes de donantes sanos se han determinado los siguientes intervalos de normalidad con el ensayo Anti-MPO IgG:

	Anti MPO (p-ANCA)
Corte	20 (AU/mL)

Los valores indicados arriba solo son valores sugeridos. Cada laboratorio debe establecer su propio rango de normalidad basado en el procedimiento utilizado por este, los controles, el equipo y la población de pacientes según los métodos Calibración propios.
Los resultados positivos deben verificarse con relación al estado clínico del paciente. Además, cada decisión relativa a la terapia debe tomarse individualmente. Se recomienda que cada laboratorio establezca sus propios intervalos normal y patológico de anti-MPO sérica.

10. LIMITACIONES DEL ENSAYO

La presencia en la muestra de complejos inmunes o de otros agregados de inmunoglobulinas puede dar lugar a reacciones no específicas con resultados falsos positivos.

11. PARÁMETROS CARACTERÍSTICOS

11.1. Especificidad

Ensayos de correlación frente a un kit similar de referencia disponible en el mercado, realizados con 32 sueros (8 positivos y 24 negativos) han mostrado una especificidad del 100%.

11.2. Sensibilidad

Ensayos de correlación frente a un kit similar de referencia disponible en el mercado, realizados con 32 sueros (8 positivos y 24 negativos) han mostrado una sensibilidad del 100%.

11.3. Límite de detección

La concentración mínima de anti-MPO que puede distinguirse del Calibrador cero es de aproximadamente 0.73 AU/mL con un límite de confianza del 98%.

11.4. Precisión

11.4.1. Intraensayo

La variabilidad dentro del mismo kit se ha determinado replicando la medición de tres sueros de control distintos. La variabilidad intraensayo es ≤ 6.1%.

11.4.2. Interensayo

La variabilidad entre distintos kits se ha determinado replicando la medición de dos sueros de control distintos con kits pertenecientes a lotes distintos. La variabilidad interensayo es ≤ 12.3%.

12. DISPOSICIONES PARA LA ELIMINACIÓN

Los reactivos deben eliminarse de acuerdo con las leyes locales.

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DiaMetra	Packaging Information Sheet	Mod. PIS000-1

IVD	DE ES FR GB IT PT	In vitro Diagnostikum Producto sanitario para diagnóstico In vitro Dispositif medical de diagnostic in vitro In vitro Diagnostic Medical Device Dispositivo medico-diagnostico in vitro Dispositivos medicos de diagnostico in vitro		DE ES FR GB IT PT	Hergestellt von Elaborado por Fabriqué par Manufacturer Produttore Produzido por
	DE ES FR GB IT PT	Achtung, Begleitdokumente Precaución, consulte los documentos adjuntos Attention, veuillez consulter les documents d'accompagnement Caution, consult accompanying documents Attenzione, consultare la documentazione allegata Atenção,consultar os documentos de acompanhamento	 yyyy-mm	DE ES FR GB IT PT	Herstellungs datum Fecha de fabricacion Date de fabrication Date of manufacture Data di produzione Data de produção
 yyyy-mm-dd	DE ES FR GB IT PT	Verwendbar bis Establa hasta (usar antes de último día del mes) Utiliser avant (dernier jour du mois indiqué) Use by (last day of the month) Utilizzare prima del (ultimo giorno del mese) Utilizar (antes ultimo dia do mês)		DE ES FR GB IT PT	Biogefährdung Riesco biológico Risque biologique Biological risk Rischio biologico Risco biológico
	DE ES FR GB IT PT	Gebrauchsanweisung beachten Consultar las instrucciones Consulter le mode d'emploi Consult instructions for use Consultare le istruzioni per l'uso Consultar instruções para uso	LOT	DE ES FR GB IT PT	Chargenbezeichnung Codigo de lote Numero de lot Batch code Codice del lotto Codigo do lote
 Σ = xx	DE ES FR GB IT PT	Ausreichend für "n" Tests Contenido suficiente para "n" tests Contenu suffisant pour "n" tests Contains sufficient for "n" tests Contenuto sufficiente per "n" saggi Contém o suficiente para "n" testes	CONT	DE ES FR GB IT PT	Inhalt Contenido del estuche Contenu du coffret Contents of kit Contenuto del kit Conteúdo do kit
Max  Min	DE ES FR GB IT PT	Temperaturbereich Límitaciôn de temperatura Limites de température de conservation Temperature limitation Limiti di temperatura Temperaturas limites de conservação	REF	DE ES FR GB IT PT	Bestellnummer Nûmero de catálogo Référéncès du catalogue Catalogue number Numero di Catalogo Número do catálogo
	DE ES FR GB IT PT	Vor direkter sonneneinstrahlung schützen Mantener alejado de la luz solar Tenir à l'écart de la lumière du soleil Keep away from sunlight Tenere lontano dalla luce solare Mantenha longe da luz solar			

SUGGERIMENTI PER LA RISOLUZIONE DEI PROBLEMI/TROUBLESHOOTING

ERRORE CAUSE POSSIBILI/ SUGGERIMENTI

Nessuna reazione colorimetrica del saggio

- mancata dispensazione del coniugato
- contaminazione del coniugato e/o del Substrato
- errori nell'esecuzione del saggio (es. Dispensazione accidentale dei reagenti in sequenza errata o provenienti da flaconi sbagliati, etc.)

Reazione troppo blanda (OD troppo basse)

- coniugato non idoneo (es. non proveniente dal kit originale)
- tempo di incubazione troppo breve, temperatura di incubazione troppa bassa

Reazione troppo intensa (OD troppo alte)

- coniugato non idoneo (es. non proveniente dal kit originale)
- tempo di incubazione troppo lungo, temperatura di incubazione troppa alta
- qualità scadente dell'acqua usata per la soluzione di lavaggio (basso grado di deionizzazione,)
- lavaggi insufficienti (coniugato non completamente rimosso)

Valori inspiegabilmente fuori scala

- contaminazione di pipette, puntali o contenitori- lavaggi insufficienti (coniugato non completamente rimosso)

CV% intrasaggio elevato

- reagenti e/o strip non portate a temperatura ambiente prima dell'uso
- il lavatore per micropiastre non lava correttamente (suggerimento: pulire la testa del lavatore)

CV% intersaggio elevato

- condizioni di incubazione non costanti (tempo o temperatura)
- controlli e campioni non dispensati allo stesso tempo (con gli stessi intervalli) (controllare la sequenza di dispensazione)
- variabilità intrinseca degli operatori

ERROR POSSIBLE CAUSES / SUGGESTIONS

No colorimetric reaction

- no conjugate pipetted reaction after addition
- contamination of conjugates and/or of substrate
- errors in performing the assay procedure (e.g. accidental pipetting of reagents in a wrong sequence or from the wrong vial, etc.)

Too low reaction (too low ODs)

- incorrect conjugate (e.g. not from original kit)
- incubation time too short, incubation temperature too low

Too high reaction (too high ODs)

- incorrect conjugate (e.g. not from original kit)
- incubation time too long, incubation temperature too high
- water quality for wash buffer insufficient (low grade of deionization)
- insufficient washing (conjugates not properly removed)

Unexplainable outliers

- contamination of pipettes, tips or containers

insufficient washing (conjugates not properly removed) too high within-run

- reagents and/or strips not pre-warmed to CV% Room Temperature prior to use
- plate washer is not washing correctly (suggestion: clean washer head)

too high between-run - incubation conditions not constant (time, CV % temperature)

- controls and samples not dispensed at the same time (with the same intervals) (check pipetting order)
- person-related variation

ERROR / POSIBLES CAUSAS / SUGERENCIAS

No se produce ninguna reacción colorimétrica del ensayo

- no se ha dispensado el conjugado
- contaminación del conjugado y/o del substrato
- errores en la ejecución del ensayo (p. ej., dispensación accidental de los reactivos en orden incorrecto o procedentes de frascos equivocados, etc.)

Reacción escasa (DO demasiado bajas)

- conjugado no idóneo (p. ej., no procedente del kit original)
- tiempo de incubación demasiado corto, temperatura de incubación demasiado baja

Reacción demasiado intensa (DO demasiado altas)

- conjugado no idóneo (p. ej., no procedente del kit original)
- tiempo de incubación demasiado largo, temperatura de incubación demasiado alta
- calidad escasa del agua usada para la solución de lavado (bajo grado de desionización)
- lavados insuficientes (el conjugado no se ha retirado completamente)

Valores inexplicablemente fuera de escala

- contaminación de pipetas, puntas o contenedores- lavados insuficientes (el conjugado no se ha retirado completamente)

CV% intraensayo elevado

- los reactivos y/o tiras no se encontraban a temperatura ambiente antes del uso
- el lavador de microplacas no funciona correctamente (sugerencia: limpiar el cabezal del lavador)

CV% interensayo elevado

- condiciones de incubación no constantes (tiempo o temperatura)
- controles y muestras no dispensados al mismo tiempo (con los mismos intervalos) (controlar la secuencia de dispensación)
- variación en función de los operadores

ERREUR CAUSES POSSIBLES / SUGGESTIONS

Aucune réaction colorimétrique de l'essai

- non distribution du conjugué
- contamination du conjugué et/ou du substrat
- erreurs dans l'exécution du dosage (par ex., distribution accidentelle des réactifs dans le mauvais ordre ou en provenance des mauvais flacons, etc.)

Réaction trop faible (DO trop basse)

- conjugué non approprié (par ex., ne provenant pas du coffret original)
- temps d'incubation trop court, température d'incubation trop basse

Réaction trop intense (DO trop élevée)

- conjugué non approprié (par ex., ne provenant pas du coffret original)
- temps d'incubation trop long, température d'incubation trop élevée
- mauvaise qualité de l'eau utilisée pour la solution de lavage (bas degré de déionisation)
- lavages insuffisants (conjugué non entièrement éliminé)

Valeurs inexplicablement hors plage

- contamination des pipettes, embouts ou récipients - lavages insuffisants (conjugué non entièrement éliminé)

CV% intra-essai élevé

- les réactifs et/ou les bandes n'ont pas atteint la température ambiante avant usage
- le laveur de microplaques ne lave pas correctement (suggestion : nettoyer la tête du laveur)

CV% inter-essai élevé

- conditions d'incubation non constantes (temps ou température)
- contrôles et échantillons non distribués en même temps (avec les mêmes intervalles) (contrôler l'ordre de distribution)
- variabilité intrinsèque des opérateurs

Anti-LC-1 ELISA (IgG)
Test instruction

ORDER NO.	ANTIBODIES AGAINST	IG CLASS	SUBSTRATE	FORMAT
EA 1307-9601 G	LC-1	IgG	Ag-coated microplate wells	96 x 01 (96)

Indications: The ELISA test kit provides a semiquantitative in vitro assay for human autoantibodies of the IgG class against liver cytosol antigen type 1 (LC-1) in serum or plasma for the diagnosis of increase in transaminases for unclear reasons and suspected autoimmune hepatitis.

Application: The determination of autoantibodies against LC-1 is another important supplementing serological parameter in the diagnosis of autoimmune liver diseases. A reliable diagnosis of AIH is indispensable since untreated AIH rapidly turns into liver cirrhosis.

Principle of the test: The test kit contains microplate strips, each with 8 break-off reagent wells coated with LC-1. In the first reaction step, diluted patient samples are incubated in the wells. If samples are positive, specific IgG antibodies (also IgA and IgM) bind to the antigens. To detect the bound antibodies, a second incubation is carried out using an enzyme-labelled anti-human IgG (enzyme conjugate) catalysing a colour reaction.

Contents of the test kit:

Component	Colour	Format	Symbol
1. Microplate wells coated with antigens 12 microplate strips each containing 8 individual break-off wells in a frame, ready for use	---	12 x 8	STRIPS
2. Calibrator (IgG, human), ready for use	dark red	1 x 2.0 ml	CAL
3. Positive control (IgG, human), ready for use	blue	1 x 2.0 ml	POS CONTROL
4. Negative control (IgG, human), ready for use	green	1 x 2.0 ml	NEG CONTROL
5. Enzyme conjugate peroxidase-labelled anti-human IgG (rabbit), ready for use	green	1 x 12 ml	CONJUGATE
6. Sample buffer ready for use	light blue	1 x 100 ml	SAMPLE BUFFER
7. Wash buffer 10x concentrate	colourless	1 x 100 ml	WASH BUFFER 10x
8. Chromogen/substrate solution TMB/H_2O_2, ready for use	colourless	1 x 12 ml	SUBSTRATE
9. Stop solution 0.5 M sulphuric acid, ready for use	colourless	1 x 12 ml	STOP SOLUTION
10. Test instruction	---	1 booklet	
11. Quality control certificate	---	1 protocol	

LOT Lot description
IVD In vitro diagnostic medical device
CE
Storage temperature
Unopened usable until

Storage and stability: The test kit has to be stored at a temperature between +2°C to +8°C. Do not freeze. Unopened, all test kit components are stable until the indicated expiry date.

Waste disposal: Patient samples, calibrators, controls and incubated microplate strips should be handled as infectious waste. All reagents must be disposed of in accordance with local disposal regulations.

Preparation and stability of the reagents

Note: All reagents must be brought to room temperature (+18°C to +25°C) approx. 30 minutes before use. After first use, the reagents are stable until the indicated expiry date if stored at +2°C to +8°C and protected from contamination, unless stated otherwise below.

- **Coated wells:** Ready for use. Tear open the resealable protective wrapping of the microplate at the recesses above the grip seam. Do not open until the microplate has reached room temperature to prevent the individual strips from moistening. Immediately replace the remaining wells of a partly used microplate in the protective wrapping and tightly seal with the integrated grip seam (Do not remove the desiccant bag).
  Once the protective wrapping has been opened for the first time, the wells coated with antigens can be stored in a dry place and at a temperature between +2°C and +8°C for 4 months.

- **Calibrator and controls:** Ready for use. The reagents must be mixed thoroughly before use.

- **Enzyme conjugate:** Ready for use. The enzyme conjugate must be mixed thoroughly before use.

- **Sample buffer:** Ready for use.

- **Wash buffer:** The wash buffer is a 10x concentrate. If crystallisation occurs in the concentrated buffer, warm it to +37°C and mix well before diluting. The quantity required should be removed from the bottle using a clean pipette and diluted with deionised or distilled water (1 part reagent plus 9 parts distilled water).
  For example: For 1 microplate strip, 5 ml concentrate plus 45 ml water.
  The working strength wash buffer is stable for 4 weeks when stored at +2°C to +8°C and handled properly.

- **Chromogen/substrate solution:** Ready for use. Close the bottle immediately after use, as the contents are sensitive to light ✳. The chromogen/substrate solution must be clear on use. Do not use the solution if it is blue coloured.

- **Stop solution:** Ready for use.

Warning: The calibrator and controls of human origin have tested negative for HBsAg, anti-HCV, anti-HIV-1 and anti-HIV-2. Nonetheless, all materials should be treated as being a potential infection hazard and should be handled with care. Some of the reagents contain the agent sodium azide in a non-declarable concentration. Avoid skin contact.

Preparation and stability of the patient samples

Samples: Human serum or EDTA, heparin or citrate plasma.

Stability: Patient samples to be investigated can generally be stored at +2°C to +8°C for up to 14 days. Diluted samples should be incubated within one working day.

Sample dilution: Patient samples are diluted **1:101** in sample buffer. For example: dilute 10 µl sample in 1.0 ml sample buffer and mix well by vortexing (sample pipettes are not suitable for mixing).

NOTE: The calibrator and controls are prediluted and ready for use, do not dilute them.

Incubation

Sample incubation: (1st step)
Transfer 100 µl of the calibrator, positive or negative control or diluted patient samples into the individual microplate wells according to the pipetting protocol. Incubate for **30 minutes** at room temperature (+18°C to +25°C).

Washing:
Manual: Empty the wells and subsequently wash 3 times using 300 µl of working strength wash buffer for each wash.
Automatic: Wash the reagent wells 3 times with 450 µl of working strength wash buffer (program setting: e.g. TECAN Columbus Washer "Overflow Mode").

Leave the wash buffer in each well for 30 to 60 seconds per washing cycle, then empty the wells. After washing (manual and automated tests), thoroughly dispose of all liquid from the microplate by tapping it on absorbent paper with the openings facing downwards to remove all residual wash buffer.

Note: Residual liquid (> 10 µl) remaining in the reagent wells after washing can interfere with the substrate and lead to false low extinction values. Insufficient washing (e.g., less than 3 wash cycles, too small wash buffer volumes, or too short residence times) can lead to false high extinction values.
Free positions on the microplate strip should be filled with blank wells of the same plate format as that of the parameter to be investigated.

Conjugate incubation: (2nd step)
Pipette 100 µl of enzyme conjugate (peroxidase-labelled anti-human IgG) into each of the microplate wells. Incubate for **30 minutes** at room temperature (+18°C to +25°C).

Washing:
Empty the wells. Wash as described above.

Substrate incubation: (3rd step)
Pipette 100 µl of chromogen/substrate solution into each of the microplate wells. Incubate for **15 minutes** at room temperature (+18°C to +25°C) protect from direct sunlight.

Stopping:
Pipette 100 µl of stop solution into each of the microplate wells in the same order and at the same speed as the chromogen/substrate solution was introduced.

Measurement:
Photometric measurement of the colour intensity should be made at a wavelength of 450 nm and a reference wavelength between 620 nm and 650 nm **within 30 minutes of adding the stop solution**. Prior to measuring, slightly shake the microplate to ensure a homogeneous distribution of the solution.

Pipetting protocol

	1	2	3	4	5	6	7	8	9	10	11	12
A	**C**	**P 6**	**P 14**	**P 22**								
B	**pos.**	**P 7**	**P 15**	**P 23**								
C	**neg.**	**P 8**	**P 16**	**P 24**								
D	**P 1**	**P 9**	**P 17**									
E	**P 2**	**P 10**	**P 18**									
F	**P 3**	**P 11**	**P 19**									
G	**P 4**	**P 12**	**P 20**									
H	**P 5**	**P 13**	**P 21**									

The above pipetting protocol is an example of the semiquantitative determination of antibodies in 24 patient samples (P 1 to P 24).

Calibrator (C), positive (pos.) and negative (neg.) control as well as the patient samples have been incubated in one well each. The reliability of the ELISA test can be improved by duplicate determinations of each sample.
The wells can be broken off individually from the strips. This makes it possible to adjust the number of test substrates used to the number of samples to be examined and minimises reagent wastage.
Both positive and negative controls serve as internal controls for the reliability of the test procedure. They should be assayed with each test run.

Calculation of results

Semiquantitative: Results can be evaluated semiquantitatively by calculating a ratio of the extinction value of the control or patient sample over the extinction value of calibrator. Use the following formula to calculate the ratio:

$$\frac{\textbf{Extinction of the control or patient sample}}{\textbf{Extinction of calibrator}} = \textbf{Ratio}$$

EUROIMMUN recommends interpreting results as follows:

Ratio <1.0: **negative**
Ratio ≥1.0: **positive**

For duplicate determinations the mean of the two values should be taken. If the two values deviate substantially from one another, EUROIMMUN recommends retesting the samples.

For diagnosis, the clinical picture of the patient patient always needs to be taken into account along with the serological findings.

Test characteristics

Calibration: As no international reference serum exists for antibodies against LC-1, the results are given as a ratio. This represents a relative measurement for the concentration of antibodies in serum or plasma.

For every group of tests performed, the extinction values of the calibrator and the ratio of the positive and negative control sera must lie within the limits stated for the relevant test kit lot. A quality control certificate containing these reference values is included. If the values specified for the control sera are not achieved, the test results may be inaccurate and the test should be repeated.

The binding activity of the antibodies and the activity of the enzyme used are temperature-dependent. It is therefore recommended using a thermostat in all three incubation steps. The higher the room temperature (+18°C to +25°C) during the incubation steps, the greater the extinction values will be. Corresponding variations apply also to the incubation times. However, the calibrator is subject to the same influences, so that such variations will be largely compensated in the calculation of the result.

Antigen: The microplate wells were coated with recombinant LC-1. The corresponding human cDNA was expressed in insect cells using a baculovirus vector. The specific target antigen of anti-LC-1 antibodies was identified in 1999 as the enzyme formiminotransferase cyclodeaminase (Lapierre et al.).

Detection limit: The lower detection limit is defined as the mean value of an analyte-free sample plus three times the standard deviation and is the smallest detectable antibody titer. The lower detection limit of the Anti-LC-1 ELISA (IgG) is ratio 0.05.

Cross reactivity: This ELISA specifically detects autoantibodies of class IgG against LC-1. When investigating patient sera for autoantibodies against SLA (n = 2) and LKM (n = 8) no cross reactions were found.

Interference: Haemolytic, lipaemic and icteric samples showed no influence on the result up to a concentration of 10 mg/ml for haemoglobin, 20 mg/ml for triglycerides and 0.4 mg/ml for bilirubin in this ELISA.

Reproducibility: The reproducibility of the test was investigated by determining the intra- and inter-assay coefficients of variation (CV) using 3 samples. The intra-assay CVs are based on 20 determinations and the inter-assay CVs on 4 determinations performed in 6 different test runs.

Intra-assay variation, n = 20		
Sample	**Mean value (Ratio)**	**CV (%)**
1	3.1	4.9
2	4.7	2.7
3	8.0	2.4

Inter-assay variation, n = 4 x 6		
Sample	**Mean value (Ratio)**	**CV (%)**
1	2.5	10.8
2	3.8	10.2
3	6.7	9.4

Prevalence and specificity: Sera of 93 patients with autoimmune hepatitis, 183 patients with hepatitis A, hepatitis B, toxic liver diseases, steatohepatitis or primary biliary cirrhosis (PBC) and 200 healthy blood donors were examined with the EUROIMMUN Anti-LC-1 ELISA (IgG). The prevalence of antibodies against LC-1 in autoimmune hepatitis was 5.4% with a specificity of 100%.

Reference range: The levels of the anti-LC-1 antibodies (IgG) were analysed with this EUROIMMUN ELISA in a panel of 200 healthy blood donors. With a cut-off ratio of 1.0, all blood donors were anti-LC-1 negative.

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Anti-SLA/LP ELISA (IgG)
Test instruction

ORDER NO.	ANTIBODIES AGAINST	IG-CLASS	SUBSTRATE	FORMAT
EA 1302-9601 G	SLA/LP (soluble liver antigen/ liver-pancreas antigen)	IgG	Ag-coated microplate wells	96 x 01 (96)

Indications: Increase in transaminases for unclear reasons, suspected autoimmune hepatitis.

Principles of the test: The ELISA test kit provides a semiquantitative or quantitative in vitro assay for human autoantibodies of the IgG class against SLA/LP (soluble liver antigen/liver-pancreas antigen) in serum or plasma. The test kit contains microtiter strips each with 8 break-off reagent wells coated with SLA/LP. In the first reaction step, diluted patient samples are incubated in the wells. In the case of positive samples, specific IgG antibodies (also IgA and IgM) will bind to the antigens. To detect the bound antibodies, a second incubation is carried out using an enzyme-labelled anti-human IgG (enzyme conjugate) which is capable of promoting a colour reaction.

Contents of the test kit:

Component	Colour	Format	Symbol
1. Microplate wells, coated with antigens: 12 microplate strips each containing 8 individual break-off wells in a frame, ready for use	---	12 x 8	STRIPS
2. Calibrator 1 200 RU/ml (IgG, human), ready for use	dark red	1 x 2.0 ml	CAL 1
3. Calibrator 2 20 RU/ml (IgG, human), ready for use	red	1 x 2.0 ml	CAL 2
4. Calibrator 3 2 RU/ml (IgG, human), ready for use	light red	1 x 2.0 ml	CAL 3
5. Positive control (IgG, human), ready for use	blue	1 x 2.0 ml	POS CONTROL
6. Negative control (IgG, human), ready for use	green	1 x 2.0 ml	NEG CONTROL
7. Enzyme conjugate peroxidase-labelled anti-human IgG (rabbit), ready for use	green	1 x 12 ml	CONJUGATE
8. Sample buffer ready for use	light blue	1 x 100 ml	SAMPLEBUFFER
9. Wash buffer 10x concentrate	colourless	1 x 100 ml	WASHBUFFER 10x
10. Chromogen/substrate solution TMB/H_2O_2, ready for use	colourless	1 x 12 ml	SUBSTRATE
11. Stop solution 0.5 M sulphuric acid, ready for use	colourless	1 x 12 ml	STOP SOLUTION
12. Test instruction	---	1 booklet	
13. Protocol with target values	---	1 protocol	

LOT	Lot		storage temperature
IVD	In vitro determination	CE	unopened usable until

Storage and stability: The test kit has to be stored at a temperature between +2°C to +8°C. Do not freeze. Unopened, all test kit components are stable until the indicated expiry date.

Waste disposal: Patient samples, calibrators, controls and incubated microplate strips shoud be handled as infectious waste. All reagents should be disposed of according to official regulations.

Preparation and stability of the reagents

Note: All reagents must be brought to room temperature (+18°C to +25°C) around 30 minutes before use. After first use, the reagents are stable until the indicated expiry date if stored at +2°C to +8°C and protected from contamination, unless stated otherwise below.

- **Coated wells:** Ready for use. Tear open the resealable protective wrapping of the microplate at the recesses above the grip seam. Do not open until the microplate has reached room temperature to prevent the individual strips from moistening. Immediately replace the remaining wells of a partly used microplate in the protective wrapping and tightly seal with the integrated grip seam (Do not remove the desiccant bag).
  Once the protective wrapping has been opened for the first time, the wells coated with antigens can be stored in a dry place and at a temperature between +2°C and +8°C for a minimum of 4 months.

- **Calibrators and controls:** Ready for use. The reagents must be mixed thoroughly before use.

- **Enzyme conjugate:** Ready for use. The enzyme conjugate must be mixed thoroughly before use.

- **Sample buffer:** Ready for use.

- **Wash buffer:** The wash buffer is a 10x concentrate. If crystallization occurs in the concentrated buffer, warm it to 37°C and mix well before diluting. The quantity required should be removed from the bottle using a clean pipette and diluted with deionized or distilled water (1 part reagent plus 9 parts distilled water).
  For example, for 1 microplate: 5 ml concentrate plus 45 ml water.
  The ready-to-use diluted wash buffer is stable for 4 weeks when stored at +2°C to +8°C and handled properly.

- **Chromogen/substrate solution:** Ready for use. Close the bottle immediately after use, as the contents are sensitive to light. The chromogen/substrate solution must be clear on use. Do not use the solution if it is blue coloured.

- **Stop solution:** Ready for use.

Warning: Calibrators and controls used have tested negative for HBsAg, anti-HCV, anti-HIV-1 and anti-HIV-2 using enzyme immunoassays and indirect immunofluorescence methods. Nonetheless, all materials should be treated as being a potential infection hazard and should be handled with care. Some of the reagents contain the toxic agent sodium azide. Avoid contact with the skin.

Preparation and stability of the patient samples

Sample material: Human serum or EDTA, heparin or citrate plasma.

Stability: Patient samples to be investigated can generally be stored at +2°C to +8°C for up to 14 days. Diluted samples should be incubated within one working day.

Sample dilution: Patient samples are diluted **1:101** sample buffer. For example: dilute 10 µl serum in 1.0 ml sample buffer and mix well by vortexing (sample pipettes are not suitable for mixing).

NOTE: Calibrators and controls are prediluted and ready for use, do not dilute them.

Incubation

For **semiquantative analysis** incubate **calibrator 2** along with the positive and negative controls and patient samples. For **quantitative analysis** incubate **calibrators 1, 2 and 3** along with the positive and negative controls and patient samples.

Sample incubation: (1. step)
Transfer 100 µl calibrators, positive and negative controls or diluted patient samples into the individual microplate wells according to the pipetting protocol. Incubate for **30 minutes** at room temperature (+18°C to +25°C).

Washing:
Manual: Empty the wells and subsequently wash 3 times using 300 µl of working strength wash buffer for each wash.
Automatic: Wash reagent wells 3 times with 400 µl working strength wash buffer (program setting: e.g. TECAN Columbus Washer “Overflow Modus”).

Leave the wash buffer in each well for 30 to 60 seconds per washing cycle, then empty the wells. After washing (manual and automated tests), thoroughly dispose of all liquid from the microplate by tapping it on absorbent paper with the openings facing downwards to remove all residual wash buffer.

Note: Residual liquid (> 10 µl) remaining in the reagent wells after washing can interfere with the substrate and lead to falsely low extinction values. Insufficient washing (e.g., less than 3 wash cycles, too small wash buffer volumes, or too short reaction times) can lead to falsely high extinction values.
Free positions on the microplate strip should be filled with blank wells of the same plate format as that of the parameter to be investigated.

Conjugate incubation: (2. step)
Pipette 100 µl of enzyme conjugate (peroxidase-labelled anti-human IgG) into each of the microplate wells. Incubate for **30 minutes** at room temperature (+18°C bis 25°C).

Washing:
Empty the wells. Wash as described above.

Substrate incubation: (3. step)
Pipette 100 µl of chromogen/substrate solution into each of the microplate wells. Incubate for **15 minutes** at room temperature (+18°C bis 25°C) protect from direct sunlight.

Stopping the reaction:
Pipette 100 µl of stop solution into each of the microplate wells in the same order and at the same speed as the chromogen/substrate solution was introduced.

Measurement:
Photometric measurement of the colour intensity should be made at a wavelength of 450 nm and a reference wavelength of between 620 nm and 650 nm **within 30 minutes of adding the stop solution.** Prior to measuring, slightly shake the micro-plate to ensure a homogeneous distribution of the solution.

Pipetting protocol

	1	2	3	4	5	6	7	8	9	10	11	12
A	**C 2**	**P 6**	**P 14**	**P 22**			**C 1**	**P 4**	**P 12**	**P 20**		
B	**pos.**	**P 7**	**P 15**	**P 23**			**C 2**	**P 5**	**P 13**	**P 21**		
C	**neg.**	**P 8**	**P 16**	**P 24**			**C 3**	**P 6**	**P 14**	**P 22**		
D	**P 1**	**P 9**	**P 17**				**pos.**	**P 7**	**P 15**	**P 23**		
E	**P 2**	**P 10**	**P 18**				**neg.**	**P 8**	**P 16**	**P 24**		
F	**P 3**	**P 11**	**P 19**				**P 1**	**P 9**	**P 17**			
G	**P 4**	**P 12**	**P 20**				**P 2**	**P 10**	**P 18**			
H	**P 5**	**P 13**	**P 21**				**P 3**	**P 11**	**P 19**			

The pipetting protocol for microtiter strips 1-4 is an example for the **semiquantitative analysis** of 24 patient samples (P 1 to P 24).
The pipetting protocol for microtiter strips 7-10 is an example for the **quantitative analysis** of 24 patient samples (P 1 to P 24).

The calibrators (C 1 to C 3), the positive (pos.) and negative (neg.) controls, and the patient samples have each been incubated in one well. The reliability of the ELISA test can be improved by duplicate determinations for each sample.
The wells can be broken off individually from the strips. This makes it possible to adjust the number of test substrates used to the number of samples to be examined and minimizes reagent wastage.
Both positive and negative controls serve as internal controls for the reliability of the test procedure. They should be assayed with each test run.

Calculation of results

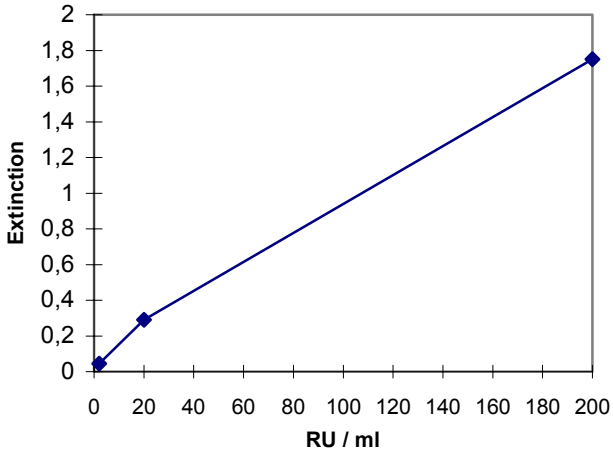
Semiquantitative: Results can be evaluated semiquantitatively by calculating a ratio of the extinction value of the control or patient sample over the extinction value of calibrator 2. Calculate the ratio according the following formula:

$$\frac{\text{Extinction of the control or patient sample}}{\text{Extinction of calibrator 2}} = \text{Ratio}$$

EUROIMMUN recommends interpreting results as follows:

Ratio <1.0:	**negative**
Ratio ≥1.0:	**positive**

Quantitative: The standard curve from which the concentration of antibodies in the serum samples can be taken is obtained by point-to-point plotting of the extinction values measured for the 3 calibration sera against the corresponding units (linear/linear). Use “point-to-point” plotting for calculation of the standard curve by computer. The following plot is an example of a typical calibration curve. Please do not use this curve for the determination of antibody concentrations in patient samples.



If the extinction of a serum sample lies above the value of calibrator 1 (200 RU/ml). The result shold be given as ">200 RU/ml". It is recommended that the sample be re-tested at a dilution of 1:400. The result in IU/ml read from the calibration curve for this sample must then be multiplied by a factor of 4.

The upper limit of the normal range **(cut-off)** recommended by EUROIMMUN is 20 relative units (RU) /ml. EUROIMMUN recommends interpreting results as follows:

<20 RU/ml:	**negative**
≥20 RU/ml:	**positive**

For duplicate determinations the mean of the two values should be taken. If the two values deviate substantially from one another the sample should be retested.

For diagnosis, the clinical symptoms of the patient should always be taken into account alongside the serelogical results.

Test characteristics

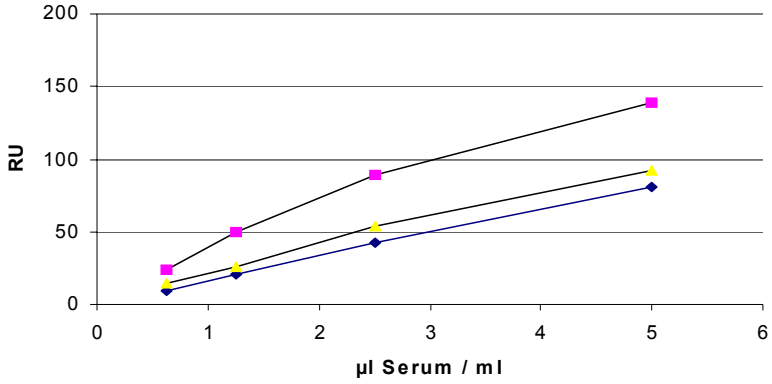
Calibration: As no international reference serum exists for antibodies against SLA/LP, the calibration is performed in relative units (RU).

For every group of tests performed, the extinction values of the calibrators and the relative units and/or ratios determined for the positive and negative controls must lie within the limits stated for the relevant test kit lot. A protocol containing these target values is included. If the values specified for the controls are not achieved, the test results may be inaccurate and the test should be repeated.

The activity of the enzyme used is temperature-dependent and the extinction values may vary if a thermostat is not used. The higher the room temperature (+18°C to +25°C) during substrate incubation, the greater will be the extinction values. Corresponding variations apply also to the incubation times. However, the calibrators are subject to the same influences, with the result that such variations will be largely compensated in the calculation of the result.

Antigen: The microplate wells were coated with recombinant SLA/LP. The corresponding human cDNA was expressed in E. coli. Identification of SLA/LP at the DNA level succeeded in 1998 by cloning the target antigen (A. W. Lohse, University Hospital of Mainz). SLA/LP is probably a cytoplasmic molecule with a molecular weight of 50 kDa which is involved in the regulation of the protein biosynthesis (an UGA-suppressor-tRNA associated protein). Previous descriptions of SLA as being the liver cytokeratins 8 and 18 or glutathione-S-transferase were apparently wrong.

Linearity: The linearity of the test was investigated by assaying serial dilutions of patient samples with high antibody concentrations. The chart below shows the typical linearity of samples on the basis of 3 patient sera. The Anti-SLA/LP ELISA (IgG) is linear in the measurement range 2 - 200 RU/ml.



Detection limit: The detection limit is defined as a value of three times the standard deviation of an analyte-free sample and is the smallest detectable antibody titer. The detection limit of the Anti-SLA/LP ELISA (IgG) is approximately 1 RU/ml.

Cross reactivity: This ELISA showed no cross reactivity.

Interference: Haemolytic, lipaemic and icteric samples showed no influence at the result up to a concentration of 10 mg/ml for hemoglobin, 20 mg/ml for triglycerides and 0.4 mg/ml for bilirubin in this ELISA.

Reproducibility: The reproducibility of the test was investigated by determining the intra- and inter-assay coefficients of variation (CV) using 3 sera with values at different points on the calibration curve. The intra-assay CVs are based on 20 determinations and the inter-assay CVs on 4 determinations performed on 6 different days.

Intra-Assay Variation, n = 20		
Serum	**Mean value (RU/ml)**	**CV (%)**
1	41	2.6
2	110	2.3
3	154	3.7

Inter-Assay Variation, n = 4 x 6		
Serum	**Mean value (RU/ml)**	**CV (%)**
1	44	4.4
2	118	3.6
3	169	3.8

Prevalence and specificity: Sera from 454 patients with autoimmune hepatitis, 165 patients with other liver diseases and 200 healthy blood donors were examined with the Anti-SLA/LP ELISA. The prevalence anti-SLA/LP antibodies in non-Japanese AIH patients was in the range between 15% and 19%. The test showed a specificity of 100%.

Patient group (n=819)	Origin of sera	n	Anti-SLA/LP-positive
Autoimmune hepatitis	Prof. Lohse, Univ. Mainz	108	21 (19%)
	Brasilian panel	154	25 (16%)
	Japanese panel	43	2 (5%)
	American panel	149	23 (15%)
AIH (anti-LKM-1 positive)	Dr. Gruber, Univ. München	18	0
Primary biliary cirrhosis	Dr. Gruber, Univ. München PD Dr. Wick, Klinikum Großhadern	30	0
Hepatitis-B infection	Labor Dr. Stöcker, Lübeck	40	0
Hepatitis-C infection	Prof. Lohse, Univ. Mainz Labor Dr. Stöcker, Lübeck	39	0
Steatohepatitis	Prof. Lohse, Univ. Mainz	24	0
Toxic liver damage	Prof. Lohse, Univ. Mainz	14	0
Blood donors	Med. Univ. Lübeck	200	0

Reference range: The levels of the anti-SLA/LP antibodies (IgG) were analyzed with this EUROIMMUN ELISA in a panel of 200 healthy blood donors. With a cut-off of 20 RU/ml, all blood donors were anti-SLA/LP negative.

Clinical significance

The determination of autoantibodies against soluble liver antigen/liver-pancreas antigen (SLA/LP) is a new and important component in the diagnostics of autoimmune diseases of the liver.

Autoimmune diseases of the liver include
- autoimmune hepatitis (AIH),
- primary biliary liver cirrhosis (PBC) and
- primary sclerosing cholangiitis (PSC).

Mainly women are affected by **autoimmune hepatitis** (**AIH**, earlier designations: lupoid hepatitis, chronically active hepatitis). The disease manifests itself through increased levels of bilirubin, liver enzymes and immunoglobulins, through typical histological changes (liver biopsies show necrosis of the parenchymal cells with lymphocyte and plasma cell infiltrates) and through the occurrence of various autoantibodies. The disease can occur from infancy until the old age, however, it affects most frequently young adults.
The incidence of AIH in western Europe is 1.9 cases per 100,000 inhabitants per year. Untreated, autoimmune hepatitis soon develops into liver cirrhosis. However, if immunosuppressive low-dosed therapy is commenced in good time and continued consistently throughout the patient's life, the patient has a normal life expectancy. For differential diagnosis, a current infection with hepatitis viruses must be ruled out by investigation of the appropriate serological parameters.

Circulating autoantibodies have gained great significance for the diagnosis of AIH. They occur in the majority of patients, but their role in the pathogenesis is questionable. Also, there is no clear correlation between the activity or the prognosis of the disease and the antibody titre. Besides antibodies against SLA/LP the following autoantibodies are associated with AIH: antibodies against cell nuclei (**ANA**), **nDNA**, smooth muscle (**SMA**, with the most important target antigen being **F-actin**), liver-kidney microsomes (**LKM-1**, target antigen: cytochrome P450 IID6) and granulocytes (**pANCA**). The **autoantibodies against SLA/LP** that can today be measured by EUROIMMUN enzyme immunoassays probably have the highest diagnostic accuracy of all antibodies involved in autoimmune hepatitis. Anti-SLA/LP occur in AIH either singly or together with other autoantibodies. Although their prevalence is only between 10% and 30%, the predictive value is almost 100%: Every positive result essentially provides evidence of autoimmune hepatitis (provided that the corresponding clinical symptoms are present).

Investigations of antibodies against SLA/LP have up until now only been possible in a few special laboratories. It was earlier suspected that the SLA antigen is identical with the liver cytokeratins 8 and 18 or with the enzyme glutathione-S-transferase. It has only recently been found that this is apparently not the case. The newly discovered target antigen SLA/LP has presently only been identified reliably at DNA level. It is probably a cytoplasmic molecule which is involved in the regulation of the protein biosynthesis (an UGA-suppressor-tRNA associated protein). The great diagnostic value of autoantibodies against SLA/LP has now been clearly proved.

Autoantibodies against cell nuclei (ANA) and against smooth muscle (SMA) are frequent in AIH, but also occur in 10% to 20% of patients with chronic viral hepatitis and in other diseases. Autoantibodies against LKM-1 can only be demonstrated in about 1% of adult AIH patients, but they are more frequent in children. Antibodies against LKM-1 can also be found 1% to 2% of patients with a positive hepatitis C serology. As opposed to all other autoantibodies, antibodies against SLA/LP are highly specific for AIH and have not been described in viral hepatitis.

Some authors classify autoimmune hepatitis in accordance with the autoantibody status: Subtype I (ANA, SMA), Subtype II (antibodies against LKM-1) and Subtype III (antibodies against SLA/LP). This classification has probably neither a clinical nor a therapeutic and prognostic significance.

The diagnosis of AIH is based on the clinical picture, the biochemical and serological test results and the histological evidence of an inflammation reaction. It requires the exclusion of other causes of chronic hepatitis, such as viruses, alcohol and drugs, and a delimitation to the other autoimmune liver diseases (primary biliary liver cirrhosis and primary sclerosing cholangiitis). The diagnosis AIH is appropriate when 4 of the following 5 main criteria are fulfilled. If all 5 criteria are present, the diagnosis is definite. The diagnosis is confirmed if the patient responds to immunosuppressive therapy.

Main criteria for the diagnosis of autoimmune hepatitis:
1. Histological evidence of hepatitis
2. Detection of autoantibodies (ANA, SMA, LKM, SLA/LP)
3. Hypergammaglobulinaemia
4. Negative HBV and HCV serology
5. Detection of the HLA antigens B8, DR3 or DR4

The serological determination of **autoantibodies against SLA/LP** provides an exact delimitation to viral hepatitis in many patients with AIH. The autoimmune serology of hepatitis has thus been enriched by a further parameter whose significance must be rated more highly than that of antibodies against cell nuclei, DNA, smooth muscle and liver-kidney microsomes (**ANA, nDNA, ASMA, LKM**). Testing for these antibodies has considerable consequences for the hepatology clinic: the consequence of incorrect treatment of AIH with interferon can be just as fatal as the immunosuppressive therapy of the virus infection.
Primary biliary cirrhosis (PBC) is a liver cirrhosis which takes the form of a non-suppurative, destructive inflammation of the biliferous ducts. It occurs in woman ten times more frequently than in men. In the foreground of the clinical picture is cholestasis. In Europe, 13 out of every 100,000 persons develop this disease each year. As a cirrhosis does not occur in every case, and mostly not until the late stage, this disease is better described as **"chronic non-suppurative destructive cholangiitis"**. Pathognomonic is the serological detection of autoantibodies against the mitochondrial fraction M2 (**AMA M2**) and against **Nuclear Dots** (acid protein SP100). Approximately 10% to 20% of PBC patients develop a secondary autoimmune hepatitis (also called **overlap syndrome**). In these cases, as in AIH, autoantibodies can frequently be detected. Antibodies against SLA/LP indicate a secondary AIH (overlap syndrome) which requires an immunosuppressive therapy.

The incidence of **primary sclerosing cholangiitis (PSC)** is quoted as being 4 cases in every 100,000 inhabitants per year. The clinical picture is also characterised by cholestasis. Diagnostic pointers are the relevant laboratory findings, the histology and ERCP (endoscopic retrograde cholangiopancreaticography). Men are mainly affected, and in half of the patients a concomitant Colitis ulcerosa is present (inversely, the prevalence of PSC in cases of Colitis ulcerosa is 4%). Most patients with PSC display autoantibodies against granulocytes (**pANCA**). Occasionally, pANCA can also occur in AIH and PBC, and their value for the differential diagnosis is limited in this respect - however, pANCA are an indication

of an autoimmune disease of the liver and can possibly be used as a means of delimitation to infectious forms of hepatitis. Even though overlaps between PSC and AIH have been reported, antibodies against SLA/LP have not been found in such patients.

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ANA Screen

per analisi di routine

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IVD

LOT
Vedere l'etichetta esterna

2°C 8°C

Σ = 96 test

REF DKO099

1. SCOPO PREVISTO

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Per uso professionale in laboratorio

Il dosaggio ANA Screen è un dispositivo diagnostico manuale *in vitro* destinato alla determinazione qualitativa degli anticorpi IgG diretti contro Sm (Smith), RNP/Sm, Scl-70, SS-A (Ro) (52kDa e 60kDa), SS-B (La), Jo1, U1-SmRNP, CENP-B, dsDNA e istoni nel siero o plasma umano. I risultati devono essere impiegati in associazione ad altri dati clinici e di laboratorio come ausilio nella diagnosi delle patologie autoimmuni sistemiche reumatiche.

2. RILEVANZA CLINICA

Gli autoanticorpi antinucleari (ANA) sono un gruppo eterogeneo di autoanticorpi che riconoscono le macromolecole nucleari e i relativi complessi; potrebbero rientrare in questa categoria anche anticorpi contro alcune proteine citoplasmatiche[1-6]. Tali anticorpi si trovano comunemente nei sieri dei pazienti affetti da patologie autoimmuni sistemiche reumatiche (SARD, systemic autoimmune rheumatic disease) e possono legarsi a DNA, RNA e proteine, nonché a complessi di acidi nucleici con proteine essenziali per diverse funzioni intracellulari come la replica e la trascrizione.

I target degli ANA possono essere suddivisi in due gruppi nell'ambito delle patologie autoimmuni sistemiche reumatiche: gli anticorpi che riconoscono il DNA, gli istoni e i nucleosomi[1,2,5,6]; e quelli che si legano ai complessi di RNA con le proteine leganti l'RNA (RBP, RNA-binding protein) e le piccole ribonucleoproteine nucleari (snRNP)[1].

Gli autoanticorpi mirati agli antigeni nucleari sono segni distintivi sierologici delle SARD e la loro rilevazione è utilizzata dai medici per supportare la diagnosi di disturbi come il LES, la sindrome di Sjögren, la sclerosi sistemica, la polimiosite e la malattia mista del tessuto connettivo. È ben noto che si tratta di disturbi complessi e caratterizzati dalla presenza di un ampio spettro di autoanticorpi. Inoltre, è stato ampiamente dimostrato che gli ANA vengono rilevati spesso nei sieri di pazienti affetti da malattie non reumatiche e soggetti sani che non sviluppano una malattia reumatica.

A causa della complessità delle SARD, i test ANA dovrebbero sempre essere considerati insieme alla valutazione dell'anamnesi del paziente, delle manifestazioni cliniche e di altri esami di laboratorio per diagnosticare queste condizioni.

3. PRINCIPIO DEL METODO

Il test ANA Screen è un dosaggio immunometrico enzimatico indiretto (ELISA) basato sul legame degli anticorpi presenti con gli antigeni Sm, RNP/Sm, SS-A (Ro), SS-B (La), Scl-70, Jo1, U1-SmRNP, CENP-B, dsDNA e istoni rivestiti sulle micropiastre. Eventuali anticorpi presenti nei calibratori, nei controlli o nei campioni prediluiti dei pazienti si legano alla superficie interna dei pozzetti. Dopo un'incubazione di 30 minuti, la micropiastra viene lavata con il tampone di lavaggio per rimuovere i componenti non reattivi del siero.

Una soluzione di coniugato con perossidasi di rafano anti-IgG umane riconosce gli anticorpi di classe IgG legati agli antigeni immobilizzati. Dopo un'incubazione di 30 minuti, il coniugato enzimatico in eccesso, che non è specificamente legato, viene lavato via con il tampone di lavaggio.

Una soluzione di substrato cromogenico contenente TMB viene erogata nei pozzetti. Dopo 15 minuti di incubazione, lo sviluppo del colore viene interrotto aggiungendo la soluzione di arresto. A questo punto, la soluzione diventa gialla. Il livello di colore è direttamente proporzionale alla concentrazione di anticorpi IgG presenti nel campione originale.

4. REAGENTI, MATERIALI E STRUMENTAZIONE

4.1. Reagenti e materiali forniti nel kit

1. Calibratore (1 fiala, 1,2 mL)
   Tampone fosfato 0,1 M, NaN_3 < 0,1%, siero umano
   CAL1 **REF. DCE002/09906-0**
2. Controlli (2 fiale, 1,2 mL ciascuna, pronte all'uso)
   Tampone fosfato 0,1 M, NaN_3 < 0,1%, siero umano
   Controllo negativo **REF DCE045/09901-0**
   Controllo positivo **REF DCE045/09902-0**
3. Diluente per campione (1 fiala, 100 mL)
   Tampone fosfato 0,1 M, NaN_3 < 0,1%
   REF DCE053-0
4. Coniugato (1 fiala, 15 mL)
   Coniugato anti h-IgG con perossidasi di rafano (HRP), BSA 0,1%, ProClin™ < 0,0015%
   REF DCE002/09902-0
5. Micropiastra rivestita (1 micropiastra frangibile)
   Micropiastra rivestita con antigeni ANA
   REF DCE002/09903-0
6. Substrato TMB (1 fiala, 15 mL)
   H_2O_2-TMB (0,26 g/L) *(evitare qualsiasi contatto con la pelle)*
   REF DCE004-0
7. Soluzione di arresto (1 fiala, 15 mL)
   Acido solforico 0,15 M *(evitare qualsiasi contatto con la pelle)*
   REF DCE005-0
8. Soluzione di arresto conc. 10X (1 fiala, 50 mL)
   Tampone fosfato 0,2 M pH 7,4 **REF DCE054-0**

4.2. Materiali richiesti ma non forniti
Acqua distillata

4.3. Materiali e strumentazione ausiliari
Erogatore automatico.
Dispositivi di pipettaggio di precisione Lettore di micropiastre (450 nm, 620-630 nm)

5. AVVERTENZE
- Questo kit è destinato all'uso *in vitro* esclusivamente da parte di professionisti. Non per uso interno o esterno in esseri umani o animali.
- Utilizzare adeguati dispositivi di protezione individuale mentre si lavora con i reagenti forniti.
- Seguire le buone prassi di laboratorio (GLP, Good Laboratory Practice) per la manipolazione di emoderivati.
- Il materiale di origine animale utilizzato nella preparazione del kit è stato ottenuto da animali certificati come sani e la proteina bovina è stata ottenuta da Paesi non infettati dalla BSE, ma tali materiali devono essere trattati come potenzialmente infettivi.
- Alcuni reagenti contengono piccole quantità di azoturo di sodio (NaN_3) o ProClin™ 300 come conservante. Evitare il contatto con pelle o mucose.
- Tutto il materiale di origine umana utilizzato nella preparazione dei reagenti è stato testato e risultato negativo per gli anticorpi dell'HIV 1 e 2, HbsAg e HCV. Nessun metodo di prova, tuttavia, può offrire la completa garanzia che HIV, HBV, HCV o altri agenti infettivi siano assenti. Pertanto, i calibratori e i controlli devono essere manipolati allo stesso modo del materiale potenzialmente infettivo.
- L'azoturo di sodio può essere tossico se ingerito o assorbito attraverso la pelle o gli occhi; inoltre, può reagire con le tubature di piombo o rame per formare azoturi metallici potenzialmente esplosivi. Se si utilizza un lavandino per rimuovere i reagenti, lavare con abbondante acqua per evitare l'accumulo di azoturi.
- Il substrato TMB contiene un irritante, che può essere dannoso se inalato, ingerito o assorbito per via cutanea. Per prevenire lesioni, evitare l'inalazione, l'ingestione o il contatto con pelle e occhi.
- La soluzione di arresto consiste in una soluzione diluita di acido solforico. L'acido solforico è velenoso e corrosivo e può essere tossico se ingerito. Per prevenire ustioni chimiche, evitare il contatto con pelle e occhi.
- Evitare l'esposizione del reagente TMB/H_2O_2 a luce solare diretta, metalli o ossidanti. Non congelare la soluzione.

6. PRECAUZIONI
- Attenersi rigorosamente alla sequenza dei passaggi di pipettaggio forniti in questo protocollo. I dati sulle prestazioni qui rappresentati sono stati ottenuti utilizzando i reagenti specifici elencati in queste istruzioni per l'uso.
- Tutti i reagenti devono essere conservati refrigerati a 2-8 °C nel contenitore originale. Tutte le eccezioni sono chiaramente indicate.
- Lasciare che tutti i componenti del kit e i campioni raggiungano la temperatura ambiente (22-28 °C) e mescolare bene prima dell'uso.
- Non scambiare i componenti di kit di lotti diversi. La data di scadenza stampata sulle etichette della confezione e delle fiale deve essere rispettata. Non utilizzare alcun componente del kit dopo la data di scadenza.
- Se si utilizzano apparecchiature automatizzate, l'utente ha la responsabilità di assicurarsi che il kit sia stato adeguatamente testato.
- La rimozione incompleta o imprecisa del liquido dai pozzetti potrebbe influenzare la precisione del dosaggio e/o aumentare il background. Per migliorare le prestazioni del kit sui sistemi automatici, si raccomanda di aumentare il numero di lavaggi.
- È importante che il tempo di reazione in ogni pozzetto sia mantenuto costante per ottenere risultati riproducibili. Il pipettaggio dei campioni non deve andare oltre i dieci minuti per evitare deviazioni del dosaggio. Se sono necessari più di 10 minuti, seguire lo stesso ordine di erogazione. Se si utilizza più di una piastra, si raccomanda di ripetere la curva dose-risposta in ogni piastra.
- L'aggiunta della soluzione di substrato TMB avvia una reazione cinetica, che viene terminata dall'aggiunta della soluzione di arresto. Pertanto, il substrato TMB e la soluzione di arresto devono essere aggiunti nella stessa sequenza per eliminare qualsiasi deviazione temporale durante la reazione.
- Osservare le linee guida per l'esecuzione del controllo di qualità nei laboratori medici analizzando i controlli e/o i sieri in pool.
- La massima precisione è richiesta per la ricostituzione e l'erogazione dei reagenti.
- I campioni microbiologicamente contaminati, altamente lipemici o emolizzati non devono essere utilizzati nel dosaggio.
- I lettori di piastre misurano verticalmente. Non toccare il fondo dei pozzetti.
- **AVVERTENZA: il reagente coniugato è progettato per garantire la massima sensibilità per la dose e può essere contaminato da agenti esterni se non utilizzato correttamente;** pertanto, si raccomanda di utilizzare materiali di consumo monouso (puntali, flaconi, vassoi, ecc.). Per le dosi divise, prelevare l'esatta quantità di coniugato necessaria e non reintrodurre alcun prodotto di scarto nel flacone originale. Inoltre, **per le dosi erogate con l'ausilio di dispositivi automatici e semiautomatici,** prima di utilizzare il coniugato, è consigliabile pulire il sistema per la gestione dei fluidi, assicurandosi che le procedure di lavaggio, deproteinizzazione e decontaminazione siano efficaci per evitare la contaminazione del coniugato. Questa procedura è altamente raccomandata quando il kit viene elaborato utilizzando analizzatori non dotati di puntali monouso.
  A tale scopo, DiaMetra fornisce un reagente di decontaminazione separato per la pulizia degli aghi.

7. CONSERVAZIONE E STABILITÀ DEI REAGENTI

Conservare il kit a 2÷8 °C, al buio.
- Il kit è stabile a 2-8 °C fino alla data di scadenza indicata sull'etichetta esterna del kit.
- Una volta aperto, il kit è stabile a 2-8 °C per 6 mesi.
- La soluzione di lavaggio diluita è stabile per 30 giorni a 2-8 °C.

Nota importante: aprire il sacchetto contenente la micropiastra rivestita solo quando è a temperatura ambiente e chiuderlo immediatamente dopo l'uso.

8. RACCOLTA E CONSERVAZIONE DEI CAMPIONI
Il dosaggio deve essere effettuato su campioni di siero (provette di campionamento standard o provette contenenti gel per la separazione del siero) o plasma (litio eparina, sodio eparina o EDTA di potassio).

Conservazione dei campioni	Durata
2-8 °C	96 ore
Cicli di congelamento/scongelamento	3 cicli

9. PROCEDURA

9.1. Preparazione dei calibratori (C_1)

I calibratori sono pronti all'uso; la concentrazione del calibratore è stampata sull'etichetta.

9.2. Preparazione della soluzione di lavaggio

Diluire il contenuto di ogni fiala della soluzione di lavaggio tamponata concentrata (10X) con acqua distillata fino a un volume finale di 500 mL prima dell'uso. Per i volumi più piccoli, rispettare il rapporto di diluizione 1:10. La soluzione di lavaggio diluita è stabile per 30 giorni a 2-8 °C.
È possibile osservare la presenza di cristalli all'interno della soluzione di lavaggio concentrata; in tal caso, mescolare a temperatura ambiente fino alla completa dissoluzione dei cristalli. Per una maggiore precisione, diluire l'intero flacone di soluzione di lavaggio concentrata a 500 mL, avendo cura anche di trasferire completamente i cristalli sciacquando il flacone, quindi mescolare fino a quando i cristalli non si dissolvono completamente.

9.3. Preparazione dei campioni

Per la determinazione degli anticorpi ANA, il siero o il plasma umano sono le matrici di campione preferite.
Tutti i campioni di siero e plasma devono essere prediluiti con diluente per campione 1:100; ad esempio, 10 µL di campione possono essere diluiti con 990 µL di diluente per campione.
I campioni a digiuno non sono necessari e non sono richieste preparazioni speciali. Prelevare il sangue tramite venipuntura in Vacutainer e separare il siero (dopo la formazione del coagulo) o il plasma dalle cellule tramite centrifugazione.

Né la bilirubina né l'emolisi hanno un effetto significativo sulla procedura.

I controlli sono pronti per l'uso.

9.4. Procedura

- **Lasciare che tutti i reagenti raggiungano la temperatura ambiente (22-28 °C) per almeno 30 minuti.** Alla fine del dosaggio, conservare immediatamente i reagenti a 2-8 °C: evitare una lunga esposizione a temperatura ambiente.
- Le strisce di micropozzetti rivestiti non utilizzate devono essere rilasciate in modo sicuro nella busta di alluminio contenente l'essiccante e conservate a 2-8 °C.
- Per evitare potenziali contaminazioni microbiche e/o chimiche, i reagenti inutilizzati non devono mai essere trasferiti nelle fiale originali.
- Poiché è necessario eseguire la determinazione in duplicato per migliorare la precisione dei risultati della prova, preparare due pozzetti per ogni punto della curva di calibrazione (C_1), due per ogni controllo, due per ogni campione, uno per il bianco.

Reagente	Calibratore	Campione/ Controlli	Bianco
Calibratore C_1	**100 µL**		
Controlli		**100 µL**	
Campione diluito		**100 µL**	
Incubare per 30 minuti a temperatura ambiente (22-28 °C). Rimuovere il contenuto da ogni pozzetto, lavare i pozzetti 3 volte con 300 µL di soluzione di lavaggio diluita. **Nota importante**: durante ogni fase di lavaggio, agitare delicatamente la piastra per 5 secondi e rimuovere la soluzione in eccesso picchiettando la piastra capovolta su un tovagliolo di carta assorbente. **Lavatore automatico**: se si utilizzano apparecchiature automatiche, lavare i pozzetti almeno 5 volte.			
Coniugato	**100 µL**	**100 µL**	
Incubare per 30 minuti a temperatura ambiente (22-28 °C). Rimuovere il contenuto da ogni pozzetto, lavare i pozzetti 3 volte con 300 µL di soluzione di lavaggio diluita. **Lavaggio**: seguire le stesse indicazioni del punto precedente.			
Substrato TMB	**100 µL**	**100 µL**	**100 µL**
Incubare per 15 minuti, al buio, a temperatura ambiente (22-28 °C).			
Soluzione di arresto	**100 µL**	**100 µL**	**100 µL**
Agitare delicatamente la micropiastra. Leggere l'assorbanza (E) a 450 nm contro una lunghezza d'onda di riferimento di 620-630 nm o contro il bianco entro 5 minuti.			

10. CONTROLLO QUALITÀ

Le buone prassi di laboratorio (GLP) richiedono l'inclusione di campioni per il controllo della qualità in ogni serie di dosaggi al fine di verificare le prestazioni del dosaggio. I controlli devono essere trattati come campioni sconosciuti e i risultati devono essere analizzati con metodi statistici appropriati.

I controlli forniti nel kit devono essere testati come se fossero sconosciuti e hanno lo scopo di agevolare la valutazione della validità dei risultati ottenuti in ogni piastra di dosaggio.

La concentrazione media di ciascun livello di controllo è documentata nel rapporto del controllo di qualità incluso in ciascun kit. Tali livelli di concentrazione media sono determinati in base a diversi dosaggi eseguiti in duplicato in più posizioni su ciascuna piastra.

DiaMetra raccomanda agli utenti di conservare le annotazioni grafiche dei valori di controllo generati con ciascun dosaggio, tra cui medie mobili, DS e CV%. Queste informazioni faciliteranno l'analisi delle tendenze dei controlli per quanto riguarda le prestazioni dei lotti di controllo attuali e pregressi rispetto ai dati forniti nel controllo di qualità. Le tendenze aiuteranno a identificare i dosaggi che generano valori di controllo significativamente diversi dal rispettivo intervallo medio.

Quando si interpretano i dati dei controlli, occorre tenere conto del fatto che il prodotto è stato progettato e sviluppato come prodotto per l'utilizzo manuale. L'intervallo riportato sul certificato del controllo di qualità deve essere appropriato per i dosaggi eseguiti manualmente e rispettando rigorosamente la procedura di dosaggio descritta sopra. Gli esperti del controllo di qualità riconoscono che, a causa delle differenze di condizioni e di prassi, si avrà sempre una variabilità nei valori medi e nella precisione delle misurazioni dei controlli eseguite da laboratori diversi[7].

11. CALCOLO DEI RISULTATI

Determinare l'assorbanza media per ogni campione duplicato.

Per ottenere il valore del campione: dividere l'assorbanza media del campione per l'assorbanza del calibratore, quindi moltiplicare per la concentrazione del calibratore, come mostrato di seguito:

$$Conc.campione\ .= \frac{campione\ OD}{OD\ C1} \times\ Conc.C1$$

La reattività non è collegata in modo lineare alla quantità di anticorpi presenti. Anche se un aumento o una diminuzione della concentrazione di anticorpi provoca un aumento o una diminuzione della reattività, il cambiamento non è proporzionale (ad es., il raddoppio della concentrazione di anticorpi non comporta un raddoppio della reattività).

Per ottenere una maggiore precisione nella determinazione degli anticorpi, si raccomanda di testare diluizioni seriali del campione. La diluizione finale che risulta positiva nel test è la concentrazione di anticorpi del paziente.

12. METROLOGIA E TRACCIABILITÀ

Il dosaggio ANA Screen è tracciabile agli standard di riferimento degli anticorpi antinucleari (ANA) dell'Autoantibody Standardisation Committee (ASC), Center for Disease Control (CDC) e del WHO anti-dsDNA 15/174.

13. INTERPRETAZIONE DEI RISULTATI

ANA Screen (AU/mL)	Interpretazione
< 25	Il campione deve essere considerato negativo
≥ 25	Il campione deve essere considerato positivo

L'indice e l'interpretazione succitati devono essere considerati solo linee guida. I risultati positivi devono essere verificati relativamente all'intero stato clinico del paziente. Inoltre, ogni decisione per la terapia deve essere presa in base alle condizioni di ciascun paziente.
È consigliabile che ogni laboratorio stabilisca i propri intervalli normali e patologici di anti-ANA nel siero.

14. CARATTERISTICHE DI AZIONE

Sono mostrati i dati più rappresentativi delle prestazioni. I risultati ottenuti nei singoli laboratori possono variare.

14.1. Sensibilità e specificità diagnostica

La sensibilità e la specificità sono state determinate basandosi sulla procedura CLSI EP-24 "Assessment of the Diagnostic Accuracy of Laboratory Tests Using Receiver Operating Characteristic Curves" utilizzando 50 campioni negativi e 53 campioni positivi eseguiti su un singolo lotto di reagenti.

		DKO099		Totale
		Positivo	Negativo	
Stato reale	Positivo	53	0	53
	Negativo	0	50	50
Totale		53	50	103

Sensibilità diagnostica: 100%

Specificità diagnostica: 100%

14.2. Precisione

La precisione del kit ANA Screen è stata determinata eseguendo un complesso studio di precisione.

Ripetibilità: un totale di 3 campioni di siero è stato analizzato utilizzando 2 lotti di reagenti in 10 repliche per campione, da parte di 3 operatori, una volta al giorno per 5 giorni. I dati di un lotto rappresentativo sono mostrati di seguito:

Risultati di ripetibilità relativi a un lotto rappresentativo:

Campione	n	Risultati +vi	Risultati -vi	Risultati % +vi	Risultati% -vi
Cut-off	150	82	68	54,7	45,3
Negativo	150	1	149	0,7	99,3
Positivo	150	150	0	100,0	0,0

I risultati di riproducibilità per i dati combinati di due lotti sono mostrati di seguito:

Campione	n	Risultati +vi	Risultati -vi	Risultati % +vi	Risultati% -vi
Cut-off	300	163	137	54,3	45,7
Negativo	300	3	297	1,0	99,0
Positivo	300	300	0	100,0	0,0

14.3. Studio su siero-plasma

Lo studio di confronto della matrice ANA Screen è stato eseguito per valutare la differenza tra i vari tipi di provette (provette per la separazione del siero (SST), per plasma in litio eparina, per plasma in sodio eparina e plasma in K2 EDTA) rispetto ai campioni di controllo (siero tappo rosso, senza additivo) secondo CLSI GP34-A "Validation and Verification of Tubes for Venous and Capillary Blood. Specimen Collection". È stato valutato un totale di 20 campioni (16 nativi, 4 artificiali).

Tipo di campione	N. campioni	Risultati rispetto al siero	
		Falso pos	Falso neg
SST	20	0	0
K2 EDTA	20	0	0
Litio-eparina	20	0	0
Sodio-eparina	20	0	0

14.4. Interferenti

Le seguenti sostanze non interferiscono con il dosaggio ANA Screen fino alle concentrazioni riportate nella tabella seguente.

Reagente potenzialmente interferente	Concentrazione di soglia
Bilirubina, coniugata	15 mg/dL
Bilirubina, non coniugata	15 mg/dL
Emoglobina	200 mg/dL
Proteine totali	10 g/dL
Trigliceridi	500 mg/dL

15. LIMITAZIONI D'USO

- Come nel caso di qualsiasi procedura diagnostica, i risultati devono essere interpretati unitamente ai dati clinici del paziente e alle altre informazioni a disposizione del medico.
- Non sono state stabilite le caratteristiche di azione di questo dosaggio nella popolazione pediatrica.
- Gli anticorpi eterofili nel siero umano possono reagire con le immunoglobuline dei reagenti, interferendo con gli immunodosaggi *in vitro*[8]. I pazienti regolarmente esposti agli animali o a prodotti derivati da siero animale possono essere soggetti a questa interferenza, quindi si potrebbero osservare valori anomali.

16. GESTIONE DEI RIFIUTI

I reagenti devono essere smaltiti in conformità alle normative locali.

17. BIBLIOGRAFIA

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18. IDENTIFICATORE DELLE REVISIONI

Le aggiunte o le modifiche alle istruzioni per l'uso sono indicate dall'evidenziazione in grigio.

19. RECLAMI SUI PRODOTTI E SUPPORTO TECNICO

Per un paziente/utente/terza parte nell'Unione Europea e nei Paesi con un regime normativo simile (Regulation 2017/746/EU on IVD Medical Devices); se, durante l'uso di questo dispositivo o come risultato del suo utilizzo, si è verificato un incidente grave, segnalarlo al produttore e/o al suo rappresentante autorizzato e all'autorità normativa nazionale.
Il produttore può essere contattato tramite il relativo servizio clienti o il team di supporto tecnico. I dettagli di contatto sono disponibili di seguito e sul sito Web dell'azienda: www.diametra.com.

Ed. 10/2021 **DCM099-7**

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CE

DiaMetra
care for quality

DCM099-7
Ed. 10/2021

ANA Screen

for routine analysis

Qualitative determination of IgG class antibodies against nuclear antigens in human serum or plasma

IVD | LOT See external label | 2°C 8°C | Σ = 96 tests | REF DKO099

1. INTENDED PURPOSE

For *In Vitro* Diagnostic Use
For Laboratory Professional Use

The ANA Screen assay is a manual *in vitro* diagnostic device intended for the qualitative determination of IgG antibodies directed against Sm (Smith), RNP/Sm, Scl-70, SS-A (Ro) (52kDa e 60kDa), SS-B (La), Jo1, U1-SmRNP, CENP-B, dsDNA and Histones in human serum or plasma. Results are to be used in conjunction with other clinical and laboratory data as an aid in the diagnosis of systemic rheumatic autoimmune diseases.

2. CLINICAL SIGNIFICANCE

Anti-nuclear autoantibodies (ANAs) are a heterogeneous group of autoantibodies that recognise nuclear macromolecules and their complexes; antibodies to certain cytoplasmic proteins might also fall into this category[1-6]. These antibodies commonly occur in the sera of patients with systemic autoimmune rheumatic diseases (SARDs) and can bind to DNA, RNA and proteins, as well as complexes of nucleic acids with proteins that are essential for different intracellular functions such as replication and transcription.

ANAs' targets can be divided into two groups in the context of systemic rheumatic autoimmune diseases: antibodies that recognise DNA, histones and nucleosomes[1,2,5,6]; and those that bind to complexes of RNA with RNA-binding proteins (RBPs) and small nuclear ribonucleoproteins (snRNPs)[1].

Autoantibodies directed to nuclear antigens are serological hallmarks of SARDs and their detection is used by clinicians to support the diagnosis of disorders such as SLE, Sjögren syndrome, systemic sclerosis, polymyositis and mixed connective tissue disease. It is well documented that these are complex disorders and characterised by the presence of a wide spectrum of autoantibodies. Furthermore, it has widely been shown that ANAs are also frequently found in the sera of patients with non-rheumatic diseases and healthy subjects that do not develop a rheumatic disease.

Due to the complexity of SARDs, ANA tests should always be considered together with the evaluation of the patient's history, clinical manifestations and other laboratory tests to diagnose these conditions.

3. PRINCIPLE OF THE METHOD

The ANA Screen test is an indirect enzyme immunometric assay (ELISA) based on the binding of present antibodies with Sm, RNP/Sm, SS-A (Ro), SS-B (La), Scl-70, Jo1, U1-SmRNP, CENP-B, dsDNA and Histones antigens coated on the microplates. Any antibodies present in calibrators, controls or prediluted patient samples bind to the inner surface of the wells. After a 30 minutes incubation the microplate is washed with wash buffer for removing non-reactive serum components.

An anti-human-IgG horseradish peroxidase conjugate solution recognises IgG class antibodies bound to the immobilised antigens. After a 30 minutes incubation any excess enzyme conjugate, which is not specifically bound is washed away with wash buffer.

A chromogenic substrate solution containing TMB is dispensed into the wells. After 15 minutes of incubation the colour development is stopped by adding the stop solution. The solution turns yellow at this point. The level of colour is directly proportional to the concentration of IgG antibodies present in the original sample.

4. REAGENTS, MATERIALS AND INSTRUMENTATION

4.1. Reagents and materials supplied in the kit

1. Calibrator (1 vial, 1.2 mL)
Phosphate buffer 0.1M, NaN_3 < 0.1%, human serum
CAL1 **REF DCE002/09906-0**
2. Controls (2 vials, 1.2 mL each, ready to use)
Phosphate buffer 0.1M, NaN_3 < 0.1%, human serum
Negative Control **REF DCE045/09901-0**
Positive Control **REF DCE045/09902-0**
3. Sample Diluent (1 vial, 100 mL)
Phosphate buffer 0.1M, NaN_3 < 0.1%
REF DCE053-0
4. Conjugate (1 vial, 15 mL)
Anti h-IgG conjugated with horseradish peroxidise (HRP), BSA 0.1%, ProClin™ < 0.0015%
REF DCE002/09902-0
5. Coated Microplate (1 breakable microplate)
Microplate coated with ANA antigens
REF DCE002/09903-0
6. TMB Substrate (1 vial, 15 mL)
H_2O_2 -TMB (0,26 g/L) *(avoid any skin contact)*
REF DCE004-0
7. Stop Solution (1 vial, 15 mL)
Sulphuric acid 0.15M *(avoid any skin contact)*
REF DCE005-0
8. 10X Conc. Wash Solution (1 vial, 50 mL)
Phosphate buffer 0.2M pH 7.4 **REF DCE054-0**

4.2. Materials required but not provided
Distilled water

4.3. Auxiliary materials and instrumentation
Automatic dispenser.
Precision Pipetting DevicesMicroplate reader (450 nm, 620-630 nm)

5. WARNINGS
- This kit is intended for *in vitro* use by professional persons only. Not for internal or external use in Humans or Animals.
- Use appropriate personal protective equipment while working with the reagents provided.
- Follow Good Laboratory Practice (GLP) for handling blood products.
- Material of animal origin used in the preparation of the kit has been obtained from animals certified as healthy and the bovine protein has been obtained from countries not infected by BSE, but these materials should be handled as potentially infectious.
- Some reagents contain small amounts of Sodium Azide (NaN_3) or ProClin™ 300 as preservative. Avoid the contact with skin or mucosa.
- All human source material used in the preparation of the reagents has been tested and found negative for antibody to HIV 1&2, HbsAg, and HCV. No test method however can offer complete assurance that HIV, HBV, HCV or other infectious agents are absent. Therefore, the Calibrators and the Controls should be handled in the same manner as potentially infectious material.
- Sodium Azide may be toxic if ingested or absorbed through the skin or eyes; moreover, it may react with lead or copper plumbing to form potentially explosive metal azides. If you use a sink to remove the reagents, wash through large amounts of water to prevent azide build-up.
- The TMB Substrate contains an irritant, which may be harmful if inhaled, ingested or absorbed through the skin. To prevent injury, avoid inhalation, ingestion or contact with skin and eyes.
- The Stop Solution consists of a diluted sulphuric acid solution. Sulphuric acid is poisonous and corrosive and can be toxic if ingested. To prevent chemical burns, avoid contact with skin and eyes.
- Avoid the exposure of reagent TMB/H_2O_2 to directed sunlight, metals or oxidants. Do not freeze the solution.

6. PRECAUTIONS
- Please adhere strictly to the sequence of pipetting steps provided in this protocol. The performance data represented here were obtained using specific reagents listed in this Instruction For Use.
- All reagents should be stored refrigerated at 2-8°C in their original container. Any exceptions are clearly indicated.
- Allow all kit components and specimens to reach room temperature (22-28°C) and mix well prior to use.
- Do not interchange kit components from different lots. The expiry date printed on box and vials labels must be observed. Do not use any kit component beyond their expiry date.
- If you use automated equipment, the user has the responsibility to make sure that the kit has been appropriately tested.
- The incomplete or inaccurate liquid removal from the wells could influence the assay precision and/or increase the background. To improve the performance of the kit on automatic systems is recommended to increase the number of washes.
- It is important that the time of reaction in each well is held constant for reproducible results. Pipetting of samples should not extend beyond ten minutes to avoid assay drift. If more than 10 minutes are needed, follow the same order of dispensation. If more than one plate is used, it is recommended to repeat the dose response curve in each plate.
- Addition of the TMB Substrate solution initiates a kinetic reaction, which is terminated by the addition of the Stop Solution. Therefore, the TMB Substrate and the Stop Solution should be added in the same sequence to eliminate any time deviation during the reaction.
- Observe the guidelines for performing quality control in medical laboratories by assaying controls and/or pooled sera.
- Maximum precision is required for reconstitution and dispensation of the reagents.
- Samples microbiologically contaminated, highly lipemic or haemolysed should not be used in the assay.
- Plate readers measure vertically. Do not touch the bottom of the wells.
- **WARNING: the conjugate reagent is designed to ensure maximum dose sensitivity and may be contaminated by external agents if not used properly;** therefore, it is recommended to use disposable consumables (tips, bottles, trays, etc.). For divided doses, take the exact amount of conjugate needed and do not re-introduce any waste product into the original bottle. In addition, **for doses dispensed with the aid of automatic and semi-automatic devices,** before using the conjugate, it is advisable to clean the fluid handling system, ensuring that the procedures of washing, deproteinization and decontamination are effective in avoiding contamination of the conjugate. This procedure is highly recommended when the kit is processed using analysers which are not equipped with disposable tips.
  For this purpose, DiaMetra supplies a separate decontamination reagent for cleaning needles.

7. REAGENT STORAGE AND STABILITY

Store the kit at 2÷8°C in the dark.
- The kit is stable at 2-8°C until the expiry date stated in the external kit label.
- Once opened, the kit is stable at 2-8°C for 6 months.
- The diluted wash solution is stable for 30 days at 2-8°C.

Important note: open the bag containing the Coated Microplate only when it is at room temperature and close it immediately after use.

8. SAMPLE COLLECTION AND STORAGE
The assay should be performed using serum (standard sampling tubes or tubes containing serum separating gel) or plasma (lithium heparin, sodium heparin or potassium EDTA) samples.

Sample Storage	Duration
2-8°C	96 hours
Freeze/thaw cycles	3 cycles

9. PROCEDURE

9.1. Preparation of the Calibrators (C_1)

Calibrators are ready to use; the concentration of the Calibrator is printed on the label.

9.2. Preparation of the Wash Solution

Dilute the contents of each vial of the buffered wash solution concentrate (10X) with distilled water to a final volume of 500 mL prior to use. For smaller volumes respect the 1:10 dilution ratio. The diluted wash solution is stable for 30 days at 2-8°C.
It is possible to observe the presence of crystals within the concentrated wash solution; in this case mix at room temperature until the complete dissolution of crystals. For greater accuracy, dilute the whole bottle of concentrated wash solution to 500 mL, taking care also to transfer crystals completely by rinsing of the bottle, then mix until crystals are completely dissolved.

9.3. Preparation of Samples

For determination of ANA antibodies, human serum or plasma are the preferred sample matrixes.
All serum and plasma samples must be prediluted with sample diluent 1:100; for example, 10 µL of sample may be diluted with 990 µL of sample diluent.
Fasting samples are not necessary and no special preparations are required. Collect blood by venepuncture into vacutainers and separate serum (after clot formation) or plasma from the cells by centrifugation.

Neither Bilirubin nor Haemolysis have significant effect on the procedure.

The Controls are ready to use.

9.4. Procedure

- **Allow all reagents to reach room temperature (22-28°C) for at least 30 minutes.** At the end of the assay store immediately the reagents at 2-8°C: avoid long exposure to room temperature.
- Unused coated microwell strips should be released securely in the foil pouch containing desiccant and stored at 2-8°C.
- To avoid potential microbial and/or chemical contamination, unused reagents should never be transferred into the original vials.
- As it is necessary to perform the determination in duplicate in order to improve accuracy of the test results, prepare two wells for each point of the calibration curve (C_1), two for each Control, two for each sample, one for Blank.

Reagent	Calibrator	Sample/ Controls	Blank
Calibrator C_1	**100 µL**		
Controls		**100 µL**	
Diluted Sample		**100 µL**	
Incubate for 30 minutes at room temperature (22-28°C). Remove the content from each well, wash the wells 3 times with 300 µL of diluted wash solution. **Important note**: during each washing step, gently shake the plate for 5 seconds and remove excess solution by tapping the inverted plate on an absorbent paper towel. **Automatic washer**: if you use automated equipment, wash the wells at least 5 times.			
Conjugate	**100 µL**	**100 µL**	
Incubate for 30 minutes at room temperature (22-28°C). Remove the content from each well, wash the wells 3 times with 300 µL of diluted wash solution. **Washing**: follow the same indications of the previous point.			
TMB Substrate	**100 µL**	**100 µL**	**100 µL**
Incubate for 15 minutes in the dark at room temperature (22-28°C).			
Stop Solution	**100 µL**	**100 µL**	**100 µL**
Shake the microplate gently. Read the absorbance (E) at 450 nm against a reference wavelength of 620-630 nm or against Blank within 5 minutes.			

10. QUALITY CONTROL

Good Laboratory Practice (GLP) requires the use of quality control specimens in each series of assays in order to check the performance of the assay. Controls should be treated as unknown samples, and the results analysed with appropriate statistical methods.

The kit controls provided in the kit should be tested as unknowns and are intended to assist in assessing the validity of results obtained with each assay plate.

The mean concentration of each control level is documented in the QC report included with each kit. These mean concentration levels are determined over several assays which are run in duplicate in multiple locations across each plate.

DiaMetra recommends the users to maintain graphic records of the control values generated with each assay run, including the running means, SDs and %CVs. This information will facilitate the controls trending analysis relating to the performance of current and historical control lots relative to the supplied Quality Control data. The trending will assist in the identification of assays which give control values significantly different from their average range.

When interpreting control data, users should note that this product was designed and developed as a manual product. The range stated on the QC certificate should be appropriate for assays that are performed manually and with strict adherence to the Assay Procedure described above. It is recognised by Quality Control professionals, that as a result of differences in conditions and practices, there will always be variability in the mean values and precision of control measurements between different laboratories[7].

11. CALCULATION OF RESULTS

Determine the mean absorbance for each duplicate sample.

To obtain sample value: divide the mean absorbance of the sample by the absorbance of the Calibrator, then multiply by the concentration of the Calibrator, as shown below:

$$Sample\ Conc. = \frac{OD\ sample}{OD\ C1} \times Conc.C1$$

Reactivity is not connected in a linear way to the amount of antibodies present. Although an increase or a decrease in the concentration of antibodies results in increased or decreased responsiveness, the change is not in proportion (e.g., doubling the concentration of antibodies does not lead to a doubling of responsiveness).

To achieve greater accuracy in the determination of antibodies it is recommended to test serial dilutions of the sample. The final dilution that is positive in the test is the antibody concentration of the patient.

12. METROLOGY AND TRACEABILITY

The ANA Screen assay is traceable to the Antinuclear Antibodies (ANA) Reference Standards by Autoantibody Standardisation Committee (ASC) - Center for Disease Control (CDC) and WHO anti-dsDNA 15/174.

13. INTERPRETATION OF RESULTS

ANA Screen (AU/ mL)	Interpretation
< 25	The sample should be considered negative
≥ 25	The sample should be considered positive

The above index and interpretation should be considered as guidelines only. Positive results should be verified concerning the entire clinical status of the patient. Also, every decision for therapy should be taken on an individual patient basis.
It is recommended that each laboratory establishes its own normal and pathological ranges of serum anti-ANA.

14. PERFORMANCE CHARACTERISTICS

Representative performance data are shown. Results obtained at individual laboratories may vary.

14.1. Diagnostic Sensitivity and Specificity

The sensitivity and specificity were determined with guidance from CLSI EP-24 "Assessment of the Diagnostic Accuracy of Laboratory Tests Using Receiver Operating Characteristic Curves" using 50 negative and 53 positive samples run on a single reagent lot.

		DKO099		Total
		Positive	Negative	
True state	Positive	53	0	53
	Negative	0	50	50
Total		53	50	103

Diagnostic sensitivity: 100%

Diagnostic specificity: 100%

14.2. Precision

Precision of the ANA Screen kit was determined by performing a complex precision study.

Repeatability: A total of A total of 3 serum samples were assayed using 2 lots of reagents in 10 replicates per sample, by 3 operators, once a day for 5 days. Data from one representative lot is shown below:

Repeatability results from one representative lot:

Sample	n	+'ve results	-'ve results	% +'ve results	% -'ve results
Cut off	150	82	68	54.7	45.3
Negative	150	1	149	0.7	99.3
Positive	150	150	0	100.0	0.0

Reproducibility results for the combined data from two lots is shown below:

Sample	n	+'ve results	-'ve results	% +'ve results	% -'ve results
Cut off	300	163	137	54.3	45.7
Negative	300	3	297	1.0	99.0
Positive	300	300	0	100.0	0.0

14.3. Serum-plasma study

The ANA Screen matrix comparison study was performed to evaluate the difference across tube types (serum separator tubes (SST), lithium heparin plasma, sodium heparin plasma and K2 EDTA plasma) versus the control samples (red top serum, without additive) following CLSI GP34-A "Validation and Verification of Tubes for Venous and Capillary Blood. Specimen Collection". A total of 20 samples (16 native, 4 contrived) were evaluated.

Sample Type	No. Samples	Results vs Serum	
		False pos	False neg
SST	20	0	0
K2 EDTA	20	0	0
Lithium heparin	20	0	0
Sodium heparin	20	0	0

14.4. Interferents

The following substances do not interfere in the ANA Screen assay when the concentrations presented in the following table are below the stated threshold.

Potentially Interfering Reagent	Threshold Concentration
Bilirubin, conjugated	15 mg/dL
Bilirubin, unconjugated	15 mg/dL
Haemoglobin	200 mg/dL
Total Protein	10 g/dL
Triglyceride	500 mg/dL

15. LIMITATIONS OF USE

- As in the case of any diagnostic procedure, results must be interpreted in conjunction with the patient's clinical presentation and other information available to the physician.
- The performance characteristics of this assay have not been established in a paediatric population.
- Heterophilic antibodies in human serum can react with reagent immunoglobulins, interfering with *in vitro* immunoassays[8]. Patients routinely exposed to animals or to animal serum products can be prone to this interference and anomalous values may be observed.

16. WASTE MANAGEMENT

Reagents must be disposed of in accordance with local regulations.

17. BIBLIOGRAPHY

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18. REVISION IDENTIFIER

Additions or changes to the IFU are indicated by grey highlighting.

19. PRODUCT COMPLAINTS AND TECHNICAL SUPPORT

For a patient/user/third party in the European Union and in countries with similar regulatory regime (Regulation 2017/746/EU on IVD Medical Devices); if, during the use of this device or as a result of its use, a serious incident has occurred, please report it to the manufacturer and/or its authorised representative and to your national regulatory authority.

The manufacturer can be contacted through their customer service or technical support team. The contact details can be found below and on the company website: www.diametra.com.

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CE

DiaMetra
care for quality

DCM099-7
Ed. 10/2021

ANA Screen

para el análisis de rutina

Determinación cualitativa de anticuerpos de clase IgG contra antígenos nucleares en suero o plasma humano

IVD

LOT
Ver etiqueta externa

2°C 8°C

Σ = 96 pruebas

REF DKO099

1. FINALIDAD PREVISTA

Para uso en diagnóstico *in vitro*
Para uso profesional de laboratorio

El ensayo ANA screen es un dispositivo manual de diagnóstico *in vitro* cuya finalidad es la determinación cualitativa de anticuerpos IgG dirigidos contra Sm (Smith), RNP/Sm, Scl-70, SS-A (Ro) (52 kDa y 60 kDa), SS-B (La), Jo1, U1-SmRNP, CENP-B, dsDNA e histonas en suero o plasma humano. Los resultados deben utilizarse como ayuda en el diagnóstico de enfermedades reumáticas autoinmunitarias sistémicas junto con otros datos clínicos y de laboratorio.

2. IMPORTANCIA CLÍNICA

Los autoanticuerpos antinucleares (ANA) son un grupo heterogéneo de autoanticuerpos que reconocen las macromoléculas nucleares y sus complejos; los anticuerpos contra ciertas proteínas citoplasmáticas también pueden entrar en esta categoría[1-6]. Estos anticuerpos suelen aparecer en el suero de pacientes con enfermedades reumáticas autoinmunitarias sistémicas (ERAS) y pueden unirse al ADN, al ARN y a las proteínas, así como a complejos de ácidos nucleicos con proteínas que son esenciales para diversas funciones intracelulares, como la replicación y la transcripción.

Las dianas de los ANA pueden dividirse en dos grupos en el contexto de las enfermedades reumáticas autoinmunitarias sistémicas: los anticuerpos que reconocen el ADN, las histonas y los nucleosomas[1,2,5,6]; y los que se unen a complejos de ARN con proteínas de unión a ARN (RBP) y ribonucleoproteínas nucleares pequeñas (snRNP)[1].

Los autoanticuerpos dirigidos a los antígenos nucleares son balizas serológicas de las ERAS y los especialistas usan su detección para respaldar el diagnóstico de trastornos como el LES, el síndrome de Sjögren, la esclerosis sistémica, la polimiositis y la enfermedad mixta del tejido conectivo. Hay mucha documentación que demuestra que se trata de trastornos complejos y caracterizados por la presencia de un amplio espectro de autoanticuerpos. Además, está suficientemente demostrado que los ANA también se encuentran con frecuencia en el suero de pacientes con enfermedades no reumáticas y de personas sanas que no desarrollan una enfermedad reumática.

Debido a la complejidad de las ERAS, siempre hay que considerar la realización de pruebas de ANA junto con la evaluación de los antecedentes médicos del paciente, las manifestaciones clínicas y otras pruebas de laboratorio para diagnosticar estas afecciones.

3. PRINCIPIO DEL MÉTODO

La prueba ANA screen es un enzimoinmunoanálisis de adsorción (ELISA) indirecto basado en la unión de los anticuerpos presentes con los antígenos Sm, RNP/Sm, SS-A (Ro), SS-B (La), Scl-70, Jo1, U1-SmRNP, CENP-B, dsDNA e histonas recubiertos en las microplacas. Cualquier anticuerpo presente en los calibradores, controles o muestras de pacientes prediluidas se une a la superficie interior de los pocillos. Tras una incubación de 30 minutos, la microplaca se lava con tampón de lavado para eliminar los componentes de suero no reactivos.

Una solución de anticuerpo contra IgG humana conjugado con peroxidasa de rábano picante reconoce los anticuerpos de clase IgG unidos a los antígenos inmovilizados. Después de 30 minutos de incubación, el exceso de conjugado enzimático que no se ha unido específicamente se elimina con tampón de lavado.

Una solución de sustrato cromogénico que contiene TMB se dispensa a los pocillos. Tras 15 minutos de incubación, se detiene el desarrollo del color añadiendo la solución de detención. La solución se vuelve amarilla en este punto. El nivel de color es directamente proporcional a la concentración de anticuerpos de IgG presentes en la muestra original.

4. REACTIVOS, MATERIALES E INSTRUMENTACIÓN

4.1. Reactivos y materiales incluidos en el kit

1. Calibrador (1 vial, 1,2 mL)
Tampón fosfato 0,1 M, NaN_3 <0,1 %, suero humano
CAL1 **REF DCE002/09906-0**
2. Controles (2 viales de 1,2 mL cada uno, listos para usar)
Tampón fosfato 0,1 M, NaN_3 <0,1 %, suero humano
Control negativo **REF DCE045/09901-0**
Control positivo **REF DCE045/09902-0**
3. Diluyente de muestras (1 vial, 100 mL)
Tampón fosfato 0,1 M, NaN_3 <0,1 %
REF DCE053-0
4. Conjugado (1 vial, 15 mL)
Anticuerpo AntiIgG-h conjugado con peroxidasa de rábano picante (HRP), BSA 0,1 %, ProClin™ <0,0015 %
REF DCE002/09902-0
5. Microplaca recubierta (1 microplaca que se puede romper)
Microplaca recubierta con antígenos ANA
REF DCE002/09903-0
6. Sustrato de TMB (1 vial, 15 mL)
H_2O_2-TMB (0,26 g/L) *(evitar el contacto con la piel)*
REF DCE004-0

7. Solución de detención (1 vial, 15 mL)
Ácido sulfúrico 0,15 M *(evitar el contacto con la piel)*
REF DCE005-0
8. Conc. 10X Solución de lavado (1 vial, 50 mL)
Tampón fosfato 0,2 M pH 7,4 **REF DCE054-0**

4.2. Materiales necesarios pero no suministrados
Agua destilada

4.3. Materiales auxiliares e instrumentación
Dispensador automático.
Dispositivo de pipeteo de precisión Lector de microplacas (450 nm, 620-630 nm)

5. ADVERTENCIAS
- Este kit está destinado al uso *in vitro* realizado exclusivamente por profesionales. No es para uso interno o externo en personas ni animales.
- Utilice el equipo de protección personal adecuado cuando trabaje con los reactivos suministrados.
- Siga las prácticas de laboratorio recomendadas (BPL) para manipular productos sanguíneos.
- El material de origen animal utilizado en la preparación del kit se ha obtenido de animales certificados como sanos y la proteína bovina se ha obtenido de países donde no hay infección de EEB, pero estos materiales deben manejarse como potencialmente infecciosos.
- Algunos reactivos contienen pequeñas cantidades de azida sódica (NaN_3) o ProClin™ 300 como conservante. Evite el contacto con la piel o las mucosas.
- Todo el material de origen humano utilizado en la preparación de los reactivos ha sido sometido a pruebas que han dado resultado negativo para los anticuerpos contra el VIH-1 y VIH-2, el HbsAg y el VHC. Sin embargo, ningún método de prueba puede ofrecer una garantía total de ausencia de VIH, VHB, VHC u otros agentes infecciosos. Por lo tanto, los calibradores y los controles deben manejarse de la misma manera que el material potencialmente infeccioso.
- La azida sódica puede ser tóxica si se ingiere o se absorbe a través de la piel o los ojos; además, puede reaccionar con las tuberías de plomo o cobre para formar azidas metálicas potencialmente explosivas. Si elimina los reactivos en un fregadero, lávelos con gran cantidad de agua para evitar la acumulación de azida.
- El sustrato de TMB contiene un irritante que puede ser perjudicial si se inhala, se ingiere o se absorbe a través de la piel. Para evitar lesiones, evite la inhalación, la ingestión o el contacto con la piel y los ojos.
- La solución de detención consiste en una solución diluida de ácido sulfúrico. El ácido sulfúrico es venenoso y corrosivo y puede ser tóxico si se ingiere. Para evitar quemaduras químicas, evite el contacto con la piel y los ojos.
- Evite la exposición del reactivo TMB/H_2O_2 a la luz solar directa, a metales o a oxidantes. No congele la solución.

6. PRECAUCIONES
- Siga estrictamente la secuencia de pasos de pipeteado que se indica en este protocolo. Los datos de rendimiento representados en este documento se obtuvieron utilizando los reactivos específicos indicados en estas instrucciones de uso.
- Todos los reactivos deben conservarse refrigerados entre 2 y 8 °C en su envase original. Las excepciones se indican claramente.
- Deje que todos los componentes del kit y las muestras alcancen la temperatura ambiente (22-28 °C) y mezcle bien antes de usarlos.
- No intercambie componentes del kit procedentes de diferentes lotes. Debe respetarse la fecha de caducidad impresa en las etiquetas de la caja y de los viales. No utilice ningún componente del kit después de su fecha de caducidad.
- Si el usuario utiliza un equipo automatizado, tiene la responsabilidad de asegurarse de que el kit ha sido debidamente probado.
- La eliminación incompleta o imprecisa del líquido de los pocillos podría alterar la precisión del ensayo y/o aumentar el fondo. Para mejorar el rendimiento del kit en sistemas automáticos se recomienda aumentar el número de lavados.
- Es importante que el tiempo de reacción en cada pocillo se mantenga constante para obtener resultados reproducibles. El pipeteo de las muestras no debe prolongarse más de diez minutos para evitar errores en el ensayo. Si se necesitan más de 10 minutos, siga el mismo orden de dispensación. Si se utiliza más de una placa, se recomienda repetir la curva dosis-respuesta en cada placa.
- La adición de la solución de sustrato de TMB inicia una reacción cinética, que finaliza al añadir la solución de detención. Por lo tanto, el sustrato de TMB y la solución de detención deben añadirse en la misma secuencia para eliminar las posibles desviaciones temporales durante la reacción.
- Respete las directrices para realizar el control de calidad en los laboratorios médicos mediante el ensayo de controles y/o sueros combinados.
- Se requiere la máxima precisión en la reconstitución y dispensación de los reactivos.
- No se deben usar en el ensayo muestras contaminadas microbiológicamente, muy lipémicas o hemolizadas.
- Los lectores de placas miden en vertical. No toque el fondo de los pocillos.
- **ADVERTENCIA: el reactivo conjugado está diseñado para garantizar la máxima sensibilidad de la dosis y puede contaminarse con agentes externos si no se utiliza correctamente;** por lo tanto, se recomienda utilizar consumibles desechables (puntas, frascos, bandejas, etc.). Para dosis divididas, tome la cantidad exacta de conjugado necesaria y no vuelva a introducir ningún producto de desecho en el frasco original. Además, **para las dosis dispensadas mediante dispositivos automáticos y semiautomáticos,** antes de utilizar el conjugado, es aconsejable limpiar el sistema de manipulación de fluidos, asegurándose de que los procedimientos de lavado, desproteinización y descontaminación sean eficaces para evitar la contaminación del conjugado. Este procedimiento es muy recomendable cuando el kit se procesa con analizadores que no están provistos de puntas desechables.
  Para ello, DiaMetra proporciona un reactivo de descontaminación independiente para la limpieza de las agujas.

7. ALMACENAMIENTO Y ESTABILIDAD DE LOS REACTIVOS

Almacene el kit entre 2 y 8° C en un lugar oscuro.
- El kit es estable a 2-8 °C hasta la fecha de caducidad indicada en su etiqueta externa.
- Una vez abierto, el kit es estable a 2-8 °C durante 6 meses.
- La solución de lavado diluida es estable durante 30 días a 2-8 °C.

Nota importante: abra la bolsa que contiene la microplaca recubierta solo cuando esté a temperatura ambiente y ciérrela inmediatamente después de su uso.

8. RECOGIDA Y ALMACENAMIENTO DE LAS MUESTRAS

El ensayo debe realizarse usando muestras de suero (tubos de muestras estándar o tubos que contienen gel de separación de suero) o plasma (heparina de litio, heparina de sodio o EDTA de potasio).

Almacenamiento de muestras	Duración
Entre 2 y 8 °C	96 horas
Ciclos de congelación/descongelación	3 ciclos

9. PROCEDIMIENTO

9.1. Preparación de los calibradores (C_1)

Los calibradores están listos para su uso; la concentración del calibrador está impresa en la etiqueta.

9.2. Preparación de la solución de lavado

Diluya el contenido de cada vial del concentrado de solución de lavado tamponada (10X) con agua destilada hasta un volumen final de 500 mL antes de su uso. Para volúmenes más pequeños, respete la relación de dilución de 1:10. La solución de lavado diluida es estable durante 30 días a 2-8 °C.
Es posible que observe la presencia de cristales dentro de la solución de lavado concentrada; en este caso, mezcle a temperatura ambiente hasta la completa disolución de los cristales. Para una mayor precisión, diluya todo el frasco de solución de lavado concentrada a 500 mL, teniendo cuidado también de transferir los cristales enjuagando completamente el frasco y luego mezclando hasta que los cristales se disuelvan completamente.

9.3. Preparación de las muestras

Para la determinación de los anticuerpos ANA, las matrices de muestra preferidas son suero o plasma humanos.
Todas las muestras de suero y plasma deben diluirse previamente con diluyente de muestra 1:100; por ejemplo, 10 µL de muestra pueden diluirse con 990 µL de diluyente de muestra.
No es necesario tomar muestras en ayunas y no se requiere ninguna preparación especial. Obtenga la sangre por venopunción en Vacutainers y separe el suero (tras la formación del coágulo) o el plasma de las células por centrifugación.

Ni la bilirrubina ni la hemólisis tienen un efecto significativo en el procedimiento.

Los controles están listos para su uso.

9.4. Procedimiento

- **Deje que todos los reactivos alcancen la temperatura ambiente (22-28 °C) durante al menos 30 minutos.** Al finalizar el ensayo, almacene inmediatamente los reactivos a 2-8 °C: evite la exposición prolongada a la temperatura ambiente.
- Las tiras de micropocillos recubiertas no utilizadas deben dejarse forma segura en el envoltorio de papel de aluminio que contiene desecante y almacenarse a 2-8 °C.
- Para evitar que se produzca una posible contaminación microbiana y/o química, los reactivos no utilizados nunca se deberán transferir a los viales originales.
- Como es necesario realizar la determinación por duplicado para mejorar la precisión de los resultados de la prueba, prepare dos pocillos para cada punto de la curva de calibración (C_1), dos por cada control, dos para cada muestra y uno para el blanco.

Reactivo	Calibrador	Muestra/ Controles	Blanco
Calibrador C_1	**100 µL**		
Controles		**100 µL**	
Muestra diluida		**100 µL**	
Incube durante 30 minutos a temperatura ambiente (22-28 °C). Retire el contenido de cada pocillo, lave los pocillos 3 veces con 300 µL de solución de lavado diluida. **Nota importante**: en cada paso de lavado, agite ligeramente la placa durante 5 segundos y elimine el exceso de solución golpeando la placa invertida sobre un paño de papel absorbente. **Lavadora automática**: si utiliza un equipo automático, lave los pocillos al menos 5 veces.			
Conjugado	**100 µL**	**100 µL**	
Incube durante 30 minutos a temperatura ambiente (22-28 °C). Retire el contenido de cada pocillo, lave los pocillos 3 veces con 300 µL de solución de lavado diluida. **Lavado**: siga las mismas indicaciones del punto anterior.			
Sustrato de TMB	**100 µL**	**100 µL**	**100 µL**
Incube durante 15 minutos en un lugar oscuro a temperatura ambiente (22-28 °C).			
Solución de detención	**100 µL**	**100 µL**	**100 µL**
Agite suavemente la microplaca. Compare la absorbancia (E) a 450 nm con la obtenida con una longitud de onda de referencia de 620-630 nm o con el blanco en un plazo de 5 minutos.			

10. CONTROL DE CALIDAD

Las prácticas de laboratorio recomendadas (BPL) requieren el uso de muestras de control de calidad en cada serie de ensayos para comprobar el rendimiento del ensayo. Los controles deberán tratarse como muestras desconocidas y los resultados deberán analizarse con métodos estadísticos adecuados.

Los controles incluidos en el kit deberán ser probados como desconocidos y están destinados a ayudar a evaluar la validez de los resultados obtenidos con cada placa de ensayo.

La concentración media de cada nivel de control se documenta en el informe de control de calidad que se incluye en cada kit. Los niveles de concentración media se determinan respecto de varios análisis, los cuales se realizan por duplicado en varios puntos diferentes de cada placa.

DiaMetra recomienda que los usuarios mantengan registros gráficos de los valores de control que se generan con cada ensayo, incluida la media de ejecución, la DE (desviación estándar) y el % CV. Esta información facilitará los ensayos de tendencia de los controles relacionados con el rendimiento de lotes de control actuales e históricos relativos a los datos de control de calidad proporcionados. La tendencia facilitará la identificación de los análisis que generan valores de control significativamente distintos de su intervalo medio.

Al interpretar los datos de control, los usuarios deberán tener en cuenta que este producto fue diseñado y desarrollado como un producto manual. El rango establecido en el certificado de control de calidad deberá ser adecuado para los ensayos que se realizan manualmente y en estricto cumplimiento del procedimiento de ensayo anteriormente descrito. Los profesionales del control de calidad reconocen que, como resultado de las diferencias en las condiciones y en las prácticas, siempre habrá variaciones entre laboratorios en los valores medios y en la precisión de las mediciones de control[7].

11. CÁLCULO DE LOS RESULTADOS

Determine la absorbancia media de cada muestra duplicada.

Para obtener el valor de la muestra: divida la absorbancia media de la muestra por la absorbancia del calibrador, y luego multiplique por la concentración del calibrador, como se indica a continuación:

$$Conc.muestra = \frac{DO\ muestra}{DO\ C1} \times Conc.C1$$

La reactividad no está relacionada de forma lineal con la cantidad de anticuerpos presentes. Aunque un aumento o una disminución de la concentración de anticuerpos da lugar a un aumento o a una disminución de la sensibilidad, el cambio no es proporcional (por ejemplo, duplicar la concentración de anticuerpos no duplica la sensibilidad).

Para lograr una mayor precisión en la determinación de los anticuerpos, se recomienda analizar diluciones seriales de la muestra. La dilución final que proporciona un resultado positivo en la prueba es la concentración de anticuerpos del paciente.

12. METROLOGÍA Y TRAZABILIDAD

El ensayo ANA screen se ha establecido respecto de los estándares de referencia de anticuerpos antinucleares (ANA) mediante el Autoantibody Standardisation Committee (ASC) - Center for Disease Control (CDC) y anti-dsDNA de la OMS 15/174.

13. INTERPRETACIÓN DE LOS RESULTADOS

ANA Screen (UA/mL)	Interpretación
<25	La muestra debe considerarse negativa.
≥ 25	La muestra debe considerarse positiva.

El índice y la interpretación anteriores deben tenerse en cuenta solamente como una guía. Los resultados positivos deben verificarse en función del estado clínico del paciente en su totalidad. Además, cada decisión terapéutica debe tomarse de forma individual para cada paciente.
Se recomienda que cada laboratorio establezca sus propios rangos normales y patológicos de anti-ANA en suero.

14. CARACTERÍSTICAS DE RENDIMIENTO

Se muestran los datos de rendimiento representativos. Los resultados obtenidos en diferentes laboratorios pueden diferir.

14.1. Sensibilidad y especificidad del diagnóstico

La sensibilidad y la especificidad se determinaron según las direcctrices del CLSI EP-24 «Assessment of the Diagnostic Accuracy of Laboratory Tests Using Receiver Operating Characteristic Curves» utilizando 50 muestras negativas y 53 muestras positivas de un mismo lote de reactivos.

		DKO099		Total
		Positivo	Negativo	
Estado real	Positivo	53	0	53
	Negativo	0	50	50
Total		53	50	103

Sensibilidad del diagnóstico: 100 %

Especificidad del diagnóstico: 100 %

14.2. Precisión

La precisión del kit del ANA screen se determinó mediante la realización de un estudio de precisión complejo.

Repetibilidad: Se analizaron un total de 3 muestras de suero utilizando 2 lotes de reactivos en 10 réplicas por muestra, por 3 operadores, una vez al día durante 5 días. A continuación se muestran los datos de un lote representativo:

Resultados de repetibilidad de un lote representativo:

Muestra	n	Result-ados +	Result-ados -	% de resultados +	% de resultados -
Umbral	150	82	68	54,7	45,3
Negativo	150	1	149	0,7	99,3
Positivo	150	150	0	100,0	0,0

A continuación se muestran los resultados de reproducibilidad de los datos combinados de dos lotes:

Muestra	n	Result-ados +	Result-ados -	% de resultados +	% de resultados -
Umbral	300	163	137	54,3	45,7
Negativo	300	3	297	1,0	99,0
Positivo	300	300	0	100,0	0,0

14.3. Estudio en suero-plasma

El estudio de comparación de matrices del ANA screen se realizó para evaluar la diferencia entre los tipos de tubos (tubos separadores de suero (SST), plasma con heparina de litio, plasma con heparina de sodio y plasma con K2 EDTA) frente a las muestras de control (suero rojo, sin aditivo) siguiendo el CLSI GP34-A «Validation and Verification of Tubes for Venous and Capillary Blood. Specimen Collection». Se evaluaron un total de 20 muestras (16 nativas, 4 artificiales).

Tipo de muestra	N.° de muestras	Resultados frente al suero	
		Falsos pos	Falsos neg
SST	20	0	0
K2 EDTA	20	0	0
Heparina de litio	20	0	0
Heparina sódica	20	0	0

14.4. Interferencias

Las sustancias siguientes no interfieren en el ensayo ANA screen cuando las concentraciones presentadas en la tabla siguiente están por debajo del umbral indicado.

Reactivos que pueden interferir	Límite máximo de concentración
Bilirrubina, conjugada	15 mg/dL
Bilirrubina, no conjugada	15 mg/dL
Hemoglobina	200 mg/dL
Proteína total	10 g/dL
Triglicéridos	500 mg/dL

15. LÍMITES DE USO

- Como en cualquier procedimiento diagnóstico, los resultados se deberán interpretar junto con los hallazgos clínicos del paciente y otra información de la que el médico disponga.
- Las características de rendimiento de este ensayo no se han establecido para una población pediátrica.
- Los anticuerpos heterofílicos en el suero humano pueden presentar reacciones con las inmunoglobulinas reactivas, que interfieren con los inmunoensayos *in vitro*[8]. Los pacientes que se exponen habitualmente a animales o a productos de suero animal pueden ser propensos a esta interferencia y puede que se observen valores anómalos.

16. GESTIÓN DE RESIDUOS

Los reactivos deben eliminarse de acuerdo con la normativa local.

17. BIBLIOGRAFÍA

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18. IDENTIFICADOR DE REVISIÓN

Las adiciones o cambios en las instrucciones de uso se han resaltado en gris.

19. RECLAMACIONES SOBRE PRODUCTOS Y ASISTENCIA TÉCNICA

Para un paciente/usuario/tercero en la Unión Europea y en países con un régimen regulatorio similar (Regulation 2017/746/EU on IVD Medical Devices); si, durante el uso de este dispositivo o como resultado de su uso, se ha producido un incidente grave, informe del mismo al fabricante y/o a su representante autorizado y al organismo regulador nacional. Se puede contactar con el fabricante a través de su servicio de atención al cliente o del equipo de asistencia técnica. Los datos de contacto se encuentran a continuación y en el sitio web de la empresa: www.diametra.com.

Ed. 10/2021 **DCM099-7**

Fabricante legal
Dia.Metra Srl
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Correo electrónico: info@diametra.com

DiaMetra	Packaging Information Sheet	Mod. PIS000-1

IVD	DE ES FR GB IT PT	In vitro Diagnostikum Producto sanitario para diagnóstico In vitro Dispositif medical de diagnostic in vitro In vitro Diagnostic Medical Device Dispositivo medico-diagnostico in vitro Dispositivos medicos de diagnostico in vitro		DE ES FR GB IT PT	Hergestellt von Elaborado por Fabriqué par Manufacturer Produttore Produzido por
	DE ES FR GB IT PT	Achtung, Begleitdokumente Precaución, consulte los documentos adjuntos Attention, veuillez consulter les documents d'accompagnement Caution, consult accompanying documents Attenzione, consultare la documentazione allegata Atenção,consultar os documentos de acompanhamento	 yyyy-mm	DE ES FR GB IT PT	Herstellungs datum Fecha de fabricacion Date de fabrication Date of manufacture Data di produzione Data de produção
 yyyy-mm-dd	DE ES FR GB IT PT	Verwendbar bis Establa hasta (usar antes de último día del mes) Utiliser avant (dernier jour du mois indiqué) Use by (last day of the month) Utilizzare prima del (ultimo giorno del mese) Utilizar (antes ultimo dia do mês)		DE ES FR GB IT PT	Biogefährdung Riesco biológico Risque biologique Biological risk Rischio biologico Risco biológico
	DE ES FR GB IT PT	Gebrauchsanweisung beachten Consultar las instrucciones Consulter le mode d'emploi Consult instructions for use Consultare le istruzioni per l'uso Consultar instruções para uso	LOT	DE ES FR GB IT PT	Chargenbezeichnung Codigo de lote Numero de lot Batch code Codice del lotto Codigo do lote
 Σ = xx	DE ES FR GB IT PT	Ausreichend für "n" Tests Contenido suficiente para "n" tests Contenu suffisant pour "n" tests Contains sufficient for "n" tests Contenuto sufficiente per "n" saggi Contém o suficiente para "n" testes	CONT	DE ES FR GB IT PT	Inhalt Contenido del estuche Contenu du coffret Contents of kit Contenuto del kit Conteúdo do kit
Max  Min	DE ES FR GB IT PT	Temperaturbereich Límitaciôn de temperatura Limites de température de conservation Temperature limitation Limiti di temperatura Temperaturas limites de conservação	REF	DE ES FR GB IT PT	Bestellnummer Nûmero de catálogo Référéncès du catalogue Catalogue number Numero di Catalogo Número do catálogo
	DE ES FR GB IT PT	Vor direkter sonneneinstrahlung schützen Mantener alejado de la luz solar Tenir à l'écart de la lumière du soleil Keep away from sunlight Tenere lontano dalla luce solare Mantenha longe da luz solar			

SUGGERIMENTI PER LA RISOLUZIONE DEI PROBLEMI/TROUBLESHOOTING

ERRORE CAUSE POSSIBILI/ SUGGERIMENTI

Nessuna reazione colorimetrica del saggio

- mancata dispensazione del coniugato
- contaminazione del coniugato e/o del Substrato
- errori nell'esecuzione del saggio (es. Dispensazione accidentale dei reagenti in sequenza errata o provenienti da flaconi sbagliati, etc.)

Reazione troppo blanda (OD troppo basse)

- coniugato non idoneo (es. non proveniente dal kit originale)
- tempo di incubazione troppo breve, temperatura di incubazione troppa bassa

Reazione troppo intensa (OD troppo alte)

- coniugato non idoneo (es. non proveniente dal kit originale)
- tempo di incubazione troppo lungo, temperatura di incubazione troppa alta
- qualità scadente dell'acqua usata per la soluzione di lavaggio (basso grado di deionizzazione,)
- lavaggi insufficienti (coniugato non completamente rimosso)

Valori inspiegabilmente fuori scala

- contaminazione di pipette, puntali o contenitori- lavaggi insufficienti (coniugato non completamente rimosso)

CV% intrasaggio elevato

- reagenti e/o strip non portate a temperatura ambiente prima dell'uso
- il lavatore per micropiastre non lava correttamente (suggerimento: pulire la testa del lavatore)

CV% intersaggio elevato

- condizioni di incubazione non costanti (tempo o temperatura)
- controlli e campioni non dispensati allo stesso tempo (con gli stessi intervalli) (controllare la sequenza di dispensazione)
- variabilità intrinseca degli operatori

ERROR POSSIBLE CAUSES / SUGGESTIONS

No colorimetric reaction

- no conjugate pipetted reaction after addition
- contamination of conjugates and/or of substrate
- errors in performing the assay procedure (e.g. accidental pipetting of reagents in a wrong sequence or from the wrong vial, etc.)

Too low reaction (too low ODs)

- incorrect conjugate (e.g. not from original kit)
- incubation time too short, incubation temperature too low

Too high reaction (too high ODs)

- incorrect conjugate (e.g. not from original kit)
- incubation time too long, incubation temperature too high
- water quality for wash buffer insufficient (low grade of deionization)
- insufficient washing (conjugates not properly removed)

Unexplainable outliers

- contamination of pipettes, tips or containers

insufficient washing (conjugates not properly removed) too high within-run

- reagents and/or strips not pre-warmed to CV% Room Temperature prior to use
- plate washer is not washing correctly (suggestion: clean washer head)

too high between-run - incubation conditions not constant (time, CV % temperature)

- controls and samples not dispensed at the same time (with the same intervals) (check pipetting order)
- person-related variation

ERROR / POSIBLES CAUSAS / SUGERENCIAS

No se produce ninguna reacción colorimétrica del ensayo

- no se ha dispensado el conjugado
- contaminación del conjugado y/o del substrato
- errores en la ejecución del ensayo (p. ej., dispensación accidental de los reactivos en orden incorrecto o procedentes de frascos equivocados, etc.)

Reacción escasa (DO demasiado bajas)

- conjugado no idóneo (p. ej., no procedente del kit original)
- tiempo de incubación demasiado corto, temperatura de incubación demasiado baja

Reacción demasiado intensa (DO demasiado altas)

- conjugado no idóneo (p. ej., no procedente del kit original)
- tiempo de incubación demasiado largo, temperatura de incubación demasiado alta
- calidad escasa del agua usada para la solución de lavado (bajo grado de desionización)
- lavados insuficientes (el conjugado no se ha retirado completamente)

Valores inexplicablemente fuera de escala

- contaminación de pipetas, puntas o contenedores- lavados insuficientes (el conjugado no se ha retirado completamente)

CV% intraensayo elevado

- los reactivos y/o tiras no se encontraban a temperatura ambiente antes del uso
- el lavador de microplacas no funciona correctamente (sugerencia: limpiar el cabezal del lavador)

CV% interensayo elevado

- condiciones de incubación no constantes (tiempo o temperatura)
- controles y muestras no dispensados al mismo tiempo (con los mismos intervalos) (controlar la secuencia de dispensación)
- variación en función de los operadores

ERREUR CAUSES POSSIBLES / SUGGESTIONS

Aucune réaction colorimétrique de l'essai

- non distribution du conjugué
- contamination du conjugué et/ou du substrat
- erreurs dans l'exécution du dosage (par ex., distribution accidentelle des réactifs dans le mauvais ordre ou en provenance des mauvais flacons, etc.)

Réaction trop faible (DO trop basse)

- conjugué non approprié (par ex., ne provenant pas du coffret original)
- temps d'incubation trop court, température d'incubation trop basse

Réaction trop intense (DO trop élevée)

- conjugué non approprié (par ex., ne provenant pas du coffret original)
- temps d'incubation trop long, température d'incubation trop élevée
- mauvaise qualité de l'eau utilisée pour la solution de lavage (bas degré de déionisation)
- lavages insuffisants (conjugué non entièrement éliminé)

Valeurs inexplicablement hors plage

- contamination des pipettes, embouts ou récipients - lavages insuffisants (conjugué non entièrement éliminé)

CV% intra-essai élevé

- les réactifs et/ou les bandes n'ont pas atteint la température ambiante avant usage
- le laveur de microplaques ne lave pas correctement (suggestion : nettoyer la tête du laveur)

CV% inter-essai élevé

- conditions d'incubation non constantes (temps ou température)
- contrôles et échantillons non distribués en même temps (avec les mêmes intervalles) (contrôler l'ordre de distribution)
- variabilité intrinsèque des opérateurs

CE

DiaMetra
care for quality

DCM095-7
Ed. 09/2018

Anti dsDNA IgG

per analisi di routine

Determinazione quantitativa degli anticorpi IgG contro il dsDNA nel siero o plasma umano.

IVD

LOT Vedere etichetta esterna

2°C 8°C

Σ = 96 test

REF DKO095

DESTINAZIONE D'USO

Il kit Anti dsDNA IgG è un test immunoenzimatico (ELISA) indiretto in fase solida sviluppato per la determinazione quantitativa degli anticorpi di classe IgG diretti contro il dsDNA, presenti nel siero o plasma umano.
Il kit Anti dsDNA IgG è destinato al solo uso di laboratorio.

1. SIGNIFICATO CLINICO

Il test Anti dsDNA IgG è usato per la diagnosi iniziale del Systemic Lupus Erythematosus (SLE) e per le differenti diagnosi delle malattie dello SLE.
Oltre alla determinazione di alti titoli di Anticorpi Antinucleo (ANA), la determinazione degli autoanticorpi contro dsDNA è uno dei criteri ACR (American College of Rheumatology) per la diagnosi del Systemic Lupus Erythematosus (SLE). La determinazione della concentrazione degli anticorpi può essere utilizzata per monitorare il successo terapeutico e predire eventuali attacchi della malattia (SLE)

2. PRINCIPIO DEL METODO

Il test Anti dsDNA IgG si basa sul legame degli anticorpi IgG del siero o plasma con il dsDNA adsorbito sulla micropiastra. Nella prima fase gli anticorpi nei calibratori, nei controlli o nei campioni prediluiti dei pazienti si legano sulla superficie interna dei pozzetti. Dopo un periodo di incubazione la micropiastra viene lavata con tampone di lavaggio per la rimozione delle componenti del siero che non hanno reagito. Nella seconda fase una soluzione di immunoglobuline anti-human IgG coniugate con perossidasi di rafano (HRP) riconosce gli anticorpi di classe IgG legati agli antigeni dsDNA immobilizzati. Dopo un periodo di incubazione, l'eccesso di coniugato enzimatico che non si è legato specificamente viene rimosso mediante tampone di lavaggio. Infine si aggiunge ai pozzetti una soluzione substrato cromogenica contenente TMB. Dopo 15 minuti di incubazione si blocca lo sviluppo del colore mediante aggiunta della soluzione di stop. Il colore della soluzione diventa giallo. La quantità di colore sviluppato è direttamente proporzionale alla concentrazione di anticorpi IgG anti dsDNA presenti nel campione originale.
La concentrazione di anticorpi IgG anti dsDNA presenti nel campione è calcolata sulla base di una curva di calibrazione.

3. REAGENTI, MATERIALI E STRUMENTAZIONE

3.1. Reattivi e materiali forniti nel kit

1. Calibrators (5 flaconi, 1,2 mL ciascuno, pronti all'uso)
Tampone fosfato 0,1 M, NaN_3 < 0,1%, siero umano

CAL0	**REF DCE002/9506-0**
CAL1	**REF DCE002/9507-0**
CAL2	**REF DCE002/9508-0**
CAL3	**REF DCE002/9509-0**
CAL4	**REF DCE002/9510-0**

2. Controls (2 flaconi, 1,2 mL ciascuno, pronti all'uso)
Tampone fosfato 0,1 M NaN_3 < 0,1%, siero umano

Controllo Negativo	**REF DCE045/9501-0**
Controllo Positivo	**REF DCE045/9502-0**

3. Sample diluent (1 flacone, 100 mL)
Tampone fosfato 0,1 M NaN_3 < 0,1%
REF DCE053/9553-0

4. Conjugate (1 flacone, 15 mL)
Anti h-IgG coniugato con perossidasi di rafano (HRP), BSA 0,1%, Proclin < 0,0015% **REF DCE002/9502-0**

5. Coated Microplate (1 micropiastra breakable)
dsDNA adsorbito su micropiastra
REF DCE002/9503-0

6. TMB Substrate (1 flacone, 15 mL)
H_2O_2-TMB (0,26 g/L) *(evitare il contatto con la pelle)*
REF DCE004-0

7. Stop Solution (1 flacone, 15 mL)
Acido solforico 0,15M *(evitare il contatto con la pelle)*
REF DCE005-0

8. 10X Conc. Wash Solution (1 flacone, 50 mL)
Tampone fosfato 0,2M pH 7.4 **REF DCE054-0**

3.2. Reattivi necessari non forniti nel kit

Acqua distillata.

3.3. Materiale e strumentazione ausiliare

Dispensatori automatici.
Lettore per micropiastre (450 nm, 620-630 nm).

Note
Conservare tutti i reattivi a 2÷8°C, al riparo dalla luce. Aprire la busta del Reattivo 5 (Coated Microplate) solo dopo averla riportata a temperatura ambiente e chiuderla subito dopo il prelievo delle strip da utilizzare; una volta aperta è stabile fino alla data di scadenza del kit.

4. AVVERTENZE

- Questo test kit è per uso in vitro, da eseguire da parte di personale esperto. Non per uso interno o esterno su esseri Umani o Animali.
- Usare i previsti dispositivi di protezione individuale mentre si lavora con i reagenti forniti.
- Seguire le Buone Pratiche di Laboratorio (GLP) per la manipolazione di prodotti derivati da sangue.
- Tutti i reattivi di origine umana usati nella preparazione dei reagenti sono stati testati e sono risultati negativi per la presenza di anticorpi anti-HIV 1&2, per HbsAg e per anticorpi anti-HCV. Tuttavia nessun test offre la certezza completa dell'assenza di HIV, HBV, HCV o di altri agenti infettivi. Pertanto, i Calibratori ed i Controlli devono essere maneggiati come materiali potenzialmente infettivi.
- Materiali di origine animale usati per la preparazione di questo kit sono stati ottenuti da animali sani e le proteine bovine sono state ottenute da paesi non affetti da BSE, ma comunque questi materiali dovrebbero essere usati come potenzialmente contagiosi.
- Alcuni reagenti contengono piccole quantità di Sodio Azide (NaN_3) o di Proclin 300^R come conservante. Evitare il contatto con la pelle e le mucose.
- La Sodio Azide può essere tossica se ingerita o assorbita attraverso la cute o gli occhi; inoltre, può reagire con le tubature di piombo o rame formando azidi metalliche potenzialmente esplosive. Se si usa un lavandino per eliminare i reagenti, lasciar scorrere grandi quantità di acqua per prevenire la formazione di azidi.
- Il TMB Substrato contiene un irritante, che può essere dannoso se inalato, ingerito o assorbito attraverso la cute. Per prevenire lesioni, evitare l'inalazione, l'ingestione o il contatto con la cute e con gli occhi.
- La Stop Solution è costituita da una soluzione di acido solforico diluito. L'acido solforico è velenoso e corrosivo e può essere tossico se ingerito. Per prevenire possibili ustioni chimiche, evitare il contatto con la cute e con gli occhi.
- Evitare l'esposizione del reagente TMB/H_2O_2 a luce solare diretta, metalli o ossidanti. Non congelare la soluzione.

5. PRECAUZIONI

- Si prega di attenersi rigorosamente alla sequenza dei passaggi indicata in questo protocollo. I risultati presentati qui sono stati ottenuti usando specifici reagenti elencati in queste Istruzioni per l'Uso.
- Tutti i reattivi devono essere conservati a temperatura controllata di 2-8°C nei loro contenitori originali. Eventuali eccezioni sono chiaramente indicate. I reagenti sono stabili fino alla data di scadenza se conservati e trattati seguendo le istruzioni fornite.
- Prima dell'uso lasciare tutti i componenti dei kit e i campioni a temperatura ambiente (22-28°C) e mescolare accuratamente.
- Non scambiare componenti dei kit di lotti diversi. Devono essere osservate le date di scadenza riportate sulle etichette della scatola e di tutte le fiale. Non utilizzare componenti oltre la data di scadenza.
- **ATTENZIONE**: **il reagente Coniugato è stato studiato per garantire la massima sensibilità di dosaggio, e pertanto, se non opportunamente usato, può essere contaminato da agenti esterni**; si raccomanda pertanto di utilizzare consumabili (puntali, flaconi, vaschette ecc.) usa e getta. Per dosaggi frazionati, prelevare l'esatta quantità di coniugato necessaria ed evitare di re-introdurre l'eventuale scarto nel flacone originale. Inoltre, **per dosaggi effettuati con l'ausilio di strumentazione automatica e semi-automatica**, si consiglia, prima di utilizzare il coniugato, di effettuare uno step di pulizia della fluidica, assicurandosi che le procedure di lavaggio, deproteinizzazione e decontaminazione siano efficaci nell'evitare la contaminazione del coniugato; questa procedura è fortemente raccomandata quando il kit è processato con analizzatori non dotati di puntali monouso.
  A tale scopo Dia.Metra rende disponibile separatamente un reattivo decontaminante per il lavaggio degli aghi.
- Qualora si utilizzi strumentazione automatica, è responsabilità dell'utilizzatore assicurarsi che il kit sia stato opportunamente validato.
- Un lavaggio incompleto o non accurato dei pozzetti può causare una scarsa precisione e/o un'elevato background. Per migliorare le prestazioni del kit su strumentazione automatica, si consiglia di aumentare il numero di lavaggi.
- Per la riproducibilità dei risultati, è importante che il tempo di reazione di ogni pozzetto sia lo stesso. Per evitare il time shifting durante la dispensazione degli reagenti, il tempo di dispensazione dei pozzetti non dovrebbe estendersi oltre i 10 minuti. Se si protrae oltre, si raccomanda di seguire lo stesso ordine di dispensazione. Se si utilizza più di una piastra, si raccomanda di ripetere la curva di calibrazione in ogni piastra.
- L'aggiunta del TMB Substrato dà inizio ad una reazione cinetica, la quale termina con l'aggiunta della Stop Solution. L'aggiunta del TMB Substrato e della Stop Solution deve avvenire nella stessa sequenza per evitare tempi di reazione differenti.
- Osservare le linee guida per l'esecuzione del controllo di qualità nei laboratori clinici testando controlli e/o pool di sieri.
- Osservare la massima precisione nella ricostituzione e dispensazione dei reagenti.
- Non usare campioni microbiologicamente contaminati, altamente lipemici o emolizzati.

- I lettori di micropiastre leggono l'assorbanza verticalmente. Non toccare il fondo dei pozzetti.

6. PROCEDIMENTO

6.1. Preparazione dei Calibratori (C_0…C_4)

I Calibratori sono pronti all'uso, sono tarati in accordo allo standard internazionale WHO Wo/80, ed hanno approssimativamente le seguenti concentrazioni:

	C_0	C_1	C_2	C_3	C_4
IU/mL	0	12.5	25	50	200

NB: le concentrazioni esatte dei Calibratori sono lotto-dipendenti, e sono riportate sul Certificato di Analisi e sulle etichette.
Una volta aperti sono stabili per 6 mesi a 2÷8°C.

6.2. Preparazione del campione

Le matrici di elezione per la determinazione degli anticorpi anti dsDNA sono siero o plasma umano. **Tutti i campioni di siero o plasma devono essere prediluiti 1:100 con sample diluent; per esempio 10 µL di campione possono essere diluiti con 990 µL di sample diluent**.
I pazienti non devono necessariamente essere a digiuno e non è richiesta alcuna preparazione particolare. Raccogliere il sangue mediante prelievo venoso in un vacutainer e separare il siero (dopo la formazione del coagulo) o il plasma dalle cellule per centrifugazione.
I campioni possono essere conservati refrigerati a 2-8 °C per almeno 5 giorni. Per periodi di conservazione più lunghi fino a 6 mesi i campioni dovrebbero essere congelati a -20°C. Per evitare ripetuti congelamenti e scongelamenti, i campioni dovrebbero essere aliquotati. Emolisi e presenza di bilirubina non hanno effetti evidenti sulla determinazione.
I Controlli sono pronti all'uso.

6.3. Preparazione della Wash Solution

Prima dell'uso, diluire il contenuto di ogni fiala di soluzione di lavaggio tamponata concentrata (10x) con acqua distillata fino al volume di 500 mL. Per preparare volumi minori rispettare il rapporto di diluizione di 1:10. La soluzione di lavaggio diluita è stabile a 2-8°C per almeno 30 giorni.
Nella wash solution concentrata è possibile osservare la presenza di cristalli; in tal caso agitare a temperatura ambiente fino a completa dissoluzione dei cristalli; per una maggiore precisione diluire tutto il flacone della soluzione di lavaggio concentrata a 500 mL, avendo cura di trasferire anche i cristalli, poi agitare fino a completa dissoluzione dei cristalli.

6.4. Procedimento

- **Portare tutti i reagenti a temperatura ambiente (22-28°C) per almeno 30 minuti.** Al termine del dosaggio riporre immediatamente tutti i reagenti a 2-8°C: evitare lunghi periodi a temperatura ambiente.
- Le strisce di pozzetti non utilizzate devono essere rimesse immediatamente nella busta richiudibile contenente il materiale essicante e conservate a 2-8°C.
- Per evitare potenziali contaminazioni microbiche e/o chimiche non rimettere i reagenti inutilizzati nei flaconi originali.
- Al fine di aumentare l'accuratezza dei risultati del test è necessario operare in doppio, allestendo due pozzetti per ogni punto della curva di calibrazione (C_0-C_4), due per ogni Controllo, due per ogni Campione e uno per il Bianco.

Reagente	Calibratore	Campione /Controlli	Bianco
Calibratore C_0-C_4	**100 µL**		
Controlli		**100 µL**	
Campione diluito		**100 µL**	
Incubare 45 minuti a 37°C. Rimuovere il contenuto da ogni pozzetto: lavare i pozzetti per 3 volte con 300 µL di wash solution diluita. **Nota importante**: ad ogni step di lavaggio, agitare delicatamente la piastra per 5 secondi e successivamente rimuovere l'eccesso di soluzione di lavaggio sbattendo delicatamente la micropiastra capovolta su fogli di carta assorbente. **Lavaggi automatici**: se si utilizza strumentazione automatica effettuare almeno 5 lavaggi.			
Conjugate	**100 µL**	**100 µL**	
Incubare 45 minuti a 37°C. Rimuovere il contenuto da ogni pozzetto; lavare i pozzetti per 3 volte con 300 µL di wash solution diluita (se si usa strumentazione automatica effettuare almeno 5 lavaggi). **Lavaggi**: seguire le stesse indicazioni del punto precedente.			
TMB Substrate	**100 µL**	**100 µL**	**100 µL**
Incubare 15 minuti al buio a temperatura ambiente (22-28°C).			
Stop Solution	**100 µL**	**100 µL**	**100 µL**
Agitare delicatamente la piastra. Leggere l'assorbanza (E) a 450 nm contro una lunghezza d'onda di riferimento di 620-630 nm oppure contro il Bianco entro 5 minuti.			

7. RISULTATI

7.1. Curva di calibrazione

Per il test Anti dsDNA IgG il metodo di scelta per il trattamento dei risultati è una elaborazione 4 parametri con assi lin-log per densità ottica e concentrazione rispettivamente. Inoltre si possono utilizzare un'approssimazione spline e coordinate log-log. Tuttavia si raccomanda di utilizzare una curva Lin-Log.

Innanzitutto occorre calcolare la media delle densità ottiche relative ai calibratori. Utilizzare un foglio di carta lin-log e tracciare le densità ottiche medie di ogni calibratore verso la rispettiva concentrazione. Disegnare la curva che approssima nel modo migliore tutti i punti di calibrazione. I punti dei calibratori possono anche essere collegati con segmenti di linea retta. La concentrazione dei campioni incogniti può essere determinata per interpolazione dalla curva di calibrazione.

8. VALORI DI RIFERIMENTO

Intervalli di normalità per il test Anti dsDNA IgG:

	Anti dsDNA IgG (IU/mL)
Normale	< 25
Elevato	> 25

È importante tenere presente che la determinazione di un range di valori attesi in un dato metodo per una popolazione "normale" è dipendente da molteplici fattori, quali la specificità e sensibilità del metodo in uso, e la popolazione in esame. Perciò ogni laboratorio dovrebbe considerare i range indicati dal Fabbricante come un'indicazione generale e produrre range di valori attesi propri basati sulla popolazione indigena dove il laboratorio risiede.
I risultati positivi dovrebbero essere verificati relativamente allo stato clinico del paziente. Inoltre, ogni decisione relativa alla terapia dovrebbe essere presa individualmente. Si raccomanda che ogni laboratorio stabilisca i suoi propri intervalli normale e patologico di anticorpi Anti dsDNA sierici.

9. PARAMETRI CARATTERISTICI

9.1. Specificità

Test di correlazione contro un analogo kit commerciale di riferimento, effettuati su 100 sieri (50 positivi e 50 negativi) hanno mostrato una specificità > 99%.

9.2. Sensibilità

Test di correlazione contro un analogo kit commerciale di riferimento, effettuati su 100 sieri (50 positivi e 50 negativi) hanno mostrato una sensibilità > 99%.

9.3. Limite di Rilevabilità

La minor concentrazione di anticorpi IgG diretti contro il dsDNA che può essere distinta dal Calibratore 0 è di 0,135 IU/mL.

10. DISPOSIZIONI PER LO SMALTIMENTO

I reagenti devono essere smaltiti in accordo con le leggi locali.

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CE

DiaMetra
care for quality

DCM095-7
Ed. 09/2018

Anti dsDNA IgG

for routine analysis

Quantitative determination of IgG class antibodies against dsDNA in human serum or plasma

IVD | **LOT** See external label | 2°C – 8°C | Σ = 96 tests | **REF DKO095**

INTENDED USE

Anti dsDNA IgG kit is an indirect solid phase enzyme immunometric assay (ELISA) designed for the quantitative measurement of IgG class antibodies directed against dsDNA in human serum or plasma.
Anti dsDNA IgG is intended for laboratory use only.

1. CLINICAL SIGNIFICANCE

Anti dsDNA IgG test is used for initial diagnosis of Systemic Lupus Erythematosus (SLE) and for diagnosis of SLE different diseases. Besides the determination of high titers of antinuclear antibody (ANA), the determination of autoantibodies against dsDNA is one of the ACR criteria (American College of Rheumatology) for the diagnosis of Systemic Lupus Erythematosus (SLE). The determination of the concentration of antibodies can be used to monitor treatment success and predict possible attacks of the disease (SLE)

2. PRINCIPLE

Anti dsDNA IgG test is based on the binding of serum or plasma IgG antibodies on dsDNA coated on the microplates. In the first step the antibodies in calibrators, controls or prediluted patient samples bind into the inner surface of the wells. After an incubation the microplate is washed with a wash buffer for removing non-reactive serum components.
In the second step an anti human IgG horseradish peroxidase conjugated solution recognizes the IgG class antibodies bound to the immobilized dsDNA antigens. After an incubation any excess of enzyme conjugate, which is not specifically bound, is washed away with the wash buffer.
Then a chromogenic substrate solution containing TMB is dispensed into the wells. After 15 minutes of incubation the color development is stopped by adding the stop solution. The solution color changes into yellow. The amount of color is directly proportional to the concentration of the Anti dsDNA IgG antibodies present in the original sample.
The concentration of the anti dsDNA IgG antibodies in the sample are calculated through a calibration curve.

3. REAGENTS, MATERIALS AND INSTRUMENTATION

3.1. Reagents and materials supplied in the kit

1. Calibrators (5 vials, 1.2 mL each, ready to use)
Phosphate buffer 0.1 M, NaN_3 < 0.1%, human serum

CAL0	**REF DCE002/9506-0**
CAL1	**REF DCE002/9507-0**
CAL2	**REF DCE002/9508-0**
CAL3	**REF DCE002/9509-0**
CAL4	**REF DCE002/9510-0**

2. Controls (2 vials, 1.2 mL each, ready to use)
Phosphate buffer 0.1 M, NaN_3 < 0.1%, human serum

Negative Control	**REF DCE045/9501-0**
Positive Control	**REF DCE045/9502-0**

3. Sample Diluent (1 vial, 100 mL)
Phosphate buffer 0.1 M, NaN_3 < 0.1%
REF DCE053/9553-0

4. Conjugate (1 vial, 15 mL)
Anti h-IgG conjugated with peroxidase, BSA 0.1%, Proclin < 0.0015% **REF DCE002/9502-0**

5. Coated Microplate (1 breakable microplate)
Microplate coated with dsDNA **REF DCE002/9503-0**

6. TMB Substrate (1 vial, 15 mL)
H_2O_2 -TMB (0,26 g/L) *(avoid any skin contact)*
REF DCE004-0

7. Stop Solution (1 vial, 15 mL)
Sulphuric acid 0.15M *(avoid any skin contact)*
REF DCE005-0

8. 10X Conc. Wash Solution (1 vial, 50 mL)
Phosphate buffer 0.2M pH 7.4 **REF DCE054-0**

3.2. Reagents necessary not supplied

Distilled water.

3.3. Auxiliary materials and instrumentation

Automatic dispenser.
Microplates reader (450 nm, 620-630 nm)

Notes

Store all reagents between 2÷8°C in the dark.
Open the bag of reagent 5 (Coated Microplate) only when it is at room temperature and close it immediately after use; once opened, it is stable until expiry date of the kit.

4. WARNINGS

- This kit is intended for in vitro use by professional persons only. Not for internal or external use in Humans or Animals.
- Use appropriate personal protective equipment while working with the reagents provided.
- Follow Good Laboratory Practice (GLP) for handling blood products.
- All human source material used in the preparation of the reagents has been tested and found negative for antibody to HIV 1&2, HbsAg, and HCV. No test method however can offer complete assurance that HIV, HBV, HCV or other infectious agents are absent. Therefore, Calibrators and Controls should be handled in the same manner as potentially infectious material.
- Material of animal origin used in the preparation of the kit has been obtained from animals certified as healthy and the bovine protein has been obtained from countries not infected by BSE, but these materials should be handled as potentially infectious.
- Some reagents contain small amounts of Sodium Azide (NaN_3) or Proclin 300^R as preservatives. Avoid the contact with skin or mucosa.
- Sodium Azide may be toxic if ingested or absorbed through the skin or eyes; moreover it may react with lead or copper plumbing to form potentially explosive metal azides. If you use a sink to remove the reagents, allow scroll through large amounts of water to prevent azide build-up.
- The TMB Substrate contains an irritant, which may be harmful if inhaled, ingested or absorbed through the skin. To prevent injury, avoid inhalation, ingestion or contact with skin and eyes.
- The Stop Solution consists of a diluted sulphuric acid solution. Sulphuric acid is poisonous and corrosive and can be toxic if ingested. To prevent chemical burns, avoid contact with skin and eyes.
- Avoid the exposure of reagent TMB/H_2O_2 to directed sunlight, metals or oxidants. Do not freeze the solution.

5. PRECAUTIONS

- Please adhere strictly to the sequence of pipetting steps provided in this protocol. The performance data represented here were obtained using specific reagents listed in this Instruction For Use.
- All reagents should be stored refrigerated at 2-8°C in their original container. Any exceptions are clearly indicated. The reagents are stable until the expiry date when stored and handled as indicated.
- Allow all kit components and specimens to reach room temperature (22-28°C) and mix well prior to use.
- Do not interchange kit components from different lots. The expiry date printed on box and vials labels must be observed. Do not use any kit component beyond their expiry date.
- **WARNING: the conjugate reagent is designed to ensure maximum dose sensitivity and may be contaminated by external agents if not used properly;** therefore, it is recommended to use disposable consumables (tips, bottles, trays, etc.). For divided doses, take the exact amount of conjugate needed and do not re-introduce any waste product into the original bottle. In addition, **for doses dispensed with the aid of automatic and semi-automatic devices,** before using the conjugate, it is advisable to clean the fluid handling system, ensuring that the procedures of washing, deproteinization and decontamination are effective in avoiding contamination of the conjugate; this procedure is highly recommended when the kit is processed using analyzers which are not equipped with disposable tips.
  For this purpose, Dia.Metra supplies a separate decontamination reagent for cleaning needles.
- If you use automated equipment, the user has the responsibility to make sure that the kit has been appropriately tested.
- The incomplete or inaccurate liquid removal from the wells could influence the assay precision and/or increase the background. To improve the performance of the kit on automatic systems is recommended to increase the number of washes.
- It is important that the time of reaction in each well is held constant for reproducible results. Pipetting of samples should not extend beyond ten minutes to avoid assay drift. If more than 10 minutes are needed, follow the same order of dispensation. If more than one plate is used, it is recommended to repeat the dose response curve in each plate
- Addition of the TMB Substrate solution initiates a kinetic reaction, which is terminated by the addition of the Stop Solution. Therefore, the TMB Substrate and the Stop Solution should be added in the same sequence to eliminate any time deviation during the reaction.
- Observe the guidelines for performing quality control in medical laboratories by assaying controls and/or pooled sera.
- Maximum precision is required for reconstitution and dispensation of the reagents.
- Samples microbiologically contaminated, highly lipemeic or haemolysed should not be used in the assay.
- Plate readers measure vertically. Do not touch the bottom of the wells.

6. PROCEDURE

6.1. Preparation of the Calibrators (C_0…C_4)

Calibration curve is ready to use and is calibrated against International Standard WHO Wo/80. The Calibrators have approximately the following concentration:

	C_0	C_1	C_2	C_3	C_4
IU/mL	0	12.5	25	50	200

NB: Calibrators concentration is lot-depending; exact concentrations are indicated on Certificate of Analysis and labels.

Once opened, the Calibrators are stable 6 months at 2-8°C.

6.2. Preparation of the Sample

For determination of anti dsDNA antibodies, human serum or plasma are the preferred sample matrixes.

All serum and plasma samples have to be prediluted with sample diluent 1:100; for example 10 µL of sample may be diluted with 990 µL of sample diluent.

The patients need not to be fasting, and no special preparations are necessary. Collect blood by venipuncture into vacutainers and separate serum (after clot formation) or plasma from the cells by centrifugation.

Samples may be stored refrigerated at 2-8°C for at least 5 days. For longer storage of up to six months samples should be stored frozen at -20°C. To avoid repeated thawing and freezing the samples should be aliquoted.

Neither Bilirubin nor Hemolysis have significant effect on the procedure.

The Controls are ready to use.

6.3. Preparation of the Wash Solution

Dilute the contents of each vial of the buffered wash solution concentrate (10x) with distilled water to a final volume of 500 mL prior to use. For smaller volumes respect the 1:10 dilution ratio. The diluted wash solution is stable for 30 days at 2-8°C.

In concentrated wash solution is possible to observe the presence of crystals; in this case mix at room temperature until the complete dissolution of crystals; for greater accuracy, dilute the whole bottle of concentrated wash solution to 500 mL, taking care to transfer completely the crystals, then mix until crystals are completely dissolved.

6.4. Procedure

- **Allow all reagents to reach room temperature (22-28°C) for at least 30 minutes.** At the end of the assay, store immediately the reagents at 2-8°C: avoid long exposure to room temperature.
- Unused coated microwell strips should be released securely in the foil pouch containing desiccant and stored at 2-8°C.
- To avoid potential microbial and/or chemical contamination, unused reagents should never be transferred into the original vials.
- As it is necessary to perform the determination in duplicate in order to improve accuracy of the test results, prepare two wells for each point of the calibration curve (C_0-C_4), two for each Control, two for each sample, one for Blank.

Reagent	Calibrator	Sample/ Controls	Blank
Calibrator C_0-C_4	**100 µL**		
Controls		**100 µL**	
Diluted Sample		**100 µL**	
Incubate 45 minutes at 37°C. Remove the content from each well; wash the wells 3 times with 300 µL of diluted wash solution. **Important note**: during each washing step, gently shake the plate for 5 seconds and remove excess solution by tapping the inverted plate on an absorbent paper towel. **Automatic washer**: if you use automated equipment, wash the wells at least 5 times.			
Conjugate	**100 µL**	**100 µL**	
Incubate 45 minutes at 37°C. Remove the content from each well, wash the wells 3 times with 300 µL of diluted wash solution (if you use automated equipment, wash the wells at least 5 times). **Washing**: follow the same indications of the previous point.			
TMB Substrate	**100 µL**	**100 µL**	**100 µL**
Incubate 15 minutes in the dark at room temperature (22-28°C).			
Stop Solution	**100 µL**	**100 µL**	**100 µL**
Shake the microplate gently. Read the absorbance (E) at 450 nm against a reference wavelength of 620-630 nm or against Blank within 5 minutes.			

7. RESULTS

7.1. Calibration curve

For the test Anti dsDNA IgG a 4-Parameter-Fit with lin-log coordinates for optical density and concentration is the data reduction method of choice. Smoothed-Spline Approximation and log-log coordinates are also suitable. However we reccomen using a Lin-Log curve.

First calculate the averaged optical densities for each calibrator well. Use lin-log graph paper and plot the averaged optical density of each calibrator versus the concentration. Draw the best fitting curve approximating the path of all calibrator points. The calibrator points may also be connected with straight line segments. The concentration of unknowns may then be estimated from the calibration curve by interpolation.

8. REFERENCE VALUES

Following ranges have been established with the Anti dsDNA IgG tests:

	Anti dsDNA IgG (IU/mL)
Normal	< 25
Elevated	> 25

Please pay attention to the fact that the determination of a range of expected values for a "normal" population in a given method is dependent on many factors, such as specificity and sensitivity of the method used and type of population under investigation. Therefore each laboratory should consider the range given by the Manufacurer as a general indication and produce their own range of expected values based on the indigenous population where the laboratory works.
Positive results should be verified concerning the entire clinical status of the patient. Also every decision for therapy should be taken individually.
It is recommended that each laboratory establishes its own normal and pathological ranges of serum Anti dsDNA.

9. PERFORMANCE AND CHARACTERISTICS

9.1. Specificity

Comparison test against a commercial reference kit, performed on 100 sera (50 of them positive sera and 50 negative sera) showed a specificity > 99%.

9.2. Sensitivity

Comparison test against a commercial reference kit, performed on 100 sera (50 of them positive sera and 50 negative sera) showed a sensibility > 99%..

9.3. Detection limit

The lowest concentration of Anti dsDNA IgG that can be distinguished from zero Calibrator is 0.135 IU/mL.

10. WASTE MANAGEMENT

Reagents must be disposed off in accordance with local regulations.

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CE

DiaMetra
care for quality

DCM095-7
Ed. 09/2018

Anti dsDNA IgG

para análisis de rutina

Determinación cuantitativa de los anticuerpos IgG contra el ADN bicatenario (dsDNA) en suero o plasma humano.

IVD | **LOT** Ver etiqueta externa | 2°C – 8°C | Σ = 96 ensayos | **REF DKO095**

USO PREVISTO

El kit Anti dsDNA IgG es un ensayo inmunoenzimático indirecto en fase sólida (ELISA) desarrollado para la determinación cuantitativa de los anticuerpos de clase IgG directos contra el dsDNA, presentes en el suero o plasma humano.
El kit Anti dsDNA IgG está destinado al uso en laboratorio exclusivamente.

1. SIGNIFICADO CLÍNICO

El ensayo Anti dsDNA IgG se usa para el diagnóstico inicial del lupus eritematoso sistémico (SLE) y para los distintos diagnósticos de las enfermedades del SLE.
Además de la determinación de altos títulos de anticuerpos antinúcleo (ANA), la determinación de los autoanticuerpos contra dsDNA es uno de los criterios ACR (*American College of Rheumatology*) para el diagnóstico del lupus eritematoso sistémico (SLE). La determinación de la concentración de los anticuerpos puede usarse para controlar el éxito terapéutico y predecir eventuales ataques de la enfermedad (SLE).

2. PRINCIPIO DEL MÉTODO

El ensayo Anti dsDNA IgG se basa en la unión de los anticuerpos IgG del suero o plasma con el dsDNA absorbido en la microplaca. Los anticuerpos en los calibradores, en los controles o en las muestras prediluidas de los pacientes se unen a la superficie interna de los pocillos. Tras de una incubación, la microplaca se lava con un tampón de lavado para retirar los componentes del suero que no han reaccionado. Una solución de inmunoglobulinas anti-IgG humana conjugadas con peroxidasa de rábano reconoce los anticuerpos de clase IgG unidos a los antígenos dsDNA inmovilizados. Tras de una incubación, el exceso de conjugado enzimático que no se ha unido específicamente se retira mediante el tampón de lavado. Se añade a los pocillos una solución de substrato cromogénico que contiene TMB. Tras 15 minutos de incubación se bloquea el desarrollo del color mediante la adición de la solución de parada. El color de la solución se vuelve amarillo. La cantidad del color desarrollado es directamente proporcional a la concentración de anticuerpos IgG anti dsDNA presentes en la muestra original.

3. REACTIVOS, MATERIALES E INSTRUMENTACIÓN

3.1. Reactivos y materiales suministrados en el kit

1. Calibradores (5 frascos, 1,2 mL cada uno, listo para usar)

Tampón fosfato 0,1 M, NaN_3 < 0,1%, suero humano

CAL0	**REF DCE002/9506-0**
CAL1	**REF DCE002/9507-0**
CAL2	**REF DCE002/9508-0**
CAL3	**REF DCE002/9509-0**
CAL4	**REF DCE002/9510-0**

2. Controles (2 frascos, 1,2 mL cada uno, listo para usar)

Tampón fosfato 0,1 M NaN_3 < 0,1%, suero humano

Control negativo	**REF DCE045/9501-0**
Control positivo	**REF DCE045/9502-0**

3. Diluyente de muestra (1 frasco, 100 mL)
Tampón fosfato 0,1 M NaN_3 < 0,1%
REF DCE053/9553-0

4. Conjugado (1 frasco, 15 mL)
Anti h-IgG conjugado con peroxidasa da rabano (HRP), BSA 0,1%, Proclin < 0,0015%
REF DCE002/9502-0

5. Microplaca recubierta (1 microplaca rompible)
dsDNA absorbido en la microplaca
REF DCE002/9503-0

6. Substrato TMB (1 frasco, 15 mL)
H_2O_2-TMB (0,26 g/L) *(evitar el contacto con la piel)*
REF DCE004-0

7. Solución de parada (1 frasco, 15 mL)
Ácido sulfúrico 0,15M *(evitar el contacto con la piel)*
REF DCE005-0

8. Solución de lavado conc. 10X (1 frasco, 50 mL)
Tampón fosfato 0,2M pH 7.4 **REF DCE054-0**

3.2. Reactivos necesarios no suministrados en el kit

Agua destilada.

3.3. Material e instrumentación auxiliares

Dispensadores automáticos.
Lector de microplacas (450 nm, 620-630 nm).

Nota

Conservar todos los reactivos a 2÷8°C, protegidos de la luz.

Abrir la bolsa del reactivo 5 (microplaca recubierta) solo cuando se encuentre a temperatura ambiente y cerrarla inmediatamente después de extraer las tiras que se vayan a utilizar; una vez abierta, permanece estable hasta la fecha de caducidad del kit.

4. ADVERTENCIAS

- Este kit de ensayo está previsto para usarse in vitro y por personal experto. No es para uso interno o externo en humanos o animales.
- Usar los equipos de protección individual previstos al trabajar con los reactivos suministrados.
- Siga las Buenas Prácticas de Laboratorio (GLP) en el manejo de las muestras sanguíneas y sus derivados.
- Todos los reactivos de origen humano usados en la preparación de los Calibradores y de los controles se han comprobado y han resultado negativos para la presencia de anticuerpos anti-VIH, para HbsAg y para anticuerpos anti-VHC. Sin embargo, ningún ensayo ofrece seguridad absoluta de la ausencia de VIH, VHB, VHC o de otros agentes infecciosos. Por lo tanto, los Calibradores y los controles positivo y negativo deben manipularse como material potencialmente infeccioso.
- Materiales de origen animal utilizadas para la elaboración de este kit se obtuvieron a partir de animales sanos y de las proteínas de bovino se obtuvieron de los países no afectados por la EEB, pero estos materiales se debe utilizar como potencialmente infecciosos.
- Algunos reactivos contienen pequeñas cantidades de Azida de Sodio (NaN_3) o Proclin 300^R como conservante. Evite el contacto con la piel y las mucosas.
- La Azida de Sodio, usada como conservante, puede ser tóxica si se ingiere o se absorbe a través de la piel o de los ojos; además, puede reaccionar con las tuberías de plomo o cobre formando azidas metálicas potencialmente explosivas. Dejar que corra gran cantidad de agua, si se usa un lavabo para eliminar los reactivos, para prevenir la formación de azidas.
- El cromógeno TMB contiene un irritante que puede ser dañino si se inhala, se ingiere o se absorbe a través de la piel. Para prevenir lesiones, evitar la inhalación, la ingestión o el contacto con la piel y con los ojos.
- La solución de parada está formada por una solución de ácido sulfúrico diluido. El ácido sulfúrico es venenoso y corrosivo, y puede ser tóxico si se ingiere. Para prevenir posibles quemaduras químicas, evitar el contacto con la piel y con los ojos.
- Evite la exposición de los reactivos TMB/H_2O_2 a la luz solar directa, metales u oxidantes. No congelar la solución.

5. PRECAUCIONES

- Respetar rigurosamente la secuencia de los pasos indicados en este protocolo.
- Todos los reactivos deben conservarse a una temperatura controlada de 2-8 °C en sus recipientes originales. Todas las excepciones están claramente marcados.
- Antes del uso, esperar hasta que todos los componentes del kit y las muestras se encuentren a temperatura ambiente (22-28°C) y mezclar cuidadosamente.
- No mezclar componentes de kits de lotes distintos. Se debe observar la fecha de caducidad indicada en la etiqueta de la caja y de todas las ampollas. No usar componentes después de la fecha de caducidad.
- **ATENCIÓN**: **se ha estudiado el reactivo conjugado para garantizar la máxima sensibilidad en la determinación y, por lo tanto, si no se usa adecuadamente, podría contaminarse por agentes externos**; se recomienda utilizar consumibles (puntas, frascos, bandejas, etc.) desechables. Para determinaciones fraccionadas, tomar la cantidad necesaria exacta de conjugando y evitar volver a introducir los posibles restos en el frasco original. Además, **para determinaciones realizadas con la ayuda de instrumentación automática y semiautomática**, se recomienda, antes de usar el conjugado, realizar una fase de limpieza de la fluídica, asegurándose de que los procedimientos de lavado, desproteinización y descontaminación resulten eficaces para evitar la contaminación del conjugado; este procedimiento se recomienda especialmente cuando el kit se procesa con analizadores que no están dotados de puntas monouso. Para tal fin, Dia.Metra pone a su disposición por separado un reactivo descontaminante para el lavado de las agujas.
- Si utiliza un equipo automático, es responsabilidad del usuario asegurar que el equipo ha sido debidamente validada.
- Un lavado incompleto o impreciso y la aspiración insuficiente del líquido de los pocillos ELISA pueden causar una precisión pobre y/o un elevado fondo. Para mejorar el rendimiento del kit en los sistemas automatizados, se recomienda aumentar el número de lavados.
- Para la reproducibilidad de los resultados, es importante que el tiempo de reacción sea igual para cada pocillo. El tiempo de dispensación de los pocillos no debe superar los 10 minutos; si se prolongara más allá de los 10 minutos, respétese el orden de dispensación. si utiliza más de una placa, se recomienda repetir la curva de calibración en cada plato.
- Al añadir el substrato TMB inicia una reacción cinética que termina al agregar la solución de parada. Tanto el sustrato como la solución de parada deben agregarse en la misma secuencia para evitar diferentes tiempos de reacción.
- Observar las directrices para la ejecución del control de calidad en los laboratorios clínicos al comprobar controles y/o pool de sueros.

- Observar la máxima precisión en la reconstitución y dispensación de los reactivos.
- No use muestras con contaminación microbiana, altamente lipémicas o hemolizadas.
- Los lectores de microplacas leen las DO verticalmente, por tanto no debe tocarse el fondo de los pocillos.

6. PROCEDIMIENTO

6.1. Preparación de los Calibradores (C_0…C_4)

Los Calibradores son listos para usar, son calibrados frente al estándar internacional WHO Wo/80 y tienen aproximadamente las siguientes concentraciones:

	C_0	C_1	C_2	C_3	C_4
IU/mL	0	12.5	25	50	200

Los niveles exactos se indican en las etiquetas y en el Certificado de análisis para cada lote específico.

Una vez abiertos, los Calibradores permanecen estables durante 6 meses a 2-8°C.

6.2. Preparación de la muestra

Las matrices de elección para la determinación de los anticuerpos anti dsDNA son suero o plasma humano. **Todas las muestras de suero o plasma deben prediluirse 1:100 con diluyente de muestras**. por ejemplo, 10 µL de muestra pueden diluirse con 990 µL de diluyente de muestras.

Los pacientes no deben necesariamente estar en ayunas y no se requiere ninguna preparación especial. Recoger la sangre mediante extracción venosa en un Vacutainer y separar el suero (tras la formación del coágulo) o el plasma de las células mediante centrifugado.

Las muestras pueden conservarse refrigeradas a 2-8 °C durante al menos 5 días. Para períodos de conservación más largos, hasta 6 meses, las muestras deberán congelarse a -20°C. Para evitar congelaciones y descongelaciones repetidas, las muestras deberían dividirse en alícuotas. La hemólisis y la presencia de bilirrubina no tienen efectos evidentes en la determinación.

Los Controles son listo para usar.

6.3. Preparación de la solución de lavado

Antes del uso, diluir el contenido de cada ampolla de solución de lavado tamponada concentrada (10x) con agua destilada hasta un volumen de 500 mL. Para preparar volúmenes menores, respetar la relación de dilución de 1:10. La solución de lavado diluida se mantiene estable a 2-8 °C durante al menos 30 días.

En la solución de lavado concentrada es posible observar la presencia de cristales. En ese caso, agitar a temperatura ambiente hasta que los cristales se disuelvan por completo. Para una mayor precisión, diluir todo el frasco de la solución de lavado concentrada a 500 mL, teniendo cuidado para transferir también los cristales y, a continuación, agitar hasta que se disuelvan por completo.

6.4. Procedimiento

- **Esperar hasta que todos los reactivos se encuentren a temperatura ambiente (22-28°C) durante al menos 30 minutos.** Al final del ensayo inmediatamente poner todos los reactivos a 2-8°C para evitar largos periodos a temperatura ambiente.
- Las tiras de pocillos no utilizados se deben guardar de inmediato
- Las tiras de pocillos no utilizados se deben guardar de inmediato en la bolsa desechable que contiene desecantes y almacenarse a 2-8°C.
- Para evitar la contaminación microbiana y/o química no regrese porciones de reactivos no usados en los viales originales.
- Para aumentar la precisión de los resultados de la prueba es necesario trabajar en duplicado: preparar dos pocillos para cada punto de la curva de calibración (C_0-C_4), dos para cada control, dos para cada muestra, uno para el blanco.

Reactivo	Calibrador	Muestra/ Controles	Blanco
Calibrador C_0-C_4	**100 µL**		
Controles		**100 µL**	
Muestra diluida		**100 µL**	
Incubar 45 minutos a 37°C. Retirar el contenido de cada pocillo y lavar los pocillos 3 veces con 300 µL de solución de lavado diluida. **Nota importante**: agite suavemente la placa durante 5 segundos en cada paso del lavado. Después del último lavado asegúrese haber eliminado completamente la solución de lavado de los pozos, invierta la placa y golpéela repetidas veces contra una servilleta de papel absorbente. **Lavados automático**: si está utilizando una lavadora automática, lavar los pocillos al menos 5 veces.			
Conjugado	**100 µL**	**100 µL**	
Incubar 45 minutos a 37°C . Retirar el contenido de cada pocillo y lavar los pocillos tres veces con 300 µL de solución de lavado diluida. **Lavados**: siga las mismas instrucciones del punto anterior.			
Substrato TMB	**100 µL**	**100 µL**	**100 µL**
Incubar 15 minutos en la oscuridad a temperatura ambiente (22-28 °C).			
Solución de parada	**100 µL**	**100 µL**	**100 µL**
Agitar la placa con cuidado. Leer la absorbancia (E) a 450 nm frente una segunda lectura de referencia a 620-630 nm o frente al blanco entre 5 minutos.			

7. RESULTADOS

7.1. Curva de calibración

Para Anti dsDNA IgG, el método de elección para el tratamiento de los resultados es un procesamiento de 4 parámetros con ejes lin-log para la densidad óptica y la concentración respectivamente. Además, se pueden usar una aproximación spline y coordenadas log-log. Sin embargo, se recomienda usar una curva Lin-Log.
En primer lugar, calcular la media de las densidades ópticas relativas a los calibradores. Usar una hoja de papel lin-log y trazar las densidades ópticas medias de cada calibrador frente a la respectiva concentración. Dibujar la curva que mejor se aproxime a todos los puntos de calibración. Los puntos de los calibradores también pueden unirse con segmentos de línea recta. La concentración de las muestras desconocidas puede determinarse por interpolación de la curva de calibración.

8. VALORES DE REFERENCIA

Se han determinado los siguientes intervalos de normalidad con el ensayo Anti dsDNA IgG:

	Anti dsDNA IgG (IU/mL)
Normal	< 25
Alto	> 25

Es importante señalar que la determinación de un rango de valores esperados en un método dado para una población "normal" depende de muchos factores, tales como la especificidad y sensibilidad del método en uso, y la población en estudio. Por lo tanto, cada laboratorio debe considerar el intervalo especificado por el fabricante como una guía general y producir su propio rango de valores calculados en base al estadístico obtenido por el laboratorio, donde reside la población local.
Los resultados positivos deben verificarse con relación al estado clínico del paciente. Además, cada decisión relativa a la terapia debe tomarse individualmente. Se recomienda que cada laboratorio establezca sus propios intervalos normal y patológico de anticuerpos anti- dsDNA séricos.

9. PARÁMETROS CARACTERÍSTICOS

9.1. Especificidad

Ensayos de correlación frente a un kit similar de referencia disponible en el mercado, realizados con 100 sueros (50 positivos y 50 negativos) han mostrado una especificidad > 99%.

9.2. Sensibilidad

Ensayos de correlación frente a un kit similar de referencia disponible en el mercado, realizados con 100 sueros (50 positivos y 50 negativos) han mostrado una sensibilidad > 99%.

9.3. Límite de detección

La concentración mínima de anti dsDNA IgG que puede distinguirse del Calibrador cero es de 0,135 IU/mL.

10. DISPOSICIONES PARA LA ELIMINACIÓN

Los reactivos deben eliminarse de acuerdo con las leyes locales.

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DiaMetra	Packaging Information Sheet	Mod. PIS000-1

IVD	DE ES FR GB IT PT	In vitro Diagnostikum Producto sanitario para diagnóstico In vitro Dispositif medical de diagnostic in vitro In vitro Diagnostic Medical Device Dispositivo medico-diagnostico in vitro Dispositivos medicos de diagnostico in vitro		DE ES FR GB IT PT	Hergestellt von Elaborado por Fabriqué par Manufacturer Produttore Produzido por
	DE ES FR GB IT PT	Achtung, Begleitdokumente Precaución, consulte los documentos adjuntos Attention, veuillez consulter les documents d'accompagnement Caution, consult accompanying documents Attenzione, consultare la documentazione allegata Atenção,consultar os documentos de acompanhamento	 yyyy-mm	DE ES FR GB IT PT	Herstellungs datum Fecha de fabricacion Date de fabrication Date of manufacture Data di produzione Data de produção
 yyyy-mm-dd	DE ES FR GB IT PT	Verwendbar bis Establa hasta (usar antes de último día del mes) Utiliser avant (dernier jour du mois indiqué) Use by (last day of the month) Utilizzare prima del (ultimo giorno del mese) Utilizar (antes ultimo dia do mês)		DE ES FR GB IT PT	Biogefährdung Riesco biológico Risque biologique Biological risk Rischio biologico Risco biológico
	DE ES FR GB IT PT	Gebrauchsanweisung beachten Consultar las instrucciones Consulter le mode d'emploi Consult instructions for use Consultare le istruzioni per l'uso Consultar instruções para uso	LOT	DE ES FR GB IT PT	Chargenbezeichnung Codigo de lote Numero de lot Batch code Codice del lotto Codigo do lote
 Σ = xx	DE ES FR GB IT PT	Ausreichend für "n" Tests Contenido suficiente para "n" tests Contenu suffisant pour "n" tests Contains sufficient for "n" tests Contenuto sufficiente per "n" saggi Contém o suficiente para "n" testes	CONT	DE ES FR GB IT PT	Inhalt Contenido del estuche Contenu du coffret Contents of kit Contenuto del kit Conteúdo do kit
Max  Min	DE ES FR GB IT PT	Temperaturbereich Límitaciôn de temperatura Limites de température de conservation Temperature limitation Limiti di temperatura Temperaturas limites de conservação	REF	DE ES FR GB IT PT	Bestellnummer Nûmero de catálogo Référéncès du catalogue Catalogue number Numero di Catalogo Número do catálogo
	DE ES FR GB IT PT	Vor direkter sonneneinstrahlung schützen Mantener alejado de la luz solar Tenir à l'écart de la lumière du soleil Keep away from sunlight Tenere lontano dalla luce solare Mantenha longe da luz solar			

SUGGERIMENTI PER LA RISOLUZIONE DEI PROBLEMI/TROUBLESHOOTING

ERRORE CAUSE POSSIBILI/ SUGGERIMENTI

Nessuna reazione colorimetrica del saggio

- mancata dispensazione del coniugato
- contaminazione del coniugato e/o del Substrato
- errori nell'esecuzione del saggio (es. Dispensazione accidentale dei reagenti in sequenza errata o provenienti da flaconi sbagliati, etc.)

Reazione troppo blanda (OD troppo basse)

- coniugato non idoneo (es. non proveniente dal kit originale)
- tempo di incubazione troppo breve, temperatura di incubazione troppa bassa

Reazione troppo intensa (OD troppo alte)

- coniugato non idoneo (es. non proveniente dal kit originale)
- tempo di incubazione troppo lungo, temperatura di incubazione troppa alta
- qualità scadente dell'acqua usata per la soluzione di lavaggio (basso grado di deionizzazione,)
- lavaggi insufficienti (coniugato non completamente rimosso)

Valori inspiegabilmente fuori scala

- contaminazione di pipette, puntali o contenitori- lavaggi insufficienti (coniugato non completamente rimosso)

CV% intrasaggio elevato

- reagenti e/o strip non portate a temperatura ambiente prima dell'uso
- il lavatore per micropiastre non lava correttamente (suggerimento: pulire la testa del lavatore)

CV% intersaggio elevato

- condizioni di incubazione non costanti (tempo o temperatura)
- controlli e campioni non dispensati allo stesso tempo (con gli stessi intervalli) (controllare la sequenza di dispensazione)
- variabilità intrinseca degli operatori

ERROR POSSIBLE CAUSES / SUGGESTIONS

No colorimetric reaction

- no conjugate pipetted reaction after addition
- contamination of conjugates and/or of substrate
- errors in performing the assay procedure (e.g. accidental pipetting of reagents in a wrong sequence or from the wrong vial, etc.)

Too low reaction (too low ODs)

- incorrect conjugate (e.g. not from original kit)
- incubation time too short, incubation temperature too low

Too high reaction (too high ODs)

- incorrect conjugate (e.g. not from original kit)
- incubation time too long, incubation temperature too high
- water quality for wash buffer insufficient (low grade of deionization)
- insufficient washing (conjugates not properly removed)

Unexplainable outliers

- contamination of pipettes, tips or containers

insufficient washing (conjugates not properly removed) too high within-run

- reagents and/or strips not pre-warmed to CV% Room Temperature prior to use
- plate washer is not washing correctly (suggestion: clean washer head)

too high between-run - incubation conditions not constant (time, CV % temperature)

- controls and samples not dispensed at the same time (with the same intervals) (check pipetting order)
- person-related variation

ERROR / POSIBLES CAUSAS / SUGERENCIAS

No se produce ninguna reacción colorimétrica del ensayo

- no se ha dispensado el conjugado
- contaminación del conjugado y/o del substrato
- errores en la ejecución del ensayo (p. ej., dispensación accidental de los reactivos en orden incorrecto o procedentes de frascos equivocados, etc.)

Reacción escasa (DO demasiado bajas)

- conjugado no idóneo (p. ej., no procedente del kit original)
- tiempo de incubación demasiado corto, temperatura de incubación demasiado baja

Reacción demasiado intensa (DO demasiado altas)

- conjugado no idóneo (p. ej., no procedente del kit original)
- tiempo de incubación demasiado largo, temperatura de incubación demasiado alta
- calidad escasa del agua usada para la solución de lavado (bajo grado de desionización)
- lavados insuficientes (el conjugado no se ha retirado completamente)

Valores inexplicablemente fuera de escala

- contaminación de pipetas, puntas o contenedores- lavados insuficientes (el conjugado no se ha retirado completamente)

CV% intraensayo elevado

- los reactivos y/o tiras no se encontraban a temperatura ambiente antes del uso
- el lavador de microplacas no funciona correctamente (sugerencia: limpiar el cabezal del lavador)

CV% interensayo elevado

- condiciones de incubación no constantes (tiempo o temperatura)
- controles y muestras no dispensados al mismo tiempo (con los mismos intervalos) (controlar la secuencia de dispensación)
- variación en función de los operadores

ERREUR CAUSES POSSIBLES / SUGGESTIONS

Aucune réaction colorimétrique de l'essai

- non distribution du conjugué
- contamination du conjugué et/ou du substrat
- erreurs dans l'exécution du dosage (par ex., distribution accidentelle des réactifs dans le mauvais ordre ou en provenance des mauvais flacons, etc.)

Réaction trop faible (DO trop basse)

- conjugué non approprié (par ex., ne provenant pas du coffret original)
- temps d'incubation trop court, température d'incubation trop basse

Réaction trop intense (DO trop élevée)

- conjugué non approprié (par ex., ne provenant pas du coffret original)
- temps d'incubation trop long, température d'incubation trop élevée
- mauvaise qualité de l'eau utilisée pour la solution de lavage (bas degré de déionisation)
- lavages insuffisants (conjugué non entièrement éliminé)

Valeurs inexplicablement hors plage

- contamination des pipettes, embouts ou récipients - lavages insuffisants (conjugué non entièrement éliminé)

CV% intra-essai élevé

- les réactifs et/ou les bandes n'ont pas atteint la température ambiante avant usage
- le laveur de microplaques ne lave pas correctement (suggestion : nettoyer la tête du laveur)

CV% inter-essai élevé

- conditions d'incubation non constantes (temps ou température)
- contrôles et échantillons non distribués en même temps (avec les mêmes intervalles) (contrôler l'ordre de distribution)
- variabilité intrinsèque des opérateurs

CE

DiaMetra
care for quality

DCM091-9
Ed. 05/2022

Anti PR-3 (c-ANCA)

per analisi di routine

Determinazione semiquantitativa degli anticorpi di classe IgG contro la proteinasi 3 (PR-3) nel siero o nel plasma umano

IVD

LOT
Vedere l'etichetta esterna

2 °C – 8 °C

Σ = 96 test

REF DKO091

1. SCOPO PREVISTO

Per uso diagnostico *in vitro*
Per uso professionale in laboratorio

Il dosaggio Anti PR-3 (c-ANCA) è un dispositivo diagnostico manuale *in vitro* destinato alla determinazione quantitativa di anticorpi di classe IgG diretti contro la proteinasi 3 (PR-3) nel siero o nel plasma umano. I risultati devono essere impiegati in associazione ad altri dati clinici e di laboratorio come ausilio nella diagnosi di determinate vasculiti autoimmuni, come ad esempio la granulomatosi di Wegener.

2. RILEVANZA CLINICA

La proteinasi 3 (PR-3 o PR3) è una piccola serina proteasi localizzata nei granuli primari e nelle vescicole secretorie del citoplasma dei neutrofili. La PR3 è immagazzinata nei granuli citoplasmatici dei neutrofili a riposo, mentre l'espressione della proteina sulla superficie cellulare aumenta durante l'attivazione dei neutrofili e durante l'apoptosi[1]. La PR3 si trova anche nelle trappole extracellulari dei neutrofili (NET), che consistono in cromatina rilasciata dai neutrofili come difesa dagli agenti patogeni.

Gli autoanticorpi contro la PR3 sono frequentemente rilevati in una classe specifica di vasculiti autoimmuni, nota come vasculite associata (AAV) agli autoanticorpi anti-citoplasma dei neutrofili (ANCA). Queste vasculiti sono caratterizzate dalla presenza di autoanticorpi contro i componenti citoplasmatici dei neutrofili, che possono avere una colorazione citoplasmatica (c-ANCA) o perinucleare (p-ANCA)[1-3].

Il meccanismo dell'autoimmunità per gli antigeni ANCA non è ancora stato valutato. I neutrofili attivati possono subire l'apoptosi creando i NET, che sono coinvolti nell'induzione dell'infiammazione. La persistenza dei NET durante l'infezione può portare a una risposta autoimmune[3,4]. L'aumento dell'espressione di MPO e PR3 nei neutrofili e nei monociti può influenzare la risposta autoimmune patogena che porta all'attivazione dei neutrofili correlata ad ANCA[3].

Le vasculiti ANCA-associate sono classificate come vasculiti dei piccoli vasi (SVV), che colpiscono prevalentemente i piccoli vasi, le arterie intraparenchimali, le arteriole, i capillari e le venule[5]. Le vasculiti ANCA-associate sono vasculiti necrotizzanti caratterizzate dalla mancanza di depositi immunitari nelle pareti dei vasi, che causano condizioni come la poliangioite microscopica (MPA) e la granulomatosi con poliangioite (GPA, precedentemente nota come sindrome di Wegner).

La frequenza del sierotipo (MPO o PR3), la manifestazione clinica e la distribuzione geografica delle vasculiti ANCA-associate variano, suggerendo un ruolo dell'ambiente e del background genetico dei pazienti affetti da questa categoria di patologie[1,3,6].

3. PRINCIPIO DEL METODO

Il test Anti PR-3 (c-ANCA) è un dosaggio immunometrico enzimatico indiretto (ELISA) in cui l'anticorpo anti-PR3 presente nel campione si lega alla proteinasi 3 neutrofila umana rivestita nella micropiastra (fase solida). Eventuali anticorpi presenti nei calibratori, nei controlli o nei campioni prediluiti dei pazienti si legano alla superficie interna dei pozzetti. Dopo un'incubazione di 60 minuti, la micropiastra viene lavata per rimuovere i componenti non reattivi del siero. Nella seconda fase, una soluzione coniugata con perossidasi di rafano (HRP) anti-IgG umane si lega agli anticorpi di classe IgG legati agli antigeni immobilizzati. Dopo un'ulteriore fase di incubazione, il coniugato enzimatico in eccesso, che non è specificamente legato, viene lavato via.

Una soluzione di substrato cromogenico contenente TMB viene quindi erogata nei pozzetti. Dopo un'ulteriore fase di incubazione, l'enzima HRP nella parte legata reagisce con il substrato (H_2O_2) e il substrato TMB, sviluppando un colore blu che cambia in giallo quando viene aggiunta la soluzione di arresto (H_2SO_4). L'intensità del colore è direttamente proporzionale alla concentrazione di anticorpi IgG nel campione.

La concentrazione di anticorpi IgG anti-PR3 nel campione viene calcolata mediante una curva di calibrazione.

4. REAGENTI, MATERIALI E STRUMENTAZIONE

Reagenti e materiali forniti nel kit

1. Calibratori (5 fiale, 1,2 mL ciascuno)

Tampone fosfato 0,1 M, NaN_3 < 0,1%, siero umano

CAL0	**REF DCE002/09106-0**
CAL1	**REF DCE002/09107-0**
CAL2	**REF DCE002/09108-0**
CAL3	**REF DCE002/09109-0**
CAL4	**REF DCE002/09110-0**

2. Controlli (2 fiale, 1,2 mL ciascuna)

Tampone fosfato 0,1 M, NaN_3 < 0,1%, siero umano

Controllo negativo	**REF DCE045/09101-0**
Controllo positivo	**REF DCE045/09102-0**

3. Diluente per campione (1 fiala, 100 mL)

Tampone fosfato 0,1 M, NaN_3 < 0,1%

REF DCE053-0

4. Coniugato (1 fiala, 15 mL)

Coniugato anti h-IgG con perossidasi di rafano (HRP), BSA 0,1%, contiene ProClin™ **REF DCE002/09102-0**

5. Micropiastra rivestita (1 micropiastra frangibile)

Micropiastra rivestita con proteinasi 3 neutrofila umana

REF DCE002/09103-0

6. Substrato TMB (1 fiala, 15 mL)

H_2O_2-TMB 0,26 g/L *(evitare qualsiasi contatto con la pelle)*

REF DCE004-0

7. Soluzione di arresto (1 fiala, 15 mL)

Acido solforico 0,15 M *(evitare qualsiasi contatto con la pelle)* **REF DCE005-0**

8. Soluzione di lavaggio conc. 10X (1 fiala, 50 mL)

Tampone fosfato 0,2 M, pH 7,4, contiene ProClin™

REF DCE054-0

Materiali richiesti ma non forniti

Acqua distillata

Materiali e strumentazione ausiliari

Erogatore automatico
Pipette di precisione
Lettore di micropiastre (450 nm, 620-630 nm)

5. AVVERTENZE

- Questo kit è destinato all'uso *in vitro* esclusivamente da parte di professionisti. Non per uso interno o esterno in esseri umani o animali.
- Utilizzare adeguati dispositivi di protezione individuale mentre si lavora con i reagenti forniti.
- Seguire le buone prassi di laboratorio (GLP, Good Laboratory Practice) per la manipolazione di emoderivati.
- Tutto il materiale di origine umana utilizzato nella preparazione dei reagenti è stato testato e risultato negativo per gli anticorpi dell'HIV 1 e 2, HbsAg e HCV. Nessun metodo di prova, tuttavia, può offrire la completa garanzia che HIV, HBV, HCV o altri agenti infettivi siano assenti. Pertanto, i calibratori e i controlli devono essere manipolati allo stesso modo del materiale potenzialmente infettivo.
- Il materiale di origine animale utilizzato nella preparazione del kit è stato ottenuto da animali certificati come sani e la proteina bovina è stata ottenuta da Paesi non infettati dalla BSE, ma tali materiali devono essere trattati come potenzialmente infettivi.
- Alcuni reagenti contengono piccole quantità di azoturo di sodio (NaN_3) o ProClin™ 300 come conservante. Evitare il contatto con pelle o mucose.
- ProClin™ 300: Classificazione CLP
  Sens. cutanea 1

  Indicazioni di pericolo
  H317: può provocare una reazione allergica cutanea.

  Indicazioni di precauzione
  P261: evitare di respirare la polvere/i fumi/i gas/la nebbia/i vapori/gli aerosol.
  P272: gli indumenti da lavoro contaminati non devono essere portati fuori dal luogo di lavoro.
  P280: indossare guanti/indumenti protettivi/proteggere gli occhi/proteggere il viso.
  P302/352 IN CASO DI CONTATTO CON LA PELLE: lavare con abbondante acqua/sapone e acqua.
  P321: trattamento specifico (vedere istruzioni su questa etichetta)
  P333/313: in caso di irritazione o eruzione cutanea: consultare un medico.
- L'azoturo di sodio può essere tossico se ingerito o assorbito attraverso la pelle o gli occhi; inoltre, può reagire con le tubature di piombo o rame per formare azoturi metallici potenzialmente esplosivi. Se si utilizza un lavandino per rimuovere i reagenti, lavare con abbondante acqua per evitare l'accumulo di azoturi.
- Il substrato TMB contiene un irritante, che può essere dannoso se inalato, ingerito o assorbito per via cutanea. Per prevenire lesioni, evitare l'inalazione, l'ingestione o il contatto con pelle e occhi.
- La soluzione di arresto consiste in una soluzione diluita di acido solforico. L'acido solforico è velenoso e corrosivo e può essere tossico se ingerito. Per prevenire ustioni chimiche, evitare il contatto con pelle e occhi.
- Evitare l'esposizione del reagente TMB/H_2O_2 a luce solare diretta, metalli o ossidanti. Non congelare la soluzione.

6. PRECAUZIONI

- Attenersi rigorosamente alla sequenza dei passaggi di pipettaggio forniti in questo protocollo. I dati sulle prestazioni qui rappresentati sono stati ottenuti utilizzando i reagenti specifici elencati in queste istruzioni per l'uso.
- Tutti i reagenti devono essere conservati refrigerati a 2-8 °C nel contenitore originale. Tutte le eccezioni sono chiaramente indicate.
- Lasciare che tutti i componenti del kit e i campioni raggiungano la temperatura ambiente (22-28 °C) e mescolare bene prima dell'uso.
- Non scambiare i componenti di kit di lotti diversi. La data di scadenza stampata sulle etichette della confezione e delle fiale deve essere rispettata. Non utilizzare alcun componente del kit dopo la data di scadenza.
- Se si utilizzano apparecchiature automatizzate, l'utente ha la responsabilità di assicurarsi che il kit sia stato adeguatamente testato.
- La rimozione incompleta o imprecisa del liquido dai pozzetti potrebbe influenzare la precisione del dosaggio e/o aumentare il background. Per migliorare le prestazioni del kit sui sistemi automatici, si raccomanda di aumentare il numero di lavaggi.

- È importante che il tempo di reazione in ogni pozzetto sia mantenuto costante per ottenere risultati riproducibili. Il pipettaggio dei campioni non deve andare oltre i dieci minuti per evitare deviazioni del dosaggio. Se sono necessari più di 10 minuti, seguire lo stesso ordine di erogazione. Se si utilizza più di una piastra, si raccomanda di ripetere la curva dose-risposta in ogni piastra.
- L'aggiunta della soluzione di substrato TMB avvia una reazione cinetica, che viene terminata dall'aggiunta della soluzione di arresto. Pertanto, il substrato TMB e la soluzione di arresto devono essere aggiunti nella stessa sequenza per eliminare qualsiasi deviazione temporale durante la reazione.
- Osservare le linee guida per l'esecuzione del controllo di qualità nei laboratori medici analizzando i controlli e/o i sieri in pool.
- La massima precisione è richiesta per la ricostituzione e l'erogazione dei reagenti.
- I campioni microbiologicamente contaminati, altamente lipemici o emolizzati non devono essere utilizzati nel dosaggio.
- I lettori di piastre misurano verticalmente. Non toccare il fondo dei pozzetti.
- **AVVERTENZA: il reagente coniugato è progettato per garantire la massima sensibilità per la dose e può essere contaminato da agenti esterni se non utilizzato correttamente;** pertanto, si raccomanda di utilizzare materiali di consumo monouso (puntali, flaconi, vassoi, ecc.). Per le dosi divise, prelevare l'esatta quantità di coniugato necessaria e non reintrodurre alcun prodotto di scarto nel flacone originale. Inoltre, **per le dosi erogate con l'ausilio di dispositivi automatici e semiautomatici,** prima di utilizzare il coniugato, è consigliabile pulire il sistema per la gestione dei fluidi, assicurandosi che le procedure di lavaggio, deproteinizzazione e decontaminazione siano efficaci per evitare la contaminazione del coniugato; questa procedura è altamente raccomandata quando il kit viene elaborato con analizzatori non dotati di puntali monouso.
  A tale scopo, DiaMetra fornisce un reagente di decontaminazione separato per la pulizia degli aghi.

7. CONSERVAZIONE E STABILITÀ DEI REAGENTI

Conservare il kit a 2-8 °C, al buio.

- Il kit è stabile a 2-8 °C fino alla data di scadenza indicata sull'etichetta esterna del kit.
- Una volta aperto, il kit è stabile a 2-8 °C per 6 mesi.
- La soluzione di lavaggio diluita è stabile per 30 giorni a 2-8 °C.

Nota importante: aprire il sacchetto contenente la micropiastra rivestita solo quando è a temperatura ambiente e chiuderlo immediatamente dopo l'uso.

8. RACCOLTA E CONSERVAZIONE DEI CAMPIONI

Il dosaggio deve essere effettuato su campioni di siero (provette di campionamento standard o provette contenenti gel per la separazione del siero) o plasma (litio eparina, sodio eparina o EDTA di potassio).

Conservazione dei campioni	Durata
2-8 °C	96 ore
Cicli di congelamento/scongelamento	3 cicli

9. PROCEDURA

Preparazione di calibratori e controlli

Poiché non è disponibile una preparazione di riferimento internazionale per gli anticorpi anti-proteinasi 3, il sistema di analisi è calibrato in unità arbitrarie relative. I calibratori sono pronti per l'uso e hanno le seguenti concentrazioni:

	C_0	C_1	C_2	C_3	C_4
AU/mL	0	10	20	40	160

I controlli sono pronti per l'uso; la concentrazione del controllo è stampata sull'etichetta.

Preparazione della soluzione di lavaggio

Diluire il contenuto della fiala "Soluzione di lavaggio conc. 10X" con acqua distillata fino a un volume finale di 500 mL prima dell'uso. Per i volumi più piccoli, rispettare il rapporto di diluizione 1:10.

È possibile osservare la presenza di cristalli all'interno della soluzione di lavaggio concentrata; in tal caso, mescolare a temperatura ambiente fino alla completa dissoluzione dei cristalli. Per una maggiore precisione, diluire l'intero flacone di soluzione di lavaggio concentrata a 500 mL, avendo cura anche di trasferire completamente i cristalli sciacquando il flacone, quindi mescolare fino a quando i cristalli non si dissolvono completamente.

Preparazione dei campioni

I campioni di test devono essere trasparenti. La contaminazione da lipemia è da evitare, ma non interferisce con questo dosaggio.
Si sconsiglia di analizzare campioni di siero o plasma inattivati con il calore.
Tutti i campioni di siero o plasma devono essere prediluiti con diluente per campione 1:100; ad esempio, 10 µL di campione devono essere diluiti con 990 µL di diluente per campione.

Procedura

- **Lasciare che tutti i reagenti raggiungano la temperatura ambiente (22-28 °C) per almeno 30 minuti.** Alla fine del dosaggio, conservare immediatamente i reagenti a 2-8 °C: evitare una lunga esposizione a temperatura ambiente.
- Le strisce di micropozzetti rivestiti non utilizzate devono essere rilasciate in modo sicuro nella busta di alluminio contenente l'essiccante e conservate a 2-8 °C.
- Per evitare potenziali contaminazioni microbiche e/o chimiche, i reagenti inutilizzati non devono mai essere trasferiti nelle fiale originali.

- Poiché è necessario eseguire la determinazione in duplicato per migliorare la precisione dei risultati del test, preparare due pozzetti per ogni punto della curva di calibrazione (C_0-C_4), due per ogni controllo, due per ogni campione, uno per il bianco.

Reagente	Calibratore	Campione/ Controlli	Bianco
Calibratore C_0-C_4	**100 µL**		
Controlli		**100 µL**	
Campione diluito		**100 µL**	
Incubare per 60 minuti a temperatura ambiente (22- 28 °C). Rimuovere il contenuto da ogni pozzetto, lavare i pozzetti 3 volte con 300 µL di soluzione di lavaggio diluita. **Nota importante**: durante ogni fase di lavaggio, agitare delicatamente la piastra per 5 secondi e rimuovere la soluzione in eccesso picchiettando la piastra capovolta su un tovagliolo di carta assorbente. **Lavatore automatico**: se si utilizzano apparecchiature automatiche, lavare i pozzetti almeno 5 volte.			
Coniugato	**100 µL**	**100 µL**	
Incubare per 30 minuti a temperatura ambiente (22- 28 °C). Rimuovere il contenuto da ogni pozzetto, lavare i pozzetti 3 volte con 300 µL di soluzione di lavaggio diluita. **Lavaggio**: seguire le stesse indicazioni del punto precedente.			
Substrato TMB	**100 µL**	**100 µL**	**100 µL**
Incubare per 15 minuti, al buio, a temperatura ambiente (22-28 °C).			
Soluzione di arresto	**100 µL**	**100 µL**	**100 µL**
Agitare delicatamente la micropiastra Leggere l'assorbanza (E) a 450 nm contro una lunghezza d'onda di riferimento di 620-630 nm o contro il bianco entro 5 minuti.			

10. CONTROLLO QUALITÀ

Le buone prassi di laboratorio (GLP) richiedono l'inclusione di campioni per il controllo della qualità in ogni serie di dosaggi al fine di verificare le prestazioni del dosaggio. I controlli devono essere trattati come campioni sconosciuti e i risultati devono essere analizzati con metodi statistici appropriati.

I controlli forniti nel kit devono essere testati come se fossero sconosciuti e hanno lo scopo di agevolare la valutazione della validità dei risultati ottenuti in ogni piastra di dosaggio.

La concentrazione media di ciascun livello di controllo è documentata nel rapporto del controllo di qualità incluso in ciascun kit. Tali livelli di concentrazione media sono determinati in base a diversi dosaggi eseguiti in duplicato in più posizioni su ciascuna piastra.

DiaMetra raccomanda agli utenti di conservare le annotazioni grafiche dei valori di controllo generati con ciascun dosaggio, tra cui medie mobili, DS e % CV. Queste informazioni faciliteranno l'analisi delle tendenze dei controlli per quanto riguarda le prestazioni dei lotti di controllo attuali e pregressi rispetto ai dati forniti nel controllo di qualità. Le tendenze aiuteranno a identificare i dosaggi che generano valori di controllo significativamente diversi dal rispettivo intervallo medio.

Quando si interpretano i dati dei controlli, occorre tenere conto del fatto che il prodotto è stato progettato e sviluppato come prodotto per l'utilizzo manuale. L'intervallo riportato sul certificato del controllo di qualità deve essere appropriato per i dosaggi eseguiti manualmente e rispettando rigorosamente la procedura di dosaggio descritta sopra. Gli esperti del controllo di qualità riconoscono che, a causa delle differenze di condizioni e di prassi, si avrà sempre una variabilità nei valori medi e nella precisione delle misurazioni dei controlli eseguite da laboratori diversi[7].

Affinché i risultati del test siano considerati validi, devono essere soddisfatti tutti i criteri elencati di seguito. Se una qualsiasi di queste condizioni non è soddisfatta, il test deve essere considerato non valido e il dosaggio deve essere ripetuto:

- L'assorbanza del controllo positivo IgG PR-3 prediluito deve essere maggiore dell'assorbanza del controllo negativo prediluito.
- I controlli negativi e positivi hanno lo scopo di monitorare l'errore sostanziale del reagente e non garantiscono la precisione del cut-off del dosaggio.
- Questo test è valido solo se la densità ottica a 450 nm per il controllo negativo e il controllo positivo nonché per il calibratore C_0-C_4 rientra nel rispettivo intervallo indicato sul Certificato di controllo qualità allegato a ciascun kit del test: se uno qualsiasi di questi criteri non è soddisfatto, i risultati non sono validi e il test deve essere ripetuto.

11. CALCOLO DEI RISULTATI

Sono disponibili vari pacchetti software di elaborazione dei dati, che possono essere utilizzati per generare la curva di calibrazione media e per calcolare le concentrazioni medie di campioni e controlli sconosciuti. È necessario un adattamento della curva logistica a 4 parametri (4PL) **che includa il calibratore 0.** È possibile utilizzare un adattamento uniforme della curva spline o un grafico doppio-logaritmico che includa il calibratore 0. Gli altri algoritmi di adattamento della curva non sono raccomandati.

In alternativa, è possibile preparare una curva di calibrazione su carta millimetrata semilogaritmica tracciando un grafico con l'assorbanza media sull'asse delle ordinate e la concentrazione dell'analita sull'asse delle ascisse. Nella curva di calibrazione deve essere incluso il calibratore 0. Leggere il valore medio dell'assorbanza di ciascun campione sconosciuto dalla curva.

12. INTERVALLO DI MISURAZIONE

L'intervallo di misurazione del dosaggio (AMR) è 3-160 AU/mL.
Qualsiasi valore inferiore 3 AU/mL deve essere indicato come "< 3 AU/mL". Qualsiasi valore superiore a 160 AU/mL deve essere indicato come "> 160 AU/mL".

13. METROLOGIA E TRACCIABILITÀ

I calibratori di questo kit sono tracciabili al siero umano di riferimento n. 16 per anti-proteinasi 3, Centres for Disease Control (CDC) n. catalogo Ab IS2721.

14. VALORI ATTESI

I seguenti intervalli sono stati determinati utilizzando il dosaggio Anti PR-3 (c-ANCA) e sono forniti unicamente a scopo informativo. Il 90% degli intervalli di riferimento per adulti apparentemente sani è stato calcolato attraverso un metodo non parametrico secondo le linee guida tratte dal documento CLSI C28-A3 "Defining, Establishing and Verifying Reference Intervals in the Clinical Laboratory".

N. di soggetti	73
Media (AU/mL)	8,71
DS (AU/mL)	3,21
Mediana (AU/mL)	8,12
Intervallo di riferimento (AU/mL)	4,19-14,40

Gli intervalli sopraindicati devono essere considerati solo come linee guida; si raccomanda a ogni laboratorio di stabilire i propri intervalli di valori attesi sulla base della propria popolazione di pazienti.

15. INTERPRETAZIONE DEI RISULTATI

Concentrazione	Interpretazione
< 16 AU/mL	Il campione deve essere considerato negativo
16-20 AU/mL	Il campione deve essere classificato equivoco e la ripetizione dei test/campionamenti deve essere eseguita secondo le pratiche interne
≥ 20 AU/mL	Il campione deve essere considerato positivo

L'indice e l'interpretazione sopra indicati devono essere considerati esclusivamente come linee guida. I risultati positivi devono essere verificati relativamente all'intero stato clinico del paziente. Inoltre, ogni decisione per la terapia deve essere presa in base alle condizioni di ciascun paziente.

È consigliabile che ogni laboratorio stabilisca i propri intervalli normali e patologici di anti-PR3.

16. CARATTERISTICHE DI AZIONE

Sono mostrati i dati più rappresentativi delle prestazioni. I risultati ottenuti nei singoli laboratori possono variare.

Capacità di rilevamento
Il limite del bianco (LoB), il limite di rilevamento (LoD) e il limite della determinazione quantitativa (LoQ) sono stati definiti basandosi sulla procedura CLSI EP17-A, "Protocols for Determination of Limits of Detection and Limits of Quantitation" utilizzando 6 bianchi e 6 campioni a basso livello.

Sensibilità	Concentrazione
Limite del bianco (LoB)	0,6 AU/mL
Limite di rilevamento (LoD)	1,6 AU/mL
Limite della determinazione quantitativa (LoQ)	3,2 AU/mL

Esattezza
L'esattezza è stata dimostrata attraverso un test di recupero del dosaggio Anti PR-3 (c-ANCA) con il siero di riferimento umano n.16 per anti-proteinasi 3, Centres for Disease Control and Prevention (CDC).

Precisione
La precisione del dosaggio Anti PR-3 (c-ANCA) è stata determinata eseguendo un complesso studio di precisione.

Ripetibilità: un totale di 6 campioni di è stato analizzato in 5 repliche, una volta al giorno per 5 giorni da 3 operatori.
I dati di un lotto rappresentativo sono mostrati di seguito:

Campione	n	Conc. media (AU/mL)	Intra-test (ripetibilità)	
			DS	% CV
1	75	16,51	0,96	5,8%
2	75	23,94	0,93	3,9%
3	75	40,03	1,23	3,1%
4	75	59,68	3,40	5,7%
5	75	86,96	2,80	3,2%
6	75	136,70	6,24	4,6%

Riproducibilità: un totale di 6 campioni di è stato analizzato in 5 repliche, una volta al giorno per 5 giorni da 3 operatori
I risultati per i dati combinati di due lotti sono mostrati di seguito:

Campione	n	Conc. media (AU/mL)	All'interno del laboratorio (riproducibilità)	
			DS	% CV
1	150	16,57	1,49	9,0%
2	150	23,95	1,53	6,4%
3	150	40,59	2,67	6,6%
4	150	60,11	4,15	6,9%
5	150	86,69	6,12	7,1%
6	150	137,19	9,63	7,0%

Linearità
La linearità è stata valutata in base al documento CLSI EP-06, "Evaluation of the Linearity of Quantitative Measurement Procedures". Per la concentrazione di anti-PR3 mediante il dosaggio Anti PR-3 (c-ANCA) , la procedura di misurazione mostra linearità per l'intervallo compreso tra 1,73 e 162,62 AU/mL entro la deviazione ammissibile di linearità (ADL) di ±15%.

Sensibilità e specificità diagnostica
La sensibilità e la specificità sono state determinate in base al documento CLSI EP-24 "Assessment of the Diagnostic Accuracy of Laboratory Tests Using Receiver Operating Characteristic Curves" utilizzando 73 campioni negativi e 50 positivi eseguiti su due lotti di reagenti.

		DKO091		Totale
		Positivo	Negativo	
Stato reale	Positivo	50	0	**50**
	Negativo	2	71	**73**
Totale		**52**	**71**	**123**

Sensibilità diagnostica: 100%

Specificità diagnostica: 97,3%

Interferenti
Le seguenti sostanze non interferiscono con un bias > ±15% nel dosaggio Anti PR-3 (c-ANCA) quando le concentrazioni sono inferiori alla soglia dichiarata presentata nella tabella seguente.

Reagente potenzialmente interferente	Concentrazione di soglia
Bilirubina, coniugata	15 mg/dL
Bilirubina, non coniugata	15 mg/dL
Emoglobina	200 mg/dL
Proteine totali	10 g/dL
Trigliceridi	500 mg/dL

Studio su siero-plasma
È stato condotto uno studio di confronto tra matrici del dosaggio Anti PR-3 (c-ANCA) per valutare la differenza tra i tipi di provette (provette per la separazione del siero (SST), per plasma in litio eparina, per plasma in sodio eparina e plasma in K2 EDTA) rispetto ai campioni di controllo (siero tappo rosso, senza additivo) secondo le linee guida CLSI EP9-A3. È stato valutato un totale di 20 campioni (16 nativi, 4 additivati o diluiti) per coprire l'intervallo da 2,80 a 74,40 AU/mL. L'analisi di regressione lineare è stata effettuata su dati comparativi:

Tipo di campione	Pendenza [IC 95%]	Intercetta (AU/mL) [IC 95%]	Coefficiente di correlazione (r)
SST	1,0 [da 0,97 a 1,08]	0,14 [da -1,17 a 1,45]	0,99
Litio eparina	1,1 [da 1,06 a 1,21]	-0,46 [da -2,09 a 1,17]	0,99
Sodio eparina	1,1 [da 1,05 a 1,14]	-0,91 [da -1,88 a 0,06]	1,00
EDTA	1,1 [da 1,0 a 1,12]	0,00 [da -1,79 a 1,78]	0,99

17. LIMITAZIONI D'USO

- Come nel caso di qualsiasi procedura diagnostica, i risultati devono essere interpretati unitamente ai dati clinici del paziente e alle altre informazioni a disposizione del medico.
- Non sono state stabilite le caratteristiche di azione di questo dosaggio nella popolazione pediatrica.
- Gli anticorpi eterofili nel siero umano possono reagire con le immunoglobuline dei reagenti, interferendo con gli immunodosaggi *in vitro*[11]. I pazienti regolarmente esposti agli animali o a prodotti derivati da siero animale possono essere soggetti a questa interferenza, quindi si potrebbero osservare valori anomali.

18. GESTIONE DEI RIFIUTI

I reagenti devono essere smaltiti in conformità alle normative locali.

19. BIBLIOGRAFIA

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20. IDENTIFICATORE DELLE REVISIONI

Le aggiunte o le modifiche alle istruzioni per l'uso sono indicate dall'evidenziazione in grigio.

21. RECLAMI SUI PRODOTTI E SUPPORTO TECNICO

Per un paziente/utente/terza parte nell'Unione Europea e nei Paesi con un regime normativo simile (Regolamento 2017/746/UE relativo ai dispositivi medico-diagnostici *in vitro*); se, durante l'uso di questo dispositivo o come risultato del suo utilizzo, si è verificato un incidente grave, segnalarlo al produttore e/o al suo rappresentante autorizzato e all'autorità normativa nazionale.
Il produttore può essere contattato tramite il relativo servizio clienti o il team di supporto tecnico. I dettagli di contatto sono disponibili di seguito e sul sito Web dell'azienda: www.diametra.com.

Ed. 05/2022 **DCM091-9**

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DiaMetra
care for quality

DCM091-9
Ed. 05/2022

Anti PR-3 (c-ANCA)

for routine analysis

Semi quantitative determination of IgG class antibodies against proteinase 3 (PR-3) in human serum or plasma

IVD | LOT See external label | 2°C 8°C | Σ = 96 tests | REF DKO091

1. INTENDED PURPOSE

For *In Vitro* Diagnostic Use
For Laboratory Professional Use

Anti PR-3 (c-ANCA) assay is a manual *in vitro* diagnostic device intended for the quantitative determination of IgG class autoantibodies against proteinase 3 (PR-3) in human serum or plasma. Results are to be used in conjunction with other clinical and laboratory data as an aid in the diagnosis of certain autoimmune vasculitides such as Wegener´s granulomatosis.

2. CLINICAL SIGNIFICANCE

Proteinase 3 (PR-3 or PR3) is a small serine protease located in the primary granules and secretory vesicles in the cytoplasm of neutrophils. PR3 is stored in the cytoplasmatic granules in resting neutrophils, whilst the expression of the protein on cell surface increases during the activation of neutrophils and during apoptosis[1]. PR3 is also found in neutrophil extracellular traps (NETs), that consist of chromatin released by neutrophils as defence against pathogens.

Autoantibodies against the PR3 are frequently detected in a specific class of autoimmune vasculitides known as Anti-neutrophil cytoplasmatic autoantibodies (ANCA) associated vasculitis (AAV). These vasculitides are characterised by the presence of autoantibodies against cytoplasmatic components of neutrophils that may have, cytoplasmatic staining (c-ANCA) or a perinuclear staining (p-ANCA)[1-3].

The mechanism of autoimmunity for ANCA antigens is not still being evaluated. Activated neutrophils can undergo apoptosis creating NETs, which are involved in the induction of inflammation. Persistence of NETs during the infection can lead to an autoimmune response[3,4]. Increased expression of MPO and PR3 in neutrophils and monocytes can influence the pathogenic autoimmune response leading to ANCA-neutrophils activation[3].

ANCA-associated vasculitides is classified as small vessel vasculitis (SVV), which predominantly affect the small vessels, intraparenchymal arteries, arterioles, capillaries and venules[5]. ANCA-associated vasculitides are necrotising vasculitis characterised by a lack of immune deposits in vessel walls causing conditions such as Microscopic polyangiitis (MPA) and Granulomatosis with polyangiitis (GPA, formerly known as Wegner's syndrome).

The frequency of serotype (MPO or PR3), clinical manifestation and geographic distribution of ANCA-associated vasculitides varies, suggesting a role of the environment and the genetic background of patients affected by this category of pathologies[1,3,6].

3. PRINCIPLE OF THE METHOD

The Anti PR-3 (c-ANCA) assay is an indirect enzyme immunometric assay (ELISA) where anti- PR3 (antibody) in the sample binds to human neutrophil proteinase 3 coated into the microplate (solid phase). In the first step the antibodies in calibrators, controls or prediluted patient samples bind to the inner surface of the wells. After a 60-minute incubation, the microplate is washed to remove non-reactive serum components. In the second step an anti-human-IgG horseradish peroxidase (HRP) conjugated solution binds to the IgG class antibodies bound to the immobilised antigens. After a further incubation step, any excess enzyme conjugate which is not specifically bound is washed away.

A chromogenic substrate solution containing TMB is then dispensed into the wells. After a further incubation step, the enzyme HRP in the bound fraction reacts with the Substrate (H_2O_2) and the TMB Substrate and develops a blue colour that changes into yellow when the Stop Solution (H_2SO_4) is added. The colour intensity is directly proportional to the concentration of IgG antibodies in the sample.

The concentration of anti- PR3 IgG antibodies in the sample is calculated through a calibration curve.

4. REAGENTS, MATERIALS AND INSTRUMENTATION

4.1. Reagents and materials supplied in the kit

1. Calibrators (5 vials, 1.2 mL each)
Phosphate buffer 0.1M, NaN_3 < 0.1%, human serum

CAL0	REF DCE002/09106-0
CAL1	REF DCE002/09107-0
CAL2	REF DCE002/09108-0
CAL3	REF DCE002/09109-0
CAL4	REF DCE002/09110-0

2. Controls (2 vials, 1.2 mL each)
Phosphate buffer 0.1M, NaN_3 < 0.1%, human serum
Negative control **REF DCE045/09101-0**
Positive control **REF DCE045/09102-0**

3. Sample Diluent (1 vial, 100 mL)
Phosphate buffer 0.1 M, NaN_3 < 0.1%
REF DCE053-0

4. Conjugate (1 vial, 15 mL)
Anti h-IgG conjugated with horseradish peroxidase (HRP), BSA 0.1%, contains ProClin™ **REF DCE002/09102-0**

5. Coated Microplate (1 breakable microplate)
Microplate coated with human neutrophil proteinase 3
REF DCE002/09103-0

6. TMB Substrate (1 vial, 15 mL)
H_2O_2-TMB 0.26 g/L *(avoid any skin contact)*
REF DCE004-0

7. Stop Solution (1 vial, 15 mL)
Sulphuric acid 0.15M *(avoid any skin contact)*
REF DCE005-0

8. 10X Conc. Wash Solution (1 vial, 50 mL)
Phosphate buffer 0.2M, pH 7.4, contains ProClin™
REF DCE054-0

4.2. Materials required but not provided
Distilled water

4.3. Auxiliary materials and instrumentation
Automatic dispenser
Precision Pipetting Devices
Microplate reader (450 nm, 620-630 nm)

5. WARNINGS
- This kit is intended for *in vitro* use by professional persons only. Not for internal or external use in Humans or Animals.
- Use appropriate personal protective equipment while working with the reagents provided.
- Follow Good Laboratory Practice (GLP) for handling blood products.
- All human source material used in the preparation of the reagents has been tested and found negative for antibody to HIV 1&2, HbsAg, and HCV. No test method however can offer complete assurance that HIV, HBV, HCV or other infectious agents are absent. Therefore, the Calibrators and the Controls should be handled in the same manner as potentially infectious material.
- Material of animal origin used in the preparation of the kit has been obtained from animals certified as healthy and the bovine protein has been obtained from countries not infected by BSE, but these materials should be handled as potentially infectious.
- Some reagents contain small amounts of Sodium Azide (NaN_3) or ProClin™ 300 as preservative. Avoid contact with skin or mucosa.
- ProClin™ 300: Classification under CLP
  Skin Sens. 1

  Hazard statements
  H317: May cause an allergic skin reaction

  Precautionary statements
  P261: Avoid breathing dust / fumes / gas / mist / vapours / spray.
  P272: Contaminated work clothing should not be allowed out of the workplace.
  P280: Wear protective gloves / protective clothing / eye protection / face protection.
  P302/352 IF ON SKIN: Wash with plenty of water / soap and water.
  P321: Specific treatment (see instructions on the label)
  P333/313: If skin irritation or rash occurs: get medical attention.
- Sodium Azide may be toxic if ingested or absorbed through the skin or eyes; moreover, it may react with lead or copper plumbing to form potentially explosive metal azides. If you use a sink to remove the reagents, wash through large with amounts of water to prevent azide build-up.
- The TMB Substrate contains an irritant, which harmful if inhaled, ingested or absorbed through the skin. To prevent injury, avoid inhalation, ingestion or contact with skin and eyes.
- The Stop Solution consists of a diluted sulphuric acid solution. Sulphuric acid is poisonous, corrosive and can be toxic if ingested. To prevent chemical burns, avoid contact with skin and eyes.
- Avoid the exposure of reagent TMB/H_2O_2 to direct sunlight, metals or oxidants. Do not freeze the solution.

6. PRECAUTIONS
- Please adhere strictly to the sequence of pipetting steps provided in this protocol. The performance data represented here were obtained using specific reagents listed in this Instruction For Use.
- All reagents should be stored refrigerated at 2-8°C in their original container. Any exceptions are clearly indicated.
- Allow all kit components and specimens to reach room temperature (22-28°C) and mix well prior to use.
- Do not interchange kit components from different lots. The expiry date printed on box and vials labels must be observed. Do not use any kit component beyond their expiry date.
- If you use automated equipment, the user has the responsibility to make sure that the kit has been appropriately tested.
- The incomplete or inaccurate liquid removal from the wells could influence the assay precision and/or increase the background. To improve the performance of the kit on automatic systems is recommended to increase the number of washes.
- It is important that the time of reaction in each well is held constant for reproducible results. Pipetting of samples should not extend beyond ten minutes to avoid assay drift. If more than 10 minutes are needed, follow the same order of dispensation. If more than one plate is used, it is recommended to repeat the dose response curve in each plate
- Addition of the TMB Substrate solution initiates a kinetic reaction, which is terminated by the addition of the Stop Solution. Therefore, the TMB Substrate and the Stop Solution should be added in the same sequence to eliminate any time deviation during the reaction.
- Observe the guidelines for performing quality control in medical laboratories by assaying controls and/or pooled sera.
- Maximum precision is required for reconstitution and dispensation of the reagents.

- Samples microbiologically contaminated, highly lipemic or haemolysed should not be used in the assay.
- Plate readers measure vertically. Do not touch the bottom of the wells.
- **WARNING: the conjugate reagent is designed to ensure maximum dose sensitivity and may be contaminated by external agents if not used properly;** therefore, it is recommended to use disposable consumables (tips, bottles, trays, etc.). For divided doses, take the exact amount of conjugate needed and do not re-introduce any waste product into the original bottle. In addition, **for doses dispensed with the aid of automatic and semi-automatic devices,** before using the conjugate, it is advisable to clean the fluid handling system, ensuring that the procedures of washing, deproteinisation and decontamination are effective in avoiding contamination of the conjugate; this procedure is highly recommended when the kit is processed using analysers which are not equipped with disposable tips.
  For this purpose, Diametra supplies a separate decontamination reagent for cleaning needles.

7. REAGENT STORAGE AND STABILITY

Store the kit at 2 – 8°C in the dark.

- The kit is stable at 2 – 8°C until the expiry date stated on the external kit label.
- Once opened, the kit is stable at 2 – 8°C for 6 months.
- The diluted wash solution is stable for 30 days at 2-8°C.

Important note: open the bag containing the Coated Microplate only when it is at room temperature and close it immediately after use.

8. SAMPLE COLLECTION AND STORAGE

The assay should be performed using serum (standard sampling tubes or tubes containing serum separating gel) or plasma (lithium heparin, sodium heparin, or potassium EDTA) samples.

Sample Storage	Duration
2 – 8 °C	96 hours
Freeze/thaw cycles	3 cycles

9. PROCEDURE

9.1. Preparation of Calibrators and Controls

Since no international reference preparation for Anti-proteinase 3 antibodies is available, the assay system is calibrated in relative arbitrary units. The Calibrators are ready to use and have the following concentration:

	C_0	C_1	C_2	C_3	C_4
AU/mL	0	10	20	40	160

The controls are ready to use; the concentration of the control is printed on the label.

9.2. Preparation of the Wash Solution

Dilute the content of the vial “10X Conc. Wash Solution” with distilled water to a final volume of 500 mL prior to use. For smaller volumes respect the 1:10 dilution ratio.

It is possible to observe the presence of crystals within the concentrated wash solution; in this case mix at room temperature until the complete dissolution of crystals. For greater accuracy, dilute the whole bottle of concentrated wash solution to 500 mL, taking care also to transfer crystals completely by rinsing of the bottle, then mix until crystals are completely dissolved.

9.3. Preparation of Samples

Test samples should be clear. Contamination by lipemia is best avoided but does not interfere with this assay.
Testing of heat-inactivated serum or plasma samples is not recommended.
All serum or plasma samples must be prediluted 1:100 with sample diluent; for example, 10 µL of sample should be diluted with 990 µL of sample diluent.

9.4. Procedure

- **Allow all reagents to reach room temperature (22-28°C) for at least 30 minutes.** At the end of the assay, immediately store the reagents at 2-8°C: avoiding long exposure to room temperature.
- Unused coated microwell strips should be released securely in the foil pouch containing desiccant and stored at 2-8°C.
- To avoid potential microbial and/or chemical contamination, unused reagents should never be transferred into the original vials.
- As it is necessary to perform the determination in duplicate in order to improve accuracy of the test results, prepare two wells for each point of the calibration curve (C_0-C_4), two for each Control, two for each sample, one for Blank.

Reagent	Calibrator	Sample/ Controls	Blank
Calibrator C_0-C_4	**100 µL**		
Controls		**100 µL**	
Diluted Sample		**100 µL**	
Incubate 60 minutes at room temperature (22-28°C). Remove the content from each well, wash the wells 3 times with 300 µL of diluted wash solution. **Important note**: during each washing step, gently shake the plate for 5 seconds and remove excess solution by tapping the inverted plate on an absorbent paper towel. **Automatic washer**: if you use automated equipment, wash the wells at least 5 times.			
Conjugate	**100 µL**	**100 µL**	
Incubate 30 minutes at room temperature (22-28°C). Remove the contents from each well, wash the wells 3 times with 300 µL of diluted wash solution. **Washing**: follow the same indications of the previous point.			
TMB Substrate	**100 µL**	**100 µL**	**100 µL**
Incubate 15 minutes in the dark at room temperature (22-28°C).			
Stop Solution	**100 µL**	**100 µL**	**100 µL**
Shake the microplate gently Read the absorbance (E) at 450 nm against a reference wavelength of 620-630 nm or against Blank within 5 minutes.			

10. QUALITY CONTROL

Good Laboratory Practice (GLP) requires the use of quality control specimens in each series of assays in order to check the performance of the assay. Controls should be treated as unknown samples, and the results analysed with appropriate statistical methods.

The kit controls provided in the kit should be tested as unknowns and are intended to assist in assessing the validity of results obtained with each assay plate.

The mean concentration of each control level is documented in the QC report included with each kit. These mean concentration levels are determined over several assays which are run in duplicate in multiple locations across each plate.

DiaMetra recommends the users to maintain graphic records of the control values generated with each assay run, including the running means, SDs and %CVs. This information will facilitate the controls trending analysis relating to the performance of current and historical control lots relative to the supplied Quality Control data. The trending will assist in the identification of assays which give control values significantly different from their average range.

When interpreting control data, users should note that this product was designed and developed as a manual product. The range stated on the QC certificate should be appropriate for assays that are performed manually and with strict adherence to the Assay Procedure described above. It is recognised by Quality Control professionals, that as a result of differences in conditions and practices, there will always be variability in the mean values and precision of control measurements between different laboratories[7].

In order for the test results to be considered valid, all of the criteria listed below must be met. If any of these are not met, the test should be considered invalid and the assay repeated:

- The absorbance of the prediluted PR-3 IgG Positive Control must be greater than the absorbance of the prediluted Negative Control.
- The negative and positive controls are intended to monitor for substantial reagent failure and they will not ensure precision at the assay cut-off.
- This test is only valid if the optical density at 450 nm for Negative Control and Positive Control as well as for the Calibrator C_0-C_4 complies with the respective range indicated on the Quality Control Certificate enclosed to each test kit: if any of these criteria is not met, the results are invalid and the test should be repeated.

11. CALCULATION OF RESULTS

A variety of data reduction software packages are available, which may be employed to generate the mean calibration curve and to calculate the mean concentrations of unknown samples and controls. A 4-parameter logistic (4PL) curve fit, **including Calibrator 0 is required.** A smoothed spline and log-log fit including Calibrator 0 can be used. Other curve fitting algorithms are not recommended.

Alternatively, a calibration curve may be prepared on semi-log graph paper by plotting mean absorbance on the Y-axis against concentration of analyte on the X-axis. Calibrator 0 should be included in the calibration curve. Read the mean absorbance value of each unknown sample off the curve.

12. MEASURING RANGE

The assay measuring range (AMR) is 3 – 160 AU/mL.
Any value that reads below 3 AU/mL should be reported as "< 3 AU/mL". Any value that reads above 160 AU/mL should be reported as "> 160 AU/mL".

13. METROLOGY AND TRACEABILITY

The calibrators of this kit are traceable to the Centres for Disease Control and Prevention (CDC) Human Reference Serum #16 for Anti-Proteinase 3 Ab Catalogue #IS2721.

14. EXPECTED VALUES

The following ranges were determined using the Anti PR-3 (c-ANCA) assay and are provided for information only. The 90 % reference interval for apparently healthy adults were

calculated by a non-parametric method following guidance from CLSI C28-A3 “Defining, Establishing and Verifying Reference Intervals in the Clinical Laboratory”.

No. of subjects	73
Mean (AU/mL)	8.71
SD (AU/mL)	3.21
Median (AU/mL)	8.12
Reference Interval (AU/mL)	4.19 – 14.40

The above ranges should be considered as guidelines only; it is recommended that each laboratory establish its own expected range based upon its own patient population.

15. INTERPRETATION OF RESULTS

Concentration	Interpretation
< 16 AU/mL	The sample should be considered negative
16 – 20 AU/mL	The sample should be graded equivocal and repeat testing / sampling should be performed according to internal practices
≥ 20 AU/mL	The sample should be considered positive

The above index and interpretation should be considered as guidelines only. Positive results should be verified concerning the entire clinical status of the patient. Also, every decision for therapy should be taken on an individual patient basis.

It is recommended that each laboratory establishes its own normal and pathological ranges of anti- PR3.

16. PERFORMANCE CHARACTERISTICS

Representative performance data are shown. Results obtained at individual laboratories may vary.

16.1. Detection Capability

The limit of blank (LoB), limit of detection (LoD) and limit of quantitation (LoQ) were determined with guidance from CLSI EP17-A, “Protocols for Determination of Limits of Detection and Limits of Quantitation” using 6 blanks and 6 low level samples.

Sensitivity	Concentration
Limit of Blank (LoB)	0.6 AU/mL
Limit of Detection (LoD)	1.6 AU/mL
Limit of Quantitation (LoQ)	3.2 AU/mL

16.2. Trueness

Trueness has been demonstrated through a recovery test of the Anti PR-3 (c-ANCA) assay with the human Anti-Proteinase 3 reference serum #16 from the Centres for Disease Control and Prevention (CDC).

16.3. Precision

Precision of the Anti PR-3 (c-ANCA) assay was determined by performing a complex precision study.

Repeatability: A total of 6 samples were assayed in 5 replicates, once a day for 5 days by 3 operators.
Data from one representative lot is shown below:

Sample	n	Mean Conc. (AU/mL)	Within run (Repeatability)	
			SD	CV%
1	75	16.51	0.96	5.8%
2	75	23.94	0.93	3.9%
3	75	40.03	1.23	3.1%
4	75	59.68	3.40	5.7%
5	75	86.96	2.80	3.2%
6	75	136.70	6.24	4.6%

Reproducibility: A total of 6 samples were assayed in 5 replicates, once a day for 5 days by 3 operators
Results for the combined data from two lots is shown below:

Sample	n	Mean Conc. (AU/mL)	Within Laboratory (Reproducibility)	
			SD	CV%
1	150	16.57	1.49	9.0%
2	150	23.95	1.53	6.4%
3	150	40.59	2.67	6.6%
4	150	60.11	4.15	6.9%
5	150	86.69	6.12	7.1%
6	150	137.19	9.63	7.0%

16.4. Linearity

Linearity was evaluated based on CLSI EP-06, “Evaluation of the Linearity of Quantitative Measurement Procedures”. For anti- PR3 concentration by Anti PR-3 (c-ANCA) assay, the measurement procedure shows linearity for the interval from 1.73 to 162.62 AU/mL within the allowable deviation of linearity (ADL) of ±15 %.

16.5. Diagnostic Sensitivity and Specificity

The sensitivity and specificity were determined with guidance from CLSI EP-24 “Assessment of the Diagnostic Accuracy of Laboratory Tests Using Receiver Operating Characteristic Curves” using 73 negative and 50 positive samples run on a single reagent lot.

		DKO091		Total
		Positive	Negative	
True state	Positive	50	0	**50**
	Negative	2	71	**73**
Total		**52**	**71**	**123**

Diagnostic sensitivity: 100%

Diagnostic specificity: 97.3%

16.6. Interferents

The following substances do not interfere with a bias of > ±15% in the Anti PR-3 (c-ANCA) assay when the concentrations are below the stated threshold presented in the following table.

Potentially Interfering Reagent	Threshold Concentration
Bilirubin, conjugated	15 mg/dL
Bilirubin, unconjugated	15 mg/dL
Haemoglobin	200 mg/dL
Total Protein	10 g/dL
Triglycerides	500 mg/dL

16.7. Serum-plasma study

The Anti PR-3 (c-ANCA) assay matrix comparison study was performed to evaluate the difference across tube types (serum separator tubes (SST), lithium heparin plasma, sodium heparin plasma and K2 EDTA plasma) versus the control samples (red top serum, without additive) following CLSI EP9-A3 guidelines. A total of 20 samples (16 native, 4 spiked or diluted) to cover the range of 2.80 to 74.40 AU/mL were evaluated. Linear regression analysis was performed on the comparative data:

Sample type	Slope [95% CI]	Intercept (AU/mL) [95% CI]	Correlation coefficient (r)
SST	1.0 [0.97 to 1.08]	0.14 [-1.17 to 1.45]	0.99
Lithium Heparin	1.1 [1.06 to 1.21]	-0.46 [-2.09 to 1.17]	0.99
Sodium Heparin	1.1 [1.05 to 1.14]	-0.91 [-1.88 to 0.06]	1.00
EDTA	1.1 [1.0 to 1.12]	0.00 [-1.79 to 1.78]	0.99

17. LIMITATIONS OF USE

- As in the case of any diagnostic procedure, results must be interpreted in conjunction with the patient's clinical presentation and other information available to the physician.
- The performance characteristics of this assay have not been established in a paediatric population.
- Heterophilic antibodies in human serum can react with reagent immunoglobulins, interfering with *in vitro* immunoassays[11]. Patients routinely exposed to animals or to animal serum products can be prone to this interference and anomalous values may be observed.

18. WASTE MANAGEMENT

Reagents must be disposed of in accordance with local regulations.

19. BIBLIOGRAPHY

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20. REVISION IDENTIFIER

Additions or changes to the IFU are indicated by grey highlighting.

21. PRODUCT COMPLAINTS AND TECHNICAL SUPPORT

For a patient/user/third party in the European Union and in countries with similar regulatory regime (Regulation 2017/746/EU on IVD Medical Devices); if, during the use of this device or as a result of its use, a serious incident has occurred, please report it to the manufacturer and/or its authorised representative and to your national regulatory authority.

The manufacturer can be contacted through their customer service or technical support team. The contact details can be found below and on the company website: www.diametra.com.

Ed. 05/2022 **DCM091-9**

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CE

DiaMetra
care for quality

DCM091-9
Ed. 05/2022

Anti PR-3 (c-ANCA)

para el análisis de rutina

Determinación semicuantitativa de anticuerpos de clase IgG contra la proteinasa 3 (PR-3) en suero o plasma humano

IVD

LOT
Ver etiqueta externa

2°C 8°C

Σ = 96 pruebas

REF DKO091

1. FINALIDAD PREVISTA

Para uso en diagnóstico *in vitro*
Para uso profesional de laboratorio

El ensayo Anti PR-3 (c-ANCA) es un dispositivo manual de diagnóstico *in vitro* destinado a la determinación cuantitativa de anticuerpos de clase IgG dirigidos contra la proteinasa 3 (PR-3) en suero o plasma humano. Los resultados deben utilizarse como ayuda en el diagnóstico de determinadas vasculitis autoinmunes como la granulomatosis de Wegener junto con otros datos clínicos y de laboratorio.

2. IMPORTANCIA CLÍNICA

La proteinasa 3 (PR-3 o PR3) es una pequeña serina proteasa localizada en los gránulos primarios y en las vesículas secretoras del citoplasma de los neutrófilos. La PR3 se almacena en los gránulos citoplasmáticos en los neutrófilos en reposo, mientras que la expresión de la proteína en la superficie celular aumenta durante la activación de los neutrófilos y durante la apoptosis[1]. La PR3 también se encuentra en las trampas extracelulares de neutrófilos (NET), que consisten en la cromatina liberada por los neutrófilos como defensa contra los patógenos.

Los autoanticuerpos contra la PR3 se detectan con frecuencia en una clase específica de vasculitis autoinmune conocida como autoanticuerpos citoplasmáticos antineutrófilos (ANCA) asociados a la vasculitis (AAV). Estas vasculitis se caracterizan por la presencia de autoanticuerpos contra los componentes citoplasmáticos de los neutrófilos, que pueden tener una tinción citoplasmática (c-ANCA) o una tinción perinuclear (p-ANCA)[1-3].

El mecanismo de autoinmunidad para los antígenos ANCA aún no se ha evaluado. Los neutrófilos activados pueden sufrir apoptosis creando NET, que están implicadas en la inducción de la inflamación. La persistencia de las NET durante la infección puede dar lugar a una respuesta autoinmune[3,4]. El aumento de la expresión de MPO y PR3 en los neutrófilos y monocitos puede influir en la respuesta autoinmune patógena que conduce a la activación de los neutrófilos ANCA[3].

Las vasculitis asociadas a ANCA se clasifican como vasculitis de pequeños vasos (SVV), que afectan predominantemente a los vasos pequeños, arterias intraparenquimatosas, arteriolas, capilares y vénulas[5]. Las vasculitis asociadas a los ANCA son vasculitis necrosantes caracterizadas por la ausencia de depósitos inmunitarios en las paredes de los vasos que causan afecciones como la poliangeítis microscópica (MPA) y la granulomatosis con poliangeítis (GPA, antes conocida como síndrome de Wegner).

La frecuencia del serotipo (MPO o PR3), la manifestación clínica y la distribución geográfica de las vasculitis asociadas a ANCA varían, lo que sugiere un papel del entorno y de los antecedentes genéticos de los pacientes afectados por esta categoría de patologías[1,3,6].

3. PRINCIPIO DEL MÉTODO

El ensayo Anti PR-3 (c-ANCA) es un ensayo enzimático inmunométrico indirecto (ELISA) en el que el anticuerpo anti PR3 de la muestra se une a la proteinasa 3 de neutrófilos humanos que recubre la microplaca (fase sólida). En el primer paso, los anticuerpos presentes en los calibradores, controles o muestras de pacientes prediluidas se unen a la superficie interior de los pocillos. Tras una incubación de 60 minutos, la microplaca se lava para eliminar los componentes de suero no reactivos. En el segundo paso, una solución anti-IgG humana conjugada con peroxidasa de rábano (HRP) se une a los anticuerpos de clase IgG unidos a los antígenos inmovilizados. Tras un paso adicional de incubación, el exceso de conjugado enzimático que no se ha unido específicamente se elimina mediante lavado.

Una solución de sustrato cromogénico que contiene TMB se dispensa a continuación a los pocillos. Tras un paso más de incubación, la enzima HRP de la fracción ligada reacciona con el sustrato (H_2O_2) y el sustrato de TMB, y desarrolla un color azul que cambia a amarillo cuando se añade la solución de detención (H_2SO_4). La intensidad del color es directamente proporcional a la concentración de los anticuerpos IgG de la muestra.

La concentración de anticuerpos IgG anti PR3 en la muestra se calcula mediante una curva de calibración.

4. REACTIVOS, MATERIALES E INSTRUMENTACIÓN

Reactivos y materiales incluidos en el kit

1. Calibradores (5 viales de 1,2 mL cada uno)

Tampón fosfato 0,1 M, NaN_3 <0,1 %, suero humano

CAL0 **REF DCE002/09106-0**
CAL1 **REF DCE002/09107-0**
CAL2 **REF DCE002/09108-0**
CAL3 **REF DCE002/09109-0**
CAL4 **REF DCE002/09110-0**

2. Control (2 viales de 1,2 mL cada uno)

Tampón fosfato 0,1 M, NaN_3 <0,1 %, suero humano

Control negativo **REF DCE045/09101-0**
Control positivo **REF DCE045/09102-0**

3. Diluyente de muestras (1 vial, 100 mL)

Tampón fosfato 0,1 M, NaN_3 <0,1 %

REF DCE053-0

4. Conjugado (1 vial, 15 mL)

IgG antihumano conjugado con peroxidasa de rábano picante (HRP), BSA 0,1 %, contiene ProClin™

REF DCE002/09102-0

5. Microplaca recubierta (1 microplaca que se puede romper)

Microplaca recubierta con proteinasa humana de neutrófilo 3

REF DCE002/09103-0

6. Sustrato de TMB (1 vial, 15 mL)

H_2O_2-TMB 0,26 g/L *(evitar el contacto con la piel)*

REF DCE004-0

7. Solución de detención (1 vial, 15 mL)

Ácido sulfúrico 0,15 M *(evitar el contacto con la piel)*

REF DCE005-0

8. Conc. 10X Solución de lavado (1 vial, 50 mL)

Tampón fosfato 0,2 M pH 7,4, contiene ProClin™

REF DCE054-0

Materiales necesarios pero no suministrados

Agua destilada

Materiales auxiliares e instrumentación

Dispensador automático
Dispositivos de pipetas de precisión
Lector de microplacas (450 nm, 620-630 nm)

5. ADVERTENCIAS

- Este kit está destinado al uso *in vitro* realizado exclusivamente por profesionales. No es para uso interno o externo en personas ni animales.
- Utilice el equipo de protección personal adecuado cuando trabaje con los reactivos suministrados.
- Siga las prácticas de laboratorio recomendadas (BPL) para manipular productos sanguíneos.
- Todo el material de origen humano utilizado en la preparación de los reactivos ha sido sometido a pruebas que han dado resultado negativo para los anticuerpos contra el VIH-1 y VIH-2, el HbsAg y el VHC. Sin embargo, ningún método de prueba puede ofrecer una garantía total de ausencia de VIH, VHB, VHC u otros agentes infecciosos. Por lo tanto, los calibradores y los controles deben manejarse de la misma manera que el material potencialmente infeccioso.
- El material de origen animal utilizado en la preparación del kit se ha obtenido de animales certificados como sanos y la proteína bovina se ha obtenido de países donde no hay infección de EEB, pero estos materiales deben manejarse como potencialmente infecciosos.
- Algunos reactivos contienen pequeñas cantidades de azida sódica (NaN_3) o ProClin™ 300 como conservante. Evite el contacto con la piel o las mucosas.
- ProClin™ 300: Clasificación según el Reglamento CLP
  Sensibilidad cutánea 1

  Declaraciones de riesgo
  H317: Puede provocar una reacción alérgica en la piel

  Declaraciones preventivas
  P261: Evitar inhalar polvos / humos / gases / nebulizaciones / vapores / aerosoles.
  P272: Se debe prohibir el uso de ropa de trabajo contaminada fuera del lugar de trabajo.
  P280: Usar guantes de protección, ropa de protección y protección para los ojos y la cara.
  P302/352 EN CASO DE CONTACTO CON LA PIEL: Lavar con agua y jabón abundantes.
  P321: Tratamiento específico (consulte las instrucciones en esta etiqueta).
  P333/313: En caso de irritación o erupción cutánea: consultar a un médico.
- La azida sódica puede ser tóxica si se ingiere o se absorbe a través de la piel o los ojos; además, puede reaccionar con las tuberías de plomo o cobre para formar azidas metálicas potencialmente explosivas. Si elimina los reactivos en un fregadero, lávelos con gran cantidad de agua para evitar la acumulación de azida.
- El sustrato de TMB contiene un irritante que es perjudicial si se inhala, se ingiere o se absorbe a través de la piel. Para evitar lesiones, evite la inhalación, la ingestión o el contacto con la piel y los ojos.
- La solución de detención consiste en una solución diluida de ácido sulfúrico. El ácido sulfúrico es venenoso, corrosivo y puede ser tóxico si se ingiere. Para evitar quemaduras químicas, evite el contacto con la piel y los ojos.
- Evite la exposición del reactivo TMB/H_2O_2 a la luz solar directa, a metales o a oxidantes. No congele la solución.

6. PRECAUCIONES

- Siga estrictamente la secuencia de pasos de pipeteado que se indica en este protocolo. Los datos de rendimiento representados en este documento se obtuvieron utilizando los reactivos específicos indicados en estas instrucciones de uso.
- Todos los reactivos deben conservarse refrigerados entre 2 y 8 °C en su envase original. Las excepciones se indican claramente.
- Deje que todos los componentes del kit y las muestras alcancen la temperatura ambiente (22-28 °C) y mezcle bien antes de usarlos.
- No intercambie componentes del kit procedentes de diferentes lotes. Debe respetarse la fecha de caducidad impresa en las etiquetas de la caja y de los viales. No utilice ningún componente del kit después de su fecha de caducidad.
- Si el usuario utiliza un equipo automatizado, tiene la responsabilidad de asegurarse de que el kit ha sido debidamente probado.
- La eliminación incompleta o imprecisa del líquido de los pocillos podría alterar la precisión del ensayo y/o aumentar el fondo. Para mejorar el rendimiento del kit

en sistemas automáticos se recomienda aumentar el número de lavados.

- Es importante que el tiempo de reacción en cada pocillo se mantenga constante para obtener resultados reproducibles. El pipeteo de las muestras no debe prolongarse más de diez minutos para evitar errores en el ensayo. Si se necesitan más de 10 minutos, siga el mismo orden de dispensación. Si se utiliza más de una placa, se recomienda repetir la curva dosis-respuesta en cada placa.
- La adición de la solución de sustrato de TMB inicia una reacción cinética, que finaliza al añadir la solución de detención. Por lo tanto, el sustrato de TMB y la solución de detención deben añadirse en la misma secuencia para eliminar las posibles desviaciones temporales durante la reacción.
- Respete las directrices para realizar el control de calidad en los laboratorios médicos mediante el ensayo de controles y/o sueros combinados.
- Se requiere la máxima precisión en la reconstitución y dispensación de los reactivos.
- No se deben usar en el ensayo muestras contaminadas microbiológicamente, muy lipémicas o hemolizadas.
- Los lectores de placas miden en vertical. No toque el fondo de los pocillos.
- **ADVERTENCIA: el reactivo conjugado está diseñado para garantizar la máxima sensibilidad de la dosis y puede contaminarse con agentes externos si no se utiliza correctamente;** por lo tanto, se recomienda utilizar consumibles desechables (puntas, frascos, bandejas, etc.). Para dosis divididas, tome la cantidad exacta de conjugado necesaria y no vuelva a introducir ningún producto de desecho en el frasco original. Además, **para las dosis dispensadas mediante dispositivos automáticos y semiautomáticos,** antes de utilizar el conjugado, es aconsejable limpiar el sistema de manipulación de fluidos, asegurándose de que los procedimientos de lavado, desproteinización y descontaminación sean eficaces para evitar la contaminación del conjugado; este procedimiento es muy recomendable cuando el kit se procesa con analizadores que no están equipados con puntas desechables.
  Para ello, DiaMetra proporciona un reactivo de descontaminación independiente para la limpieza de las agujas.

7. ALMACENAMIENTO Y ESTABILIDAD DE LOS REACTIVOS

Almacene el kit a 2-8 ºC en un lugar oscuro.

- El kit es estable a 2-8 ºC hasta la fecha de caducidad indicada en su etiqueta externa.
- Una vez abierto, el kit es estable a 2-8 °C durante 6 meses.
- La solución de lavado diluida es estable durante 30 días a 2-8 °C.

Nota importante: abra la bolsa que contiene la microplaca recubierta solo cuando esté a temperatura ambiente y ciérrela inmediatamente después de su uso.

8. RECOGIDA Y ALMACENAMIENTO DE LAS MUESTRAS

El ensayo debe realizarse usando muestras de suero (tubos de muestras estándar o tubos que contienen gel de separación de suero) o plasma (heparina de litio, heparina de sodio o EDTA de potasio).

Almacenamiento de muestras	Duración
2-8 ºC	96 horas
Ciclos de congelación/descongelación	3 ciclos

9. PROCEDIMIENTO

Preparación de calibradores y controles

Dado que no se dispone de una preparación de referencia internacional para los anticuerpos antiproteína 3, el sistema de ensayo se calibra en unidades arbitrarias relativas. Los calibradores están listos para utilizarse y tienen las siguientes concentraciones:

	C_0	C_1	C_2	C_3	C_4
AU/mL	0	10	20	40	160

Los controles están listos para su uso; la concentración del control está impresa en la etiqueta.

Preparación de la solución de lavado

Diluir el contenido del vial «10X Conc. Wash Solution» con agua destilada hasta un volumen final de 500 mL antes de usarlo. Para volúmenes más pequeños, respete la relación de dilución de 1:10.

Es posible que observe la presencia de cristales dentro de la solución de lavado concentrada; en este caso, mezcle a temperatura ambiente hasta la completa disolución de los cristales. Para una mayor precisión, diluya todo el frasco de solución de lavado concentrada a 500 mL, teniendo cuidado también de transferir los cristales enjuagando completamente el frasco y luego mezclando hasta que los cristales se disuelvan completamente.

Preparación de las muestras

Las muestras de ensayo deben ser claras. Es mejor evitar la contaminación por lipemia, pero no interfiere con este ensayo.

No se recomienda el análisis de muestras de suero o plasma inactivadas por calor.

Todas las muestras de suero o plasma deben diluirse previamente con diluyente de muestra 1:100; por ejemplo, 10 µL de muestra pueden diluirse con 990 µL de diluyente de muestra.

Procedimiento

- **Deje que todos los reactivos alcancen la temperatura ambiente (22-28 ºC) durante al menos 30 minutos.** Al finalizar el ensayo, almacene inmediatamente los reactivos a 2-8 ºC: evite la exposición prolongada a la temperatura ambiente.
- Las tiras de micropocillos recubiertas no utilizadas deben dejarse de forma segura en el envoltorio de papel de aluminio que contiene desecante y almacenarse a 2- 8 ºC.

- Para evitar que se produzca una posible contaminación microbiana y/o química, los reactivos no utilizados nunca se deberán transferir a los viales originales.
- Como es necesario realizar la determinación por duplicado para mejorar la precisión de los resultados de la prueba, prepare dos pocillos para cada punto de la curva de calibración (C_0-C_4), dos para el control, dos para cada muestra y uno para el blanco.

Reactivo	Calibrador	Muestra/ Controles	Blanco
Calibrador C_0-C_4	**100 µL**		
Controles		**100 µL**	
Muestra diluida		**100 µL**	
Incube durante 60 minutos a temperatura ambiente (22- 28 °C). Retire el contenido de cada pocillo, lave los pocillos 3 veces con 300 µL de solución de lavado diluida. **Nota importante**: en cada paso de lavado, agite ligeramente la placa durante 5 segundos y elimine el exceso de solución golpeando la placa invertida sobre un paño de papel absorbente. **Lavadora automática**: si utiliza un equipo automático, lave los pocillos al menos 5 veces.			
Conjugado	**100 µL**	**100 µL**	
Incube durante 30 minutos a temperatura ambiente (22- 28 °C). Retire el contenido de cada pocillo, lave los pocillos 3 veces con 300 µL de solución de lavado diluida. **Lavado**: siga las mismas indicaciones del punto anterior.			
Sustrato de TMB	**100 µL**	**100 µL**	**100 µL**
Incube durante 15 minutos en un lugar oscuro a temperatura ambiente (22-28 °C).			
Solución de detención	**100 µL**	**100 µL**	**100 µL**
Agite suavemente la microplaca. Compare la absorbancia (E) a 450 nm con la obtenida con una longitud de onda de referencia de 620-630 nm o con el blanco en un plazo de 5 minutos.			

10. CONTROL DE CALIDAD

Las prácticas de laboratorio recomendadas (BPL) requieren el uso de muestras de control de calidad en cada serie de ensayos para comprobar el rendimiento del ensayo. Los controles deberán tratarse como muestras desconocidas y los resultados deberán analizarse con métodos estadísticos adecuados.

Los controles incluidos en el kit deberán ser probados como desconocidos y están destinados a ayudar a evaluar la validez de los resultados obtenidos con cada placa de ensayo.

La concentración media de cada nivel de control se documenta en el informe de control de calidad que se incluye en cada kit. Los niveles de concentración media se determinan respecto de varios análisis, los cuales se realizan por duplicado en varios puntos diferentes de cada placa.

DiaMetra recomienda que los usuarios mantengan registros gráficos de los valores de control que se generan con cada ensayo, incluida la media de ejecución, la DE (desviación estándar) y el % CV. Esta información facilitará los ensayos de tendencia de los controles relacionados con el rendimiento de lotes de control actuales e históricos relativos a los datos de control de calidad proporcionados. La tendencia facilitará la identificación de los análisis que generan valores de control significativamente distintos de su intervalo medio.

Al interpretar los datos de control, los usuarios deberán tener en cuenta que este producto fue diseñado y desarrollado como un producto manual. El rango establecido en el certificado de control de calidad deberá ser adecuado para los ensayos que se realizan manualmente y en estricto cumplimiento del procedimiento de ensayo anteriormente descrito. Los profesionales del control de calidad reconocen que, como resultado de las diferencias en las condiciones y en las prácticas, siempre habrá variaciones entre laboratorios en los valores medios y en la precisión de las mediciones de control[7].

Para que los resultados de las pruebas se consideren válidos, deben cumplirse todos los criterios enumerados a continuación. Si alguno de ellos no se cumple, la prueba debe considerarse no válida y el ensayo debe repetirse:

- La absorbancia del control positivo PR-3 IgG prediluido debe ser mayor que la absorbancia del control negativo prediluido.
- Los controles negativos y positivos están destinados a detectar fallos sustanciales de los reactivos y no garantizan la precisión en el punto de corte del ensayo.
- Esta prueba solo es válida si la densidad óptica a 450 nm del control negativo y del control positivo, así como del calibrador C_0-C_4 cumple con el rango respectivo indicado en el Certificado de control de calidad que se incluye con cada kit de prueba: si no se cumple alguno de estos criterios, los resultados no son válidos y debe repetirse la prueba.

11. CÁLCULO DE LOS RESULTADOS

Hay disponibles diversos paquetes de software de reducción de datos que se pueden utilizar para generar el promedio de la curva de calibración y para calcular el promedio de las concentraciones de muestras y controles desconocidos. Es necesario un ajuste de curva logístico de 4 parámetros (4PL), **incluido el calibrador 0.** Se puede usar un ajuste de splines suavizado y un ajuste logarítmico-logarítmico que incluya el calibrador 0. No se recomiendan otros algoritmos de ajuste de curva.

También se puede preparar una curva de calibración en papel semilogarítmico mediante el trazado de la absorbancia media en el eje Y frente a la concentración de analitos en el eje X. El calibrador 0 debe incluirse en la curva de calibración. Lea el valor de absorbancia medio de cada muestra desconocida que se encuentra fuera de la curva.

12. RANGO DE MEDICIÓN

El rango de medición del ensayo (AMR) es de 3-160 AU/mL. Cualquier valor que sea inferior a 3 AU/mL debe informarse como "<3 AU/mL". Cualquier valor que sea superior a 160 AU/mL debe informarse como ">160 AU/mL".

13. METROLOGÍA Y TRAZABILIDAD

Los calibradores de este kit son trazables al Suero de Referencia Humano n.º 16 de los Centros de Control y Prevención de Enfermedades (CDC) para el Ab Anti-Proteinasa 3 con N.º de catálogo IS2721.

14. VALORES ESPERADOS

Los rangos siguientes se determinaron usando el ensayo Anti PR-3 (c-ANCA) y se facilitan solo con fines informativos. El intervalo de referencia del 90 % para adultos aparentemente sanos se calcularon mediante un método no paramétrico siguiendo la orientación de CLSI C28-A3 "Defining, Establishing and Verifying Reference Intervals in the Clinical Laboratory".

N.º de sujetos	73
Media (AU/mL)	8,71
DE (AU/mL)	3,21
Mediana (AU/mL)	8,12
Intervalo de referencia (AU/mL)	4,19-14,40

Los rangos anteriores deberán ser considerados como directrices solamente; se recomienda que cada laboratorio establezca su propio rango previsto en función de su propia población de pacientes.

15. INTERPRETACIÓN DE LOS RESULTADOS

Concentración	Interpretación
<16 AU/mL	La muestra debe considerarse negativa.
16-20 AU/mL	La muestra debe ser calificada como equívoca y la repetición de la prueba/muestreo debe realizarse de acuerdo con las prácticas internas.
≥20 AU/mL	La muestra debe considerarse positiva.

El índice y la interpretación anteriores deben tenerse en cuenta solamente como una guía. Los resultados positivos deben verificarse en función del estado clínico del paciente en su totalidad. Además, cada decisión terapéutica debe tomarse de forma individual para cada paciente.

Se recomienda que cada laboratorio establezca sus propios rangos normales y patológicos de anti-PR3.

16. CARACTERÍSTICAS DE RENDIMIENTO

Se muestran los datos de rendimiento representativos. Los resultados obtenidos en diferentes laboratorios pueden diferir.

Capacidad de detección

El límite de blanco (LoB), el límite de detección (LoD) y el límite de cuantificación (LoQ) se determinaron con orientación del documento CLSI EP17-A, "Protocols for Determination of Limits of Detection and Limits of Quantitation, usando 6 blancos y 6 muestras de bajo nivel.

Sensibilidad	Concentración
Límite de blanco (LoB)	0,6 AU/mL
Límite de detección (LoD)	1,6 AU/mL
Límite de cuantificación (LoQ)	3,2 AU/mL

Veracidad

Se ha demostrado la veracidad mediante una prueba de recuperación del ensayo Anti PR-3 (c-ANCA) con el suero de referencia de antiproteinasa 3 humana N.º 16 de los Centros de Control y Prevención de Enfermedades (CDC).

Precisión

La precisión de Anti PR-3 (c-ANCA) se determinó mediante la realización de un estudio de precisión complejo.

Repetibilidad: Se analizaron un total de 6 muestras en 5 réplicas, una vez al día durante 5 días por 3 operadores. A continuación se muestran los datos de un lote representativo:

Muestra	n	Concentración media (AU/mL)	Intraprueba (repetibilidad)	
			DE	CV %
1	75	16,51	0,96	5,8 %
2	75	23,94	0,93	3,9 %
3	75	40,03	1,23	3,1 %
4	75	59,68	3,40	5,7 %
5	75	86,96	2,80	3,2 %
6	75	136,70	6,24	4,6 %

Reproducibilidad: Se analizaron un total de 6 muestras de suero en 5 réplicas, una vez al día durante 5 días por 3 operadores

A continuación se muestran los resultados de los datos combinados de dos lotes:

Muestra	n	Concentración media (AU/mL)	Dentro del laboratorio (reproducibilidad)	
			DE	CV %
1	150	16,57	1,49	9,0 %
2	150	23,95	1,53	6,4 %
3	150	40,59	2,67	6,6 %
4	150	60,11	4,15	6,9 %
5	150	86,69	6,12	7,1 %
6	150	137,19	9,63	7,0 %

Linealidad
La linealidad se evaluó en base a "CLSI EP-06, "Evaluation of the Linearity of Quantitative Measurement Procedures". Para la concentración de anti- PR3 mediante el ensayo Anti PR-3 (c-ANCA), el procedimiento de medición muestra linealidad para el intervalo de 1,73 a 162,62 AU/mL dentro de la desviación de linealidad permitida (ADL) de ±15 %.

Sensibilidad y especificidad del diagnóstico
La sensibilidad y la especificidad se determinaron con orientación del documento CLSI EP-24 "Assessment of the Diagnostic Accuracy of Laboratory Tests Using Receiver Operating Characteristic Curves", usando 73 muestras negativas y 50 positivas realizadas en dos lotes de reactivos.

		DKO091		Total
		Positivo	Negativo	
Estado real	Positivo	50	0	**50**
	Negativo	2	71	**73**
Total		**52**	**71**	**123**

Sensibilidad del diagnóstico: 100 %

Especificidad del diagnóstico: 97,3 %

Interferencias
Las siguientes sustancias no interfieren con un sesgo de > ±15 % en el ensayo Anti PR-3 (c-ANCA) cuando las concentraciones están por debajo del umbral indicado presentado en la siguiente tabla.

Reactivos que pueden interferir	Límite máximo de concentración
Bilirrubina, conjugada	15 mg/dL
Bilirrubina, no conjugada	15 mg/dL
Hemoglobina	200 mg/dL
Proteína total	10 g/dL
Triglicéridos	500 mg/dL

Estudio en suero-plasma
El estudio de comparación de la matriz del ensayo Anti PR-3 (c-ANCA) se realizó para evaluar la diferencia entre los tipos de tubos (tubos separadores de suero [SST], plasma de heparina de litio, plasma de heparina sódica y plasma K2 EDTA) frente a las muestras de control (tapón rojo para suero, sin aditivos) siguiendo las directrices CLSI EP9-A3. Se evaluó un total de 20 muestras (16 nativas, 4 con aditivos) para cubrir el intervalo de 2,80 a 74,40 AU/mL. Se realizó un análisis de regresión lineal sobre los datos comparativos:

Tipo de muestra	Pendiente [IC del 95 %]	Intersección (AU/mL) [IC del 95 %]	Coeficiente de correlación (r)
SST	1,0 [0,97 a 1,08]	0,14 [–1,17 a 1,45]	0,99
Heparina de litio	1,1 [1,06 a 1,21]	–0,46 [–2,09 a 1,17]	0,99
Heparina sódica	1,1 [1,05 a 1,14]	–0,91 [–1,88 a 0,06]	1,00
EDTA	1,1 [1.0 a 1.12]	0,00 [–1,79 a 1,78]	0,99

17. LÍMITES DE USO
- Como en cualquier procedimiento diagnóstico, los resultados se deberán interpretar junto con los hallazgos clínicos del paciente y otra información de la que el médico disponga.
- Las características de rendimiento de este análisis no se han establecido para una población pediátrica.
- Los anticuerpos heterofílicos en el suero humano pueden presentar reacciones con las inmunoglobulinas reactivas, que interfieren con los inmunoensayos *in vitro*[11]. Los pacientes que se exponen habitualmente a animales o a productos de suero animal pueden ser propensos a esta interferencia y puede que se observen valores anómalos.

18. GESTIÓN DE RESIDUOS
Los reactivos deben eliminarse de acuerdo con la normativa local.

19. BIBLIOGRAFÍA

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20. IDENTIFICADOR DE REVISIÓN

Las adiciones o cambios en las instrucciones de uso se han resaltado en gris.

21. RECLAMACIONES SOBRE PRODUCTOS Y ASISTENCIA TÉCNICA

Para un paciente/usuario/tercero en la Unión Europea y en países con un régimen regulatorio similar: Reglamento (UE) 2017/746 sobre los productos sanitarios para diagnóstico in vitro; si, durante el uso de este dispositivo o como resultado de su uso, se ha producido un incidente grave, informe del mismo al fabricante y/o a su representante autorizado y al organismo regulador nacional.
Puede contactar con el fabricante a través del servicio de atención al cliente o del equipo de asistencia técnica. Los datos de contacto se encuentran a continuación y en el sitio web de la empresa: www.diametra.com.

Ed. 05/2022 **DCM091-9**

Fabricante legal
Dia.Metra Srl
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06038 SPELLO (PG) Italia
Tel. +39-0742-24851
Fax +39-0742-316197

DiaMetra	Packaging Information Sheet	Mod. PIS000-1

IVD	DE ES FR GB IT PT	In vitro Diagnostikum Producto sanitario para diagnóstico In vitro Dispositif medical de diagnostic in vitro In vitro Diagnostic Medical Device Dispositivo medico-diagnostico in vitro Dispositivos medicos de diagnostico in vitro		DE ES FR GB IT PT	Hergestellt von Elaborado por Fabriqué par Manufacturer Produttore Produzido por
	DE ES FR GB IT PT	Achtung, Begleitdokumente Precaución, consulte los documentos adjuntos Attention, veuillez consulter les documents d'accompagnement Caution, consult accompanying documents Attenzione, consultare la documentazione allegata Atenção,consultar os documentos de acompanhamento	 yyyy-mm	DE ES FR GB IT PT	Herstellungs datum Fecha de fabricacion Date de fabrication Date of manufacture Data di produzione Data de produção
 yyyy-mm-dd	DE ES FR GB IT PT	Verwendbar bis Establa hasta (usar antes de último día del mes) Utiliser avant (dernier jour du mois indiqué) Use by (last day of the month) Utilizzare prima del (ultimo giorno del mese) Utilizar (antes ultimo dia do mês)		DE ES FR GB IT PT	Biogefährdung Riesco biológico Risque biologique Biological risk Rischio biologico Risco biológico
	DE ES FR GB IT PT	Gebrauchsanweisung beachten Consultar las instrucciones Consulter le mode d'emploi Consult instructions for use Consultare le istruzioni per l'uso Consultar instruções para uso	LOT	DE ES FR GB IT PT	Chargenbezeichnung Codigo de lote Numero de lot Batch code Codice del lotto Codigo do lote
 Σ = xx	DE ES FR GB IT PT	Ausreichend für "n" Tests Contenido suficiente para "n" tests Contenu suffisant pour "n" tests Contains sufficient for "n" tests Contenuto sufficiente per "n" saggi Contém o suficiente para "n" testes	CONT	DE ES FR GB IT PT	Inhalt Contenido del estuche Contenu du coffret Contents of kit Contenuto del kit Conteúdo do kit
Max  Min	DE ES FR GB IT PT	Temperaturbereich Límitaciôn de temperatura Limites de température de conservation Temperature limitation Limiti di temperatura Temperaturas limites de conservação	REF	DE ES FR GB IT PT	Bestellnummer Nûmero de catálogo Référéncès du catalogue Catalogue number Numero di Catalogo Número do catálogo
	DE ES FR GB IT PT	Vor direkter sonneneinstrahlung schützen Mantener alejado de la luz solar Tenir à l'écart de la lumière du soleil Keep away from sunlight Tenere lontano dalla luce solare Mantenha longe da luz solar			

SUGGERIMENTI PER LA RISOLUZIONE DEI PROBLEMI/TROUBLESHOOTING

ERRORE CAUSE POSSIBILI/ SUGGERIMENTI

Nessuna reazione colorimetrica del saggio

- mancata dispensazione del coniugato
- contaminazione del coniugato e/o del Substrato
- errori nell'esecuzione del saggio (es. Dispensazione accidentale dei reagenti in sequenza errata o provenienti da flaconi sbagliati, etc.)

Reazione troppo blanda (OD troppo basse)

- coniugato non idoneo (es. non proveniente dal kit originale)
- tempo di incubazione troppo breve, temperatura di incubazione troppa bassa

Reazione troppo intensa (OD troppo alte)

- coniugato non idoneo (es. non proveniente dal kit originale)
- tempo di incubazione troppo lungo, temperatura di incubazione troppa alta
- qualità scadente dell'acqua usata per la soluzione di lavaggio (basso grado di deionizzazione,)
- lavaggi insufficienti (coniugato non completamente rimosso)

Valori inspiegabilmente fuori scala

- contaminazione di pipette, puntali o contenitori- lavaggi insufficienti (coniugato non completamente rimosso)

CV% intrasaggio elevato

- reagenti e/o strip non portate a temperatura ambiente prima dell'uso
- il lavatore per micropiastre non lava correttamente (suggerimento: pulire la testa del lavatore)

CV% intersaggio elevato

- condizioni di incubazione non costanti (tempo o temperatura)
- controlli e campioni non dispensati allo stesso tempo (con gli stessi intervalli) (controllare la sequenza di dispensazione)
- variabilità intrinseca degli operatori

ERROR POSSIBLE CAUSES / SUGGESTIONS

No colorimetric reaction

- no conjugate pipetted reaction after addition
- contamination of conjugates and/or of substrate
- errors in performing the assay procedure (e.g. accidental pipetting of reagents in a wrong sequence or from the wrong vial, etc.)

Too low reaction (too low ODs)

- incorrect conjugate (e.g. not from original kit)
- incubation time too short, incubation temperature too low

Too high reaction (too high ODs)

- incorrect conjugate (e.g. not from original kit)
- incubation time too long, incubation temperature too high
- water quality for wash buffer insufficient (low grade of deionization)
- insufficient washing (conjugates not properly removed)

Unexplainable outliers

- contamination of pipettes, tips or containers

insufficient washing (conjugates not properly removed) too high within-run

- reagents and/or strips not pre-warmed to CV% Room Temperature prior to use
- plate washer is not washing correctly (suggestion: clean washer head)

too high between-run - incubation conditions not constant (time, CV % temperature)

- controls and samples not dispensed at the same time (with the same intervals) (check pipetting order)
- person-related variation

ERROR / POSIBLES CAUSAS / SUGERENCIAS

No se produce ninguna reacción colorimétrica del ensayo

- no se ha dispensado el conjugado
- contaminación del conjugado y/o del substrato
- errores en la ejecución del ensayo (p. ej., dispensación accidental de los reactivos en orden incorrecto o procedentes de frascos equivocados, etc.)

Reacción escasa (DO demasiado bajas)

- conjugado no idóneo (p. ej., no procedente del kit original)
- tiempo de incubación demasiado corto, temperatura de incubación demasiado baja

Reacción demasiado intensa (DO demasiado altas)

- conjugado no idóneo (p. ej., no procedente del kit original)
- tiempo de incubación demasiado largo, temperatura de incubación demasiado alta
- calidad escasa del agua usada para la solución de lavado (bajo grado de desionización)
- lavados insuficientes (el conjugado no se ha retirado completamente)

Valores inexplicablemente fuera de escala

- contaminación de pipetas, puntas o contenedores- lavados insuficientes (el conjugado no se ha retirado completamente)

CV% intraensayo elevado

- los reactivos y/o tiras no se encontraban a temperatura ambiente antes del uso
- el lavador de microplacas no funciona correctamente (sugerencia: limpiar el cabezal del lavador)

CV% interensayo elevado

- condiciones de incubación no constantes (tiempo o temperatura)
- controles y muestras no dispensados al mismo tiempo (con los mismos intervalos) (controlar la secuencia de dispensación)
- variación en función de los operadores

ERREUR CAUSES POSSIBLES / SUGGESTIONS

Aucune réaction colorimétrique de l'essai

- non distribution du conjugué
- contamination du conjugué et/ou du substrat
- erreurs dans l'exécution du dosage (par ex., distribution accidentelle des réactifs dans le mauvais ordre ou en provenance des mauvais flacons, etc.)

Réaction trop faible (DO trop basse)

- conjugué non approprié (par ex., ne provenant pas du coffret original)
- temps d'incubation trop court, température d'incubation trop basse

Réaction trop intense (DO trop élevée)

- conjugué non approprié (par ex., ne provenant pas du coffret original)
- temps d'incubation trop long, température d'incubation trop élevée
- mauvaise qualité de l'eau utilisée pour la solution de lavage (bas degré de déionisation)
- lavages insuffisants (conjugué non entièrement éliminé)

Valeurs inexplicablement hors plage

- contamination des pipettes, embouts ou récipients - lavages insuffisants (conjugué non entièrement éliminé)

CV% intra-essai élevé

- les réactifs et/ou les bandes n'ont pas atteint la température ambiante avant usage
- le laveur de microplaques ne lave pas correctement (suggestion : nettoyer la tête du laveur)

CV% inter-essai élevé

- conditions d'incubation non constantes (temps ou température)
- contrôles et échantillons non distribués en même temps (avec les mêmes intervalles) (contrôler l'ordre de distribution)
- variabilité intrinsèque des opérateurs

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511_3

ORG 511 Anti-RNP/Sm

INTENDED PURPOSE

Anti-RNP/Sm is an ELISA test system for the quantitative measurement of IgG class autoantibodies against RNP/Sm in human serum or plasma. This product is intended for professional in vitro diagnostic use only.

Antibodies against the RNP/Sm complex are useful in the diagnosis of mixed connective tissue disorder (MCTD, Sharp syndrome) and related autoimmune diseases. Antibodies against the 70 kDa protein of this complex are a very specific marker for Sharp syndrome. The Sm proteins are recognised by antibodies that may occur in cases of mixed connective tissue disorder and systemic lupus erythematosus. Evaluation of a test result should always take into account all clinical and laboratory diagnostic findings.

SYMBOLS USED ON LABELS

IVD	In vitro diagnostic medical device
	Manufacturer
REF	Catalogue number
Σ 96	Sufficient for 96 determinations
LOT	Batch code
	Use by
2°C 8°C	Temperature limitation
	Keep away from sunlight
	Do not reuse
	Date of manufacture
CE	CE marked according to 98/79/EC
	Consult instructions for use
511_3	Electronic Instruction For Use: version

MICROPLATE	Microplate
CALIBRATOR A	Calibrator
CALIBRATOR B	Calibrator
CALIBRATOR C	Calibrator
CALIBRATOR D	Calibrator
CALIBRATOR E	Calibrator
CALIBRATOR F	Calibrator
CONTROL +	Control positive
CONTROL −	Control negative
DILUENT	Sample Buffer P
CONJUGATE	Enzyme Conjugate
TMB	TMB Substrate
STOP	Stop solution
WASH	Wash Buffer
RTU	Ready to use

PRINCIPLE OF THE TEST

Highly purified RNP/Sm is bound to microwells.
The determination is based on an indirect enzyme linked immune reaction with the following steps:
Specific antibodies in the patient sample bind to the antigen coated on the surface of the reaction wells. After incubation, a washing step removes unbound and unspecifically bound serum or plasma components. Subesquently added enzyme conjugate binds to the immobilized antibody-antigen-complexes. After incubation, a second washing step removes unbound enzyme conjugate. After addition of substrate solution the bound enzyme conjugate hydrolyses the substrate forming a blue coloured product. Addition of an acid stopps the reaction generating a yellow end-product. The intensity of the yellow color
correlates with the concentration of the antibody-antigen-complex and can be measured photometrically at 450 nm.

WARNINGS AND PRECAUTIONS

- All reagents of this kit are intended for professional in vitro diagnostic use only.
- Components containing human serum were tested and found negative for HBsAg, HCV, HIV1 and HIV2 by FDA approved methods. No test can guarantee the absence of HBsAg, HCV, HIV1 or HIV2, and so all human serum based reagents in this kit must be handled as though capable of transmitting infection.
- Bovine serum albumin (BSA) used in components has been tested for BSE and found negative.
- Avoid contact with the substrate TMB (3,3´,5,5´-Tetramethyl-benzidine).
- Stop solution contains acid, classifiaction is non-hazardous. Avoid contact with skin.
- Control, sample buffer and wash buffer contain sodium azide 0.09% as preservative. This concentration is classified as non-hazardous.
- Enzyme conjugate contains ProClin 300 0.05% as preservative. This concentration is classified as non-hazardous.

During handling of all reagents, controls and serum samples observe the existing regulations for laboratory safety regulations and good laboratory practice:

- First aid measures: In case of skin contact, immediately wash thoroughly with water and soap. Remove contaminated clothing and shoes and wash before reuse. If system fluid comes into contact with skin, wash thoroughly with water. After contact with the eyes carefully rinse the opened eye with running water for at least 10 minutes. Get medical attention if necessary.
- Personal precautions, protective equipment and emergency procedures:

Observe laboratory safety regulations. Avoid contact with skin and eyes. Do not swallow. Do not pipette by mouth. Do not eat, drink, smoke or apply makeup in areas where specimens or kit reagents are handled. When spilled, absorb with an inert material and put the spilled material in an appropriate waste disposal.

- Exposure controls / personal protection: Wear protective gloves of nitril rubber or natural latex. Wear protective glasses. Used according to intended use no dangerous reactions known.
- Conditions to avoid: Since substrate solution is light-sensitive. Store in the dark.
- For disposal of laboratory waste the national or regional legislation has to be observed.

Observe the guidelines for performing quality control in medical laboratories by assaying control sera.

CONTENTS OF THE KIT

ORG 511	Σ 96	Sufficient for 96 determinations
MICROPLATE	1	One divisible microplate consisting of 12 modules of 8 wells each. Ready to use. Color code on module
CALIBRATOR A	1x 1.5 ml	Calibrator A 0 U/ml, containing serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use.
CALIBRATOR B	1x 1.5 ml	Calibrator B 12.5 U/ml, containing RNP/Sm antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use.
CALIBRATOR C	1x 1.5 ml	Calibrator C 25 U/ml, containing RNP/Sm antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use.
CALIBRATOR D	1x 1.5 ml	Calibrator D 50 U/ml, containing RNP/Sm antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use.
CALIBRATOR E	1x 1.5 ml	Calibrator E 100 U/ml, containing RNP/Sm antibodies in a serum/buffer matrix (PBS, BSA, NaN3 0.09%), yellow. Ready to use.
CALIBRATOR F	1x 1.5 ml	Calibrator F 200 U/ml, containing RNP/Sm antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use.
CONTROL +	1x 1.5 ml	Control positive, containing RNP/Sm antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use. The concentration is specified on the certificate of analysis.
CONTROL −	1x 1.5 ml	Control negative, containing RNP/Sm antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use. The concentration is specified on the certificate of analysis.
DILUENT	20 ml	Sample Buffer P, containing PBS, BSA, detergent, preservative sodium azide 0.09%, yellow, concentrate (5 x).
CONJUGATE	15 ml	Enzyme Conjugate containing anti-human IgG antibodies, HRP labelled; PBS, BSA, detergent, preservative PROCLIN 0.05%, light red. Ready to use.
TMB	15 ml	TMB Substrate; containing 3,3', 5,5'- Tetramethylbenzidin, colorless. Ready to use.
STOP	15 ml	Stop solution; contains acid. Ready to use.
WASH	20 ml	Wash Buffer, containing Tris, detergent, preservative sodium azide 0.09%; 50 x conc.
	1	Certificate of Analysis

MATERIALS REQUIRED

- Microplate reader capable of endpoint measurements at 450 nm; optional: reference filter at 620 nm
- Data reduction software
- Multi-channel dispenser or repeatable pipette for 100 µl
- Vortex mixer
- Pipettes for 10 µl, 100 µl and 1000 µl
- Laboratory timing device
- Distilled or deionised water
- Measuring cylinder for 1000 ml and 100 ml
- Plastic container for storage of the wash solution

This ELISA assay is suitable for use on open automated ELISA processors. Each assay has to be validated on the respective automated system. Detailed information is provided upon request.

SPECIMEN COLLECTION, STORAGE AND HANDLING

- Collect whole blood specimens using acceptable medical techniques to avoid hemolysis.
- Allow blood to clot and separate the serum or plasma by centrifugation.
- Test serum should be clear and non-hemolyzed. Contamination by hemolysis or lipemia should be avoided, but does not interfere with this assay.
- Specimens may be refrigerated at 2-8°C for up to five days or stored at -20°C up to six months.
- Avoid repetitive freezing and thawing of serum or plasma samples. This may result in variable loss of antibody activity.
- Testing of heat-inactivated sera is not recommended.

STORAGE AND STABILITY

- Store test kit at 2-8°C in the dark.
- Do not expose reagents to heat, sun, or strong light during storage and usage.
- Store microplate sealed and dessicated in the clip bag provided.
- Shelf life of the unopended test kit is 18 months from day of production. Unopened reagents are stable until expiration of the kit. See labels for individual batch.
- Diluted Wash Buffer and Sample Buffer are stable for at least 30 days when stored at 2-8°C. We recommend consumption on the same day.

PROCEDURAL NOTES

- Do not use kit components beyond their expiration dates.
- Do not interchange kit components from different lots and products.
- All materials must be at room temperature (20-28°C) prior to use.
- Prepare all reagents and samples. Once started, performe the test without interruption.
- Double determinations may be done. By this means pipetting errors may become obvious.
- Perform the assay steps only in the order indicated.
- Always use fresh sample dilutions.
- Pipette all reagents and samples into the bottom of the wells.
- To avoid carryover or contamination, change the pipette tip between samples and different kit controls.
- Wash microwells thoroughly and remove the last droplets of wash buffer.
- All incubation steps must be accurately timed.
- Do not re-use microplate wells.

PREPARATION OF REAGENTS

WASH

Dilute the contents of one vial of the buffered wash solution concentrate (50x) with distilled or deionised water to a final volume of 1000 ml prior to use.

DILUENT

Sample Buffer P: Prior to use dilute the contents (20 ml) of one vial of sample buffer 5x concentrate with distilled or deionised water to a final volume of 100 ml.

Preparation of samples

Dilute patient samples 1:100 before the assay: Put 990 µl of prediluted sample buffer in a polystyrene tube and add 10 µl of sample. Mix well. Note: Calibrators / Controls are ready to use and need not be diluted.

TEST PROCEDURE

Prepare enough microplate modules for all calibrators / controls and patient samples.

1. Pipette **100 µl** of calibrators, controls and prediluted patient samples into the wells.
   Incubate for **30 minutes** at room temperature (20-28 °C).
   Discard the contents of the microwells and **wash 3 times** with **300 µl** of wash solution.
2. Dispense **100 µ**l of enzyme conjugate into each well.
   Incubate for **15 minutes** at room temperature.
   Discard the contents of the microwells and **wash 3 times** with **300 µl** of wash solution.
3. Dispense **100 µ**l of TMB substrate solution into each well.
   Incubate for **15 minutes** at room temperature
4. **Add 100 µl** of stop solution to each well of the modules
   Incubate for **5 minutes** at room temperature.
   Read the optical density at 450 nm (reference 600-690nm) and calculate the results.
   The developed colour is stable for at least 30 minutes. Read during this time.

Example for a pipetting scheme:

	1	2	3	4	5	6	7	8	9	10	11	12
A	A	P1										
B	B	P2										
C	C	P3										
D	D											
E	E											
F	F											
G	C+											
H	C-											

P1, ... patient sample A-F calibrators C+, C- controls

VALIDATION

Test results are valid if the optical densities at 450 nm for calibrators / controls and the results for controls comply with the reference ranges indicated on the Certificate of Analysis enclosed in each test kit.
If these quality control criteria are not met the assay run is invalid and should be repeated.

CALCULATION OF RESULTS

For quantitative results plot the optical density of each calibrator versus the calibrator concentration to create a calibration curve. The concentration of patient samples may then be estimated from the calibration curve by interpolation.
Using data reduction software a 4-Parameter-Fit with lin-log coordinates for optical density and concentration is the data reduction method of choice.

PERFORMANCE CHARACTERISTICS

Calibration

The assay system is calibrated against the internationally recognized reference sera from CDC, Atlanta USA.

Measuring range

The calculation range of this ELISA assay is 0 - 200 U/ml

Expected values

In a normal range study with samples from healthy blood donors the following ranges have been established with this ELISA assay: Cut-off 25 U/ml

Interpretation of results

Negative:	< 15 U/ml
Borderline:	15 - 25 U/ml
Positive:	> 25 U/ml

Linearity

Patient samples containing high levels of specific antibody were serially diluted in sample buffer to demonstrate the dynamic range of the assay and the upper / lower end of linearity. Activity for each dilution was calculated from the calibration curve using a 4-Parameter-Fit with lin-log coordinates.

Sample	Dilution	Observed U/ml	Expected U/ml	O/E [%]
1	1:100	161.4	161.4	100
.	1:200	78.0	80.7	97
.	1:400	39.7	40.4	98
.	1:800	20.1	20.2	100
2	1:100	167.2	167.2	100
.	1:200	83.7	83.6	100
.	1:400	41.5	41.8	99
.	1:800	20.8	20.9	100

Limit of detection

Functional sensitivity was determined to be: 1 U/ml

Reproducibility

Intra-assay precision: Coefficient of variation (CV) was calculated for each of three samples from the results of 24 determinations in a single run. Results for precision-within-assay are shown in the table below.
Inter-assay precision: Coefficient of variation (CV) was calculated for each of three samples from the results of 6 determinations in 5 different runs. Results for run-to-run precision are shown in the table below.

Intra-Assay		
Sample	Mean U/ml	CV %
1	65.6	4.1
2	101.9	5.9
3	182.0	1.8

Inter-Assay		
Sample	Mean U/ml	CV %
1	33.3	4.2
2	109.0	3.1
3	176.8	2.9

Interfering substances

No interference has been observed with haemolytic (up to 1000 mg/dl) or lipemic (up to 3 g/dl triglycerides) sera or plasma, or bilirubin (up to 40 mg/dl) containing sera or plasma. Nor have any interfering effects been observed with the use of anticoagulants (Citrate, EDTA, Heparine). However for practical reasons it is recommended that grossly hemolyzed or lipemic samples should be avoided.

Study results

Study population	n	n Pos	%
SLE	70	37	52.9
MCTD	30	29	96.7
Rheumatoid arthritis	20	3	15.0
Normal human sera	100	2	2.0

		Clinical Diagnosis POS	NEG	
ORG 511	POS	66	5	
	NEG	34	115	
		100	120	220

Sensitivity: 66.0 %
Specificity: 95.8 %
Overall agreement: 82.3 %

LIMITATIONS OF THE PROCEDURE

This assay is a diagnostic aid. A definite clinical diagnosis should not be based on the results of a single test, but should be made by the physician after all clinical and laboratory findings have been evaluated concerning the entire clinical picture of the patient. Also every decision for therapy should be taken individually.

The above pathological and normal reference ranges for antibodies in patient samples should be regarded as recommendations only. Each laboratory should establishe its own ranges according to ISO 15189 or other applicable laboratory guidelines.

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Notice to the user (European Union):

Any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the EU Member State in which the user and/or the patient is established .

Change Control

Former version: *ORG 511_IFU_EN_QM113138_2013-12-16_1.2* Reason for revision: *Introduction electronic IFU on homepage*

1. Pipet **100 µl** calibrator, control or patient sample
   → Incubate for **30 minutes** at room temperature
   → Discard the contents of the wells and wash 3 times with **300 µl** wash solution
2. Pipet **100 µl** enzyme conjugate
   → Incubate for **15 minutes** at room temperature
   → Discard the contents of the wells and wash 3 times with **300 µl** wash solution
3. Pipet **100 µl** substrate solution
   → Incubate for **15 minutes** at room temperature
4. Add **100 µl** stop solution
   → Leave untouched for **5 minutes**
   → Read at **450 nm**

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633_3

ORG 633 Anti-Centromere B

INTENDED PURPOSE

Anti-Centromere B is an ELISA test system for the quantitative measurement of IgG class autoantibodies against centromere B in human serum or plasma. This product is intended for professional in vitro diagnostic use only.

The test is used as an aid in the differential diagnosis of inflammatory autoimmune diseases, e.g. CREST syndrome. Evaluation of a test result should always take into account all clinical and laboratory diagnostic findings.

SYMBOLS USED ON LABELS

IVD	In vitro diagnostic medical device
	Manufacturer
REF	Catalogue number
96	Sufficient for 96 determinations
LOT	Batch code
	Use by
2°C 8°C	Temperature limitation
	Keep away from sunlight
	Do not reuse
	Date of manufacture
CE	CE marked according to 98/79/EC
	Consult instructions for use
633_3	Electronic Instruction For Use: version

MICROPLATE	Microplate
CALIBRATOR A	Calibrator
CALIBRATOR B	Calibrator
CALIBRATOR C	Calibrator
CALIBRATOR D	Calibrator
CALIBRATOR E	Calibrator
CONTROL +	Control positive
CONTROL −	Control negative
DILUENT	Sample Buffer P
CONJUGATE	Enzyme Conjugate
TMB	TMB Substrate
STOP	Stop solution
WASH	Wash Buffer
RTU	Ready to use

PRINCIPLE OF THE TEST

Recombinant centromere protein B is bound to microwells.
The determination is based on an indirect enzyme linked immune reaction with the following steps:
Specific antibodies in the patient sample bind to the antigen coated on the surface of the reaction wells. After incubation, a washing step removes unbound and unspecifically bound serum or plasma components. Subesquently added enzyme conjugate binds to the immobilized antibody-antigen-complexes. After incubation, a second washing step removes unbound enzyme conjugate. After addition of substrate solution the bound enzyme conjugate hydrolyses the substrate forming a blue coloured product. Addition of an acid stopps the reaction generating a yellow end-product. The intensity of the yellow color
correlates with the concentration of the antibody-antigen-complex and can be measured photometrically at 450 nm.

WARNINGS AND PRECAUTIONS

- All reagents of this kit are intended for professional in vitro diagnostic use only.
- Components containing human serum were tested and found negative for HBsAg, HCV, HIV1 and HIV2 by FDA approved methods. No test can guarantee the absence of HBsAg, HCV, HIV1 or HIV2, and so all human serum based reagents in this kit must be handled as though capable of transmitting infection.
- Bovine serum albumin (BSA) used in components has been tested for BSE and found negative.
- Avoid contact with the substrate TMB (3,3´,5,5´-Tetramethyl-benzidine).
- Stop solution contains acid, classifiaction is non-hazardous. Avoid contact with skin.
- Control, sample buffer and wash buffer contain sodium azide 0.09% as preservative. This concentration is classified as non-hazardous.
- Enzyme conjugate contains ProClin 300 0.05% as preservative. This concentration is classified as non-hazardous.

During handling of all reagents, controls and serum samples observe the existing regulations for laboratory safety regulations and good laboratory practice:

- First aid measures: In case of skin contact, immediately wash thoroughly with water and soap. Remove contaminated clothing and shoes and wash before reuse. If system fluid comes into contact with skin, wash thoroughly with water. After contact with the eyes carefully rinse the opened eye with running water for at least 10 minutes. Get medical attention if necessary.
- Personal precautions, protective equipment and emergency procedures:

Observe laboratory safety regulations. Avoid contact with skin and eyes. Do not swallow. Do not pipette by mouth. Do not eat, drink, smoke or apply makeup in areas where specimens or kit reagents are handled. When spilled, absorb with an inert material and put the spilled material in an appropriate waste disposal.

- Exposure controls / personal protection: Wear protective gloves of nitril rubber or natural latex. Wear protective glasses. Used according to intended use no dangerous reactions known.
- Conditions to avoid: Since substrate solution is light-sensitive. Store in the dark.
- For disposal of laboratory waste the national or regional legislation has to be observed.

Observe the guidelines for performing quality control in medical laboratories by assaying control sera.

CONTENTS OF THE KIT

ORG 633	Σ 96	Sufficient for 96 determinations
MICROPLATE	1	One divisible microplate consisting of 12 modules of 8 wells each. Ready to use. Product code on module: ***CEN***
CALIBRATOR A	1x 1.5 ml	Calibrator A 0 U/ml, containing serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use.
CALIBRATOR B	1x 1.5 ml	Calibrator B 10 U/ml, containing centromere B antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use.
CALIBRATOR C	1x 1.5 ml	Calibrator C 30 U/ml, containing centromere B antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use.
CALIBRATOR D	1x 1.5 ml	Calibrator D 100 U/ml, containing centromere B antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use.
CALIBRATOR E	1x 1.5 ml	Calibrator E 300 U/ml, containing centromere B antibodies in a serum/buffer matrix (PBS, BSA, NaN3 0.09%), yellow. Ready to use.
CONTROL +	1x 1.5 ml	Control positive, containing centromere B antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use. The concentration is specified on the certificate of analysis.
CONTROL −	1x 1.5 ml	Control negative, containing centromere B antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use. The concentration is specified on the certificate of analysis.
DILUENT	20 ml	Sample Buffer P, containing PBS, BSA, detergent, preservative sodium azide 0.09%, yellow, concentrate (5 x).
CONJUGATE	15 ml	Enzyme Conjugate containing anti-human IgG antibodies, HRP labelled; PBS, BSA, detergent, preservative PROCLIN 0.05%, light red. Ready to use.
TMB	15 ml	TMB Substrate; containing 3,3', 5,5'- Tetramethylbenzidin, colorless. Ready to use.
STOP	15 ml	Stop solution; contains acid. Ready to use.
WASH	20 ml	Wash Buffer, containing Tris, detergent, preservative sodium azide 0.09%; 50 x conc.
	1	Certificate of Analysis

MATERIALS REQUIRED

- Microplate reader capable of endpoint measurements at 450 nm; optional: reference filter at 620 nm
- Data reduction software
- Multi-channel dispenser or repeatable pipette for 100 µl
- Vortex mixer
- Pipettes for 10 µl, 100 µl and 1000 µl
- Laboratory timing device
- Distilled or deionised water
- Measuring cylinder for 1000 ml and 100 ml
- Plastic container for storage of the wash solution

This ELISA assay is suitable for use on open automated ELISA processors. Each assay has to be validated on the respective automated system. Detailed information is provided upon request.

SPECIMEN COLLECTION, STORAGE AND HANDLING

- Collect whole blood specimens using acceptable medical techniques to avoid hemolysis.
- Allow blood to clot and separate the serum or plasma by centrifugation.
- Test serum should be clear and non-hemolyzed. Contamination by hemolysis or lipemia should be avoided, but does not interfere with this assay.
- Specimens may be refrigerated at 2-8°C for up to five days or stored at -20°C up to six months.
- Avoid repetitive freezing and thawing of serum or plasma samples. This may result in variable loss of antibody activity.
- Testing of heat-inactivated sera is not recommended.

STORAGE AND STABILITY

- Store test kit at 2-8°C in the dark.
- Do not expose reagents to heat, sun, or strong light during storage and usage.

- Store microplate sealed and dessicated in the clip bag provided.
- Shelf life of the unopended test kit is 18 months from day of production. Unopened reagents are stable until expiration of the kit. See labels for individual batch.
- Diluted Wash Buffer and Sample Buffer are stable for at least 30 days when stored at 2-8°C. We recommend consumption on the same day.

PROCEDURAL NOTES

- Do not use kit components beyond their expiration dates.
- Do not interchange kit components from different lots and products.
- All materials must be at room temperature (20-28°C) prior to use.
- Prepare all reagents and samples. Once started, performe the test without interruption.
- Double determinations may be done. By this means pipetting errors may become obvious.
- Perform the assay steps only in the order indicated.
- Always use fresh sample dilutions.
- Pipette all reagents and samples into the bottom of the wells.
- To avoid carryover or contamination, change the pipette tip between samples and different kit controls.
- Wash microwells thoroughly and remove the last droplets of wash buffer.
- All incubation steps must be accurately timed.
- Do not re-use microplate wells.

PREPARATION OF REAGENTS

WASH

Dilute the contents of one vial of the buffered wash solution concentrate (50x) with distilled or deionised water to a final volume of 1000 ml prior to use.

DILUENT

Sample Buffer P: Prior to use dilute the contents (20 ml) of one vial of sample buffer 5x concentrate with distilled or deionised water to a final volume of 100 ml.

Preparation of samples

Dilute patient samples 1:100 before the assay: Put 990 µl of prediluted sample buffer in a polystyrene tube and add 10 µl of sample. Mix well. Note: Calibrators / Controls are ready to use and need not be diluted.

TEST PROCEDURE

Prepare enough microplate modules for all calibrators / controls and patient samples.

1. Pipette **100 µl** of calibrators, controls and prediluted patient samples into the wells.
   Incubate for **30 minutes** at room temperature (20-28 °C).
   Discard the contents of the microwells and **wash 3 times** with **300 µl** of wash solution.
2. Dispense **100 µ**l of enzyme conjugate into each well.
   Incubate for **15 minutes** at room temperature.
   Discard the contents of the microwells and **wash 3 times** with **300 µl** of wash solution.
3. Dispense **100 µ**l of TMB substrate solution into each well.
   Incubate for **15 minutes** at room temperature
4. **Add 100 µl** of stop solution to each well of the modules
   Incubate for **5 minutes** at room temperature.
   Read the optical density at 450 nm (reference 600-690nm) and calculate the results.
   The developed colour is stable for at least 30 minutes. Read during this time.

Example for a pipetting scheme:

	1	2	3	4	5	6	7	8	9	10	11	12
A	A	P2										
B	B	P3										
C	C											
D	D											
E	E											
F	C+											
G	C-											
H	P1											

P1, ... patient sample A-E calibrators C+, C- controls

VALIDATION

Test results are valid if the optical densities at 450 nm for calibrators / controls and the results for controls comply with the reference ranges indicated on the Certificate of Analysis enclosed in each test kit.
If these quality control criteria are not met the assay run is invalid and should be repeated.

CALCULATION OF RESULTS

For quantitative results plot the optical density of each calibrator versus the calibrator concentration to create a calibration curve. The concentration of patient samples may then be estimated from the calibration curve by interpolation. Using data reduction software a 4-Parameter-Fit with lin-log coordinates for optical density and concentration is the data reduction method of choice.

PERFORMANCE CHARACTERISTICS

Calibration

This assay system is calibrated in relative arbitrary units, since no international reference preparation is available for this assay.

Measuring range

The calculation range of this ELISA assay is 0 - 300 U/ml

Expected values

In a normal range study with samples from healthy blood donors the following ranges have been established with this ELISA assay: Cut-off 10 U/ml

Interpretation of results

Negative: < 10 U/ml
Positive: ≥ 10 U/ml

Linearity

Patient samples containing high levels of specific antibody were serially diluted in sample buffer to demonstrate the dynamic range of the assay and the upper / lower end of linearity. Activity for each dilution was calculated from the calibration curve using a 4-Parameter-Fit with lin-log coordinates.

Sample	Dilution	Observed U/ml	Expected U/ml	O/E [%]
1	1:100	136.8	136.8	100
.	1:200	67.1	68.4	98
.	1:400	35.2	34.2	103
.	1:800	16.9	17.1	99
2	1:100	285.0	285.0	100
.	1:200	139.2	142.5	98
.	1:400	73.5	71.3	103
.	1:800	37.0	35.6	104

Limit of detection

Functional sensitivity was determined to be: 1 U/ml

Reproducibility

Intra-assay precision: Coefficient of variation (CV) was calculated for each of three samples from the results of 24 determinations in a single run. Results for precision-within-assay are shown in the table below.
Inter-assay precision: Coefficient of variation (CV) was calculated for each of three samples from the results of 6 determinations in 5 different runs. Results for run-to-run precision are shown in the table below.

Intra-Assay		
Sample	Mean U/ml	CV %
1	15.2	5.4
2	122.0	4.4
3	220.0	4.7

Inter-Assay		
Sample	Mean U/ml	CV %
1	16.4	5.4
2	125.6	5.0
3	225.4	4 2

Interfering substances

No interference has been observed with haemolytic (up to 1000 mg/dl) or lipemic (up to 3 g/dl triglycerides) sera or plasma, or bilirubin (up to 40 mg/dl) containing sera or plasma. Nor have any interfering effects been observed with the use of anticoagulants (Citrate, EDTA, Heparine). However for practical reasons it is recommended that grossly hemolyzed or lipemic samples should be avoided.

Study results

Study population	n	n Pos	%
CREST syndrome	32	31	96.9
Rheumatoid arthritis	20	1	5.0
Normal human sera	100	6	6.0

		Clinical Diagnosis POS	NEG	
ORG 633	POS	31	7	
	NEG	1	113	
		32	120	152

Sensitivity: 96.9 %
Specificity: 94.2 %
Overall agreement: 94.7 %

LIMITATIONS OF THE PROCEDURE

This assay is a diagnostic aid. A definite clinical diagnosis should not be based on the results of a single test, but should be made by the physician after all clinical and laboratory findings have been evaluated concerning the entire clinical picture of the patient. Also every decision for therapy should be taken individually.

The above pathological and normal reference ranges for antibodies in patient samples should be regarded as recommendations only. Each laboratory should establishe its own ranges according to ISO 15189 or other applicable laboratory guidelines.

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Notice to the user (European Union):

Any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the EU Member State in which the user and/or the patient is established .

Change Control

Former version: *ORG 633_IFU_EN_QM113209_2016-08-16_1.3* Reason for revision: *Introduction electronic IFU on homepage*

1. Pipet **100 µl** calibrator, control or patient sample
   → Incubate for **30 minutes** at room temperature
   → Discard the contents of the wells and wash 3 times with **300 µl** wash solution
2. Pipet **100 µl** enzyme conjugate
   → Incubate for **15 minutes** at room temperature
   → Discard the contents of the wells and wash 3 times with **300 µl** wash solution
3. Pipet **100 µl** substrate solution
   → Incubate for **15 minutes** at room temperature
4. Add **100 µl** stop solution
   → Leave untouched for **5 minutes**
   → Read at **450 nm**

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516_4

ORG 516 AMA-M2

INTENDED PURPOSE

AMA-M2 is an ELISA test system for the quantitative measurement of IgG class autoantibodies against mitochondrial M2 subtype antigen in human serum or plasma. This product is intended for professional in vitro diagnostic use only.

The test is used as an aid in the differential diagnosis of primary biliary cirrhosis (PBC). In patients with other autoimmune diseases occurrence of AMA antibodies may be related to the development or association of PBC. Evaluation of a test result should always take into account all clinical and laboratory diagnostic findings.

SYMBOLS USED ON LABELS

IVD	In vitro diagnostic medical device
	Manufacturer
REF	Catalogue number
Σ 96	Sufficient for 96 determinations
LOT	Batch code
	Use by
2°C 8°C	Temperature limitation
	Keep away from sunlight
	Do not reuse
	Date of manufacture
CE	CE marked according to 98/79/EC
	Consult instructions for use
516_4	Electronic Instruction For Use: version

MICROPLATE	Microplate
CALIBRATOR A	Calibrator
CALIBRATOR B	Calibrator
CALIBRATOR C	Calibrator
CALIBRATOR D	Calibrator
CALIBRATOR E	Calibrator
CALIBRATOR F	Calibrator
CONTROL +	Control positive
CONTROL −	Control negative
DILUENT	Sample Buffer P
CONJUGATE	Enzyme Conjugate
TMB	TMB Substrate
STOP	Stop solution
WASH	Wash Buffer
RTU	Ready to use

PRINCIPLE OF THE TEST

Highly purified mitochondrial M2 subtype (PDC-E2, BCOADC-E2, OGDC-E2) antigen is bound to microwells.
The determination is based on an indirect enzyme linked immune reaction with the following steps:
Specific antibodies in the patient sample bind to the antigen coated on the surface of the reaction wells. After incubation, a washing step removes unbound and unspecifically bound serum or plasma components. Subesquently added enzyme conjugate binds to the immobilized antibody-antigen-complexes. After incubation, a second washing step removes unbound enzyme conjugate. After addition of substrate solution the bound enzyme conjugate hydrolyses the substrate forming a blue coloured product. Addition of an acid stopps the reaction generating a yellow end-product. The intensity of the yellow color
correlates with the concentration of the antibody-antigen-complex and can be measured photometrically at 450 nm.

WARNINGS AND PRECAUTIONS

- All reagents of this kit are intended for professional in vitro diagnostic use only.
- Components containing human serum were tested and found negative for HBsAg, HCV, HIV1 and HIV2 by FDA approved methods. No test can guarantee the absence of HBsAg, HCV, HIV1 or HIV2, and so all human serum based reagents in this kit must be handled as though capable of transmitting infection.
- Bovine serum albumin (BSA) used in components has been tested for BSE and found negative.
- Avoid contact with the substrate TMB (3,3´,5,5´-Tetramethyl-benzidine).
- Stop solution contains acid, classifiaction is non-hazardous. Avoid contact with skin.
- Control, sample buffer and wash buffer contain sodium azide 0.09% as preservative. This concentration is classified as non-hazardous.
- Enzyme conjugate contains ProClin 300 0.05% as preservative. This concentration is classified as non-hazardous.

During handling of all reagents, controls and serum samples observe the existing regulations for laboratory safety regulations and good laboratory practice:

- First aid measures: In case of skin contact, immediately wash thoroughly with water and soap. Remove contaminated clothing and shoes and wash before reuse. If system fluid comes into contact with skin, wash thoroughly with water. After contact with the eyes carefully rinse the opened eye with running water for at least 10 minutes. Get medical attention if necessary.
- Personal precautions, protective equipment and emergency procedures:

Observe laboratory safety regulations. Avoid contact with skin and eyes. Do not swallow. Do not pipette by mouth. Do not eat, drink, smoke or apply makeup in areas where specimens or kit reagents are handled. When spilled, absorb with an inert material and put the spilled material in an appropriate waste disposal.

- Exposure controls / personal protection: Wear protective gloves of nitril rubber or natural latex. Wear protective glasses. Used according to intended use no dangerous reactions known.
- Conditions to avoid: Since substrate solution is light-sensitive. Store in the dark.
- For disposal of laboratory waste the national or regional legislation has to be observed.

Observe the guidelines for performing quality control in medical laboratories by assaying control sera.

CONTENTS OF THE KIT

ORG 516	Σ 96	Sufficient for 96 determinations
MICROPLATE	1	One divisible microplate consisting of 12 modules of 8 wells each. Ready to use. Product code on module: ***AMA***
CALIBRATOR A	1x 1.5 ml	Calibrator A 0 IU/ml, containing serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use.
CALIBRATOR B	1x 1.5 ml	Calibrator B 12.5 IU/ml, containing AMA-M2 antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use.
CALIBRATOR C	1x 1.5 ml	Calibrator C 25 IU/ml, containing AMA-M2 antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use.
CALIBRATOR D	1x 1.5 ml	Calibrator D 50 IU/ml, containing AMA-M2 antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use.
CALIBRATOR E	1x 1.5 ml	Calibrator E 100 IU/ml, containing AMA-M2 antibodies in a serum/buffer matrix (PBS, BSA, NaN3 0.09%), yellow. Ready to use.
CALIBRATOR F	1x 1.5 ml	Calibrator F 200 IU/ml, containing AMA-M2 antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use.
CONTROL +	1x 1.5 ml	Control positive, containing AMA-M2 antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use. The concentration is specified on the certificate of analysis.
CONTROL −	1x 1.5 ml	Control negative, containing AMA-M2 antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use. The concentration is specified on the certificate of analysis.
DILUENT	20 ml	Sample Buffer P, containing PBS, BSA, detergent, preservative sodium azide 0.09%, yellow, concentrate (5 x).
CONJUGATE	15 ml	Enzyme Conjugate containing anti-human IgG antibodies, HRP labelled; PBS, BSA, detergent, preservative PROCLIN 0.05%, light red. Ready to use.
TMB	15 ml	TMB Substrate; containing 3,3', 5,5'- Tetramethylbenzidin, colorless. Ready to use.
STOP	15 ml	Stop solution; contains acid. Ready to use.
WASH	20 ml	Wash Buffer, containing Tris, detergent, preservative sodium azide 0.09%; 50 x conc.
	1	Certificate of Analysis

MATERIALS REQUIRED

- Microplate reader capable of endpoint measurements at 450 nm; optional: reference filter at 620 nm
- Data reduction software
- Multi-channel dispenser or repeatable pipette for 100 µl
- Vortex mixer
- Pipettes for 10 µl, 100 µl and 1000 µl
- Laboratory timing device
- Distilled or deionised water
- Measuring cylinder for 1000 ml and 100 ml
- Plastic container for storage of the wash solution

This ELISA assay is suitable for use on open automated ELISA processors. Each assay has to be validated on the respective automated system. Detailed information is provided upon request.

SPECIMEN COLLECTION, STORAGE AND HANDLING

- Collect whole blood specimens using acceptable medical techniques to avoid hemolysis.
- Allow blood to clot and separate the serum or plasma by centrifugation.
- Test serum should be clear and non-hemolyzed. Contamination by hemolysis or lipemia should be avoided, but does not interfere with this assay.
- Specimens may be refrigerated at 2-8°C for up to five days or stored at -20°C up to six months.
- Avoid repetitive freezing and thawing of serum or plasma samples. This may result in variable loss of antibody activity.
- Testing of heat-inactivated sera is not recommended.

STORAGE AND STABILITY

- Store test kit at 2-8°C in the dark.
- Do not expose reagents to heat, sun, or strong light during storage and usage.
- Store microplate sealed and dessicated in the clip bag provided.
- Shelf life of the unopended test kit is 18 months from day of production. Unopened reagents are stable until expiration of the kit. See labels for individual batch.
- Diluted Wash Buffer and Sample Buffer are stable for at least 30 days when stored at 2-8°C. We recommend consumption on the same day.

PROCEDURAL NOTES

- Do not use kit components beyond their expiration dates.
- Do not interchange kit components from different lots and products.
- All materials must be at room temperature (20-28°C) prior to use.
- Prepare all reagents and samples. Once started, performe the test without interruption.
- Double determinations may be done. By this means pipetting errors may become obvious.
- Perform the assay steps only in the order indicated.
- Always use fresh sample dilutions.
- Pipette all reagents and samples into the bottom of the wells.
- To avoid carryover or contamination, change the pipette tip between samples and different kit controls.
- Wash microwells thoroughly and remove the last droplets of wash buffer.
- All incubation steps must be accurately timed.
- Do not re-use microplate wells.

PREPARATION OF REAGENTS

WASH

Dilute the contents of one vial of the buffered wash solution concentrate (50x) with distilled or deionised water to a final volume of 1000 ml prior to use.

DILUENT

Sample Buffer P: Prior to use dilute the contents (20 ml) of one vial of sample buffer 5x concentrate with distilled or deionised water to a final volume of 100 ml.

Preparation of samples

Dilute patient samples 1:100 before the assay: Put 990 µl of prediluted sample buffer in a polystyrene tube and add 10 µl of sample. Mix well. Note: Calibrators / Controls are ready to use and need not be diluted.

TEST PROCEDURE

Prepare enough microplate modules for all calibrators / controls and patient samples.

1. Pipette **100 µl** of calibrators, controls and prediluted patient samples into the wells.
   Incubate for **30 minutes** at room temperature (20-28 °C).
   Discard the contents of the microwells and **wash 3 times** with **300 µl** of wash solution.
2. Dispense **100 µ**l of enzyme conjugate into each well.
   Incubate for **15 minutes** at room temperature.
   Discard the contents of the microwells and **wash 3 times** with **300 µl** of wash solution.
3. Dispense **100 µ**l of TMB substrate solution into each well.
   Incubate for **15 minutes** at room temperature
4. **Add 100 µl** of stop solution to each well of the modules
   Incubate for **5 minutes** at room temperature.
   Read the optical density at 450 nm (reference 600-690nm) and calculate the results.
   The developed colour is stable for at least 30 minutes. Read during this time.

Example for a pipetting scheme:

	1	2	3	4	5	6	7	8	9	10	11	12
A	A	P1										
B	B	P2										
C	C	P3										
D	D											
E	E											
F	F											
G	C+											
H	C-											

P1, ... patient sample A-F calibrators C+, C- controls

VALIDATION

Test results are valid if the optical densities at 450 nm for calibrators / controls and the results for controls comply with the reference ranges indicated on the Certificate of Analysis enclosed in each test kit.
If these quality control criteria are not met the assay run is invalid and should be repeated.

CALCULATION OF RESULTS

For quantitative results plot the optical density of each calibrator versus the calibrator concentration to create a calibration curve. The concentration of patient samples may then be estimated from the calibration curve by interpolation.
Using data reduction software a 4-Parameter-Fit with lin-log coordinates for optical density and concentration is the data reduction method of choice.

PERFORMANCE CHARACTERISTICS

Calibration

The assay system is calibrated against the international reference preparation WHO 67/183 for AMA-M2 as 100 IU/ml.

Measuring range

The calculation range of this ELISA assay is 0 - 200 IU/ml

Expected values

In a normal range study with samples from healthy blood donors the following ranges have been established with this ELISA assay: Cut-off 10 IU/ml

Interpretation of results

Negative: < 10 IU/ml
Positive: ≥ 10 IU/ml

Linearity

Samples containing high levels of specific antibody were serially diluted in sample buffer to demonstrate the dynamic range of the assay and the upper / lower end of linearity. Activity for each dilution was calculated from the calibration curve using a 4-Parameter-Fit with lin-log coordinates.

Sample	Dilution	Observed IU/ml	Expected IU/ml	O/E [%]
WHO	1:100	108.5	100.0	109
.	1:200	51.2	50.0	102
.	1:400	25.2	25.0	101
.	1:800	12.8	12.5	102
.	1:1600	6.1	6.3	98
.	1:3200	3.1	3.1	99
1	1:100	49.5	49.5	100
.	1:200	25.0	24.8	101
.	1:400	12.2	12.4	99
.	1:800	5.9	6.2	95

Limit of detection

Functional sensitivity was determined to be: 1 IU/ml

Reproducibility

Intra-assay precision: Coefficient of variation (CV) was calculated for each of three samples from the results of 24 determinations in a single run. Results for precision-within-assay are shown in the table below.
Inter-assay precision: Coefficient of variation (CV) was calculated for each of three samples from the results of 6 determinations in 5 different runs. Results for run-to-run precision are shown in the table below.

Intra-Assay		
Sample	Mean IU/ml	CV %
1	39.8	7.0
2	81.3	3.8
3	177.3	3.6

Inter-Assay		
Sample	Mean IU/ml	CV %
1	40.1	6.2
2	84.6	11.8
3	180.4	3.8

Interfering substances

No interference has been observed with haemolytic (up to 1000 mg/dl) or lipemic (up to 3 g/dl triglycerides) sera or plasma, or bilirubin (up to 40 mg/dl) containing sera or plasma. Nor have any interfering effects been observed with the use of anticoagulants (Citrate, EDTA, Heparine). However for practical reasons it is recommended that grossly hemolyzed or lipemic samples should be avoided.

Study results

Study population	n	n Pos	%
Primary biliary cirrhosis (PBC)	143	139	97.2
Rheumatoid Arthritis	60	1	1.7
Normal human sera	267	18	6.7

		Clinical Diagnosis POS	NEG	
ORG 516	POS	139	19	
	NEG	4	308	
		143	327	470

Sensitivity: 97.2 %
Specificity: 94.2 %
Overall agreement: 95.1 %

LIMITATIONS OF THE PROCEDURE

This assay is a diagnostic aid. A definite clinical diagnosis should not be based on the results of a single test, but should be made by the physician after all clinical and laboratory findings have been evaluated concerning the entire clinical picture of the patient. Also every decision for therapy should be taken individually.
The above pathological and normal reference ranges for antibodies in patient samples should be regarded as recommendations only. Each laboratory should establishe its own ranges according to ISO 15189 or other applicable laboratory guidelines.

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Notice to the user (European Union):
Any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the EU Member State in which the user and/or the patient is established .

Change Control

Former version: *ORG 516_IFU_EN_QM113145_2013-12-16_2.1* Reason for revision: *Introduction electronic IFU on homepage*

1. Pipet **100 µl** calibrator, control or patient sample
   → Incubate for **30 minutes** at room temperature
   → Discard the contents of the wells and wash 3 times with **300 µl** wash solution
2. Pipet **100 µl** enzyme conjugate
   → Incubate for **15 minutes** at room temperature
   → Discard the contents of the wells and wash 3 times with **300 µl** wash solution
3. Pipet **100 µl** substrate solution
   → Incubate for **15 minutes** at room temperature
4. Add **100 µl** stop solution
   → Leave untouched for **5 minutes**
   → Read at **450 nm**

Anti-LKM-1 ELISA (IgG)
Test instruction

ORDER NO.	ANTIBODIES AGAINST	IG CLASS	SUBSTRATE	FORMAT
EA 1321-9601 G	LKM-1	IgG	Ag-coated microplate wells	96 x 01 (96)

Indications: The ELISA test kit provides a semiquantitative or quantitative in vitro assay for human antibodies of the IgG class against liver-kidney microsomes (LKM) in serum or plasma for the diagnosis of inexplainable increase in transaminases, suspected autoimmune hepatitis.

Application: According to the simplified diagnostic criteria by EM Hennes and colleagues *(International Autoimmune Hepatitis Group*) published in 2008, the detection of autoantibodies against LKM belongs to the routine investigations performed to diagnose autoimmune hepatitis. Antibodies against LKM-1 are mostly observed in children, but may be also present in adult patients with AIH. For delimitation from a virus hepatitis, the parallel determination of the other autoantibodies associated with AIH, such as ANA, pANCA, ASMA or antibodies against LC-1 and SLA/LP is recommended.

Principles of the test: The test kit contains microtiter strips each with 8 break-off reagent wells coated with LKM-1. In the first reaction step, diluted patient samples are incubated in the wells. In the case of positive samples, specific IgG antibodies (also IgA and IgM) will bind to the antigens. To detect the bound antibodies, a second incubation is carried out using an enzyme-labelled anti-human IgG (enzyme conjugate) catalysing a colour reaction.

Contents of the test kit:

Component	Colour	Format	Symbol
1. Microplate wells coated with antigens 12 microplate strips each containing 8 individual break-off wells in a frame, ready for use	---	12 x 8	STRIPS
2. Calibrator 1 200 RU/ml (IgG, human), ready for use	dark red	1 x 2.0 ml	CAL 1
3. Calibrator 2 20 RU/ml (IgG, human), ready for use	red	1 x 2.0 ml	CAL 2
4. Calibrator 3 2 RU/ml (IgG, human), ready for use	light red	1 x 2.0 ml	CAL 3
5. Positive control (IgG, human), ready for use	blue	1 x 2.0 ml	POS CONTROL
6. Negative control (IgG, human), ready for use	green	1 x 2.0 ml	NEG CONTROL
7. Enzyme conjugate peroxidase-labelled anti-human IgG (rabbit), ready for use	green	1 x 12 ml	CONJUGATE
8. Sample buffer ready for use	light blue	1 x 100 ml	SAMPLE BUFFER
9. Wash buffer 10x concentrate	colourless	1 x 100 ml	WASH BUFFER 10x
10. Chromogen/substrate solution TMB/H_2O_2, ready for use	colourless	1 x 12 ml	SUBSTRATE
11. Stop solution 0.5 M sulphuric acid, ready for use	colourless	1 x 12 ml	STOP SOLUTION
12. Test instruction	---	1 booklet	
13. Quality control certificate	---	1 protocol	

LOT Lot description
IVD In vitro diagnostic medical device
CE
Storage temperature
Unopened usable until

Preparation and stability of the reagents

Note: All reagents must be brought to room temperature (+18°C to +25°C) approx. 30 minutes before use. After first use, the reagents are stable until the indicated expiry date if stored at +2°C to +8°C and protected from contamination, unless stated otherwise below.

- **Coated wells:** Ready for use. Tear open the resealable protective wrapping of the microplate at the recesses above the grip seam. Do not open until the microplate has reached room temperature to prevent the individual strips from moistening. Immediately replace the remaining wells of a partly used microplate in the protective wrapping and tightly seal with the integrated grip seam (Do not remove the desiccant bag).
  Once the protective wrapping has been opened for the first time, the wells coated with antigens can be stored in a dry place and at a temperature between +2°C and +8°C for 4 months.

- **Calibrators and controls:** Ready for use. The reagents must be mixed thoroughly before use.

- **Enzyme conjugate:** Ready for use. The enzyme conjugate must be mixed thoroughly before use.

- **Sample buffer:** Ready for use.

- **Wash buffer:** The wash buffer is a 10x concentrate. If crystallisation occurs in the concentrated buffer, warm it to 37°C and mix well before diluting. The quantity required should be removed from the bottle using a clean pipette and diluted with deionised or distilled water (1 part reagent plus 9 parts distilled water).
  For example: For 1 microplate strip, 5 ml concentrate plus 45 ml water.
  The working strength wash buffer is stable for 4 weeks when stored at +2°C to +8°C and handled properly.

- **Chromogen/substrate solution:** Ready for use. Close the bottle immediately after use, as the contents are sensitive to light. The chromogen/substrate solution must be clear on use. Do not use the solution if it is blue coloured.

- **Stop solution:** Ready for use.

Storage and stability: The test kit has to be stored at a temperature between +2°C to +8°C. Do not freeze. Unopened, all test kit components are stable until the indicated expiry date.

Waste disposal: Patient samples, calibrators, controls and incubated microplate strips should be handled as infectious waste. All reagents must be disposed of in accordance with local disposal regulations.

Warning: The calibrators and controls of human origin have tested negative for HBsAg, anti-HCV, anti-HIV-1 and anti-HIV-2. Nonetheless, all materials should be treated as being a potential infection hazard and should be handled with care. Some of the reagents contain the agent sodium azide in a non-declarable concentration. Avoid skin contact.

Preparation and stability of the patient samples

Samples: Human serum or EDTA, heparin or citrate plasma.

Stability: Patient samples to be investigated can generally be stored at +2°C to +8°C for up to 14 days. Diluted samples should be incubated within one working day.

Sample dilution: Patient samples are diluted **1:101** in sample buffer. For example: dilute 10 µl of sample in 1.0 ml sample buffer and mix well by vortexing (sample pipettes are not suitable for mixing).

NOTE: Calibrators and controls are prediluted and ready for use, do not dilute them.

Incubation

For **semiquantative analysis** incubate **calibrator 2** along with the positive and negative controls and patient samples. For **quantitative analysis** incubate **calibrators 1, 2 and 3** along with the positive and negative controls and patient samples.

(Partly) manual test performance

Sample incubation: (1st step)
Transfer 100 µl of the calibrators, positive and negative controls or diluted patient samples into the individual microplate wells according to the pipetting protocol. Incubate for **30 minutes** at room temperature (+18°C to +25°C).

Washing:
Manual: Empty the wells and subsequently wash 3 times using 300 µl of working strength wash buffer for each wash.
Automatic: Wash reagent wells 3 times with 450 µl of working strength wash buffer (program setting: e.g. TECAN Columbus Washer “Overflow Modus”).

Leave the wash buffer in each well for 30 to 60 seconds per washing cycle, then empty the wells. After washing (manual and automated tests), thoroughly dispose of all liquid from the microplate by tapping it on absorbent paper with the openings facing downwards to remove all residual wash buffer.

Note: Residual liquid (> 10 µl) remaining in the reagent wells after washing can interfere with the substrate and lead to false low extinction values. Insufficient washing (e.g., less than 3 wash cycles, too small wash buffer volumes, or too short residence times) can lead to false high extinction values.
Free positions on the microplate strip should be filled with blank wells of the same plate format as that of the parameter to be investigated.

Conjugate incubation: (2nd step)
Pipette 100 µl of enzyme conjugate (peroxidase-labelled anti-human IgG) into each of the microplate wells. Incubate for **30 minutes** at room temperature (+18°C to +25°C).

Washing:
Empty the wells. Wash as described above.

Substrate incubation: (3rd step)
Pipette 100 µl of chromogen/substrate solution into each of the microplate wells. Incubate for **15 minutes** at room temperature (+18°C to +25°C), protect from direct sunlight.

Stopping the reaction:
Pipette 100 µl of stop solution into each of the microplate wells in the same order and at the same speed as the chromogen/substrate solution was introduced.

Measurement:
Photometric measurement of the colour intensity should be made at a **wavelength of 450 nm** and a reference wavelength between 620 nm and 650 nm **within 30 minutes of adding the stop solution**. Prior to measuring, slightly shake the microplate to ensure a homogeneous distribution of the solution.

Test performance using fully automated analysis devices

Sample dilution and test performance are carried out fully automatically using an analysis device. The incubation conditions programmed in the respective software authorised by EUROIMMUN may deviate slightly from the specifications given in the ELISA test instruction. However, these conditions were validated in respect of the combination of the EUROIMMUN Analyzer I, Analyzer I-2P or the DSX from Dynex and this EUROIMMUN ELISA. Validation documents are available on enquiry.
Automated test performance using other fully automated, open system analysis devices is possible. However, the combination should be validated by the user.

Pipetting protocol

	1	2	3	4	5	6	7	8	9	10	11	12
A	**C 2**	**P 6**	**P 14**	**P 22**			**C 1**	**P 4**	**P 12**	**P 20**		
B	**pos.**	**P 7**	**P 15**	**P 23**			**C 2**	**P 5**	**P 13**	**P 21**		
C	**neg.**	**P 8**	**P 16**	**P 24**			**C 3**	**P 6**	**P 14**	**P 22**		
D	**P 1**	**P 9**	**P 17**				**pos.**	**P 7**	**P 15**	**P 23**		
E	**P 2**	**P 10**	**P 18**				**neg.**	**P 8**	**P 16**	**P 24**		
F	**P 3**	**P 11**	**P 19**				**P 1**	**P 9**	**P 17**			
G	**P 4**	**P 12**	**P 20**				**P 2**	**P 10**	**P 18**			
H	**P 5**	**P 13**	**P 21**				**P 3**	**P 11**	**P 19**			

The pipetting protocol for microtiter strips 1-4 is an example for the **semiquantitative analysis** of 24 patient samples (P 1 to P 24).
The pipetting protocol for microtiter strips 7-10 is an example for the **quantitative analysis** of 24 patient samples (P 1 to P 24).

The calibrators (C 1 to C 3), the positive (pos.) and negative (neg.) controls, and the patient samples have each been incubated in one well. The reliability of the ELISA test can be improved by duplicate determinations for each sample.
The wells can be broken off individually from the strips. This makes it possible to adjust the number of test substrates used to the number of samples to be examined and minimises reagent wastage.
Both positive and negative controls serve as internal controls for the reliability of the test procedure. They should be assayed with each test run.

Calculation of results

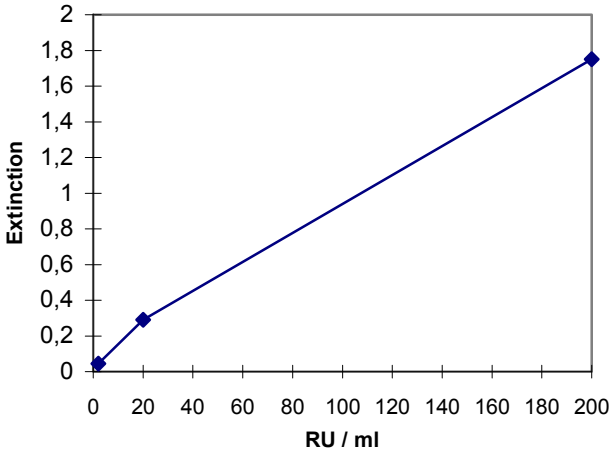
Semiquantitative: Results can be evaluated semiquantitatively by calculating a ratio of the extinction value of the control or patient sample over the extinction value of calibrator 2. Calculate the ratio according to the following formula:

$$\frac{\text{Extinction of the control or patient sample}}{\text{Extinction of calibrator 2}} = \text{Ratio}$$

EUROIMMUN recommends interpreting results as follows:

Ratio <1.0:	**negative**
Ratio ≥1.0:	**positive**

Quantitative: The standard curve from which the concentration of antibodies in the serum samples can be taken is obtained by point-to-point plotting of the extinction values measured for the 3 calibration sera against the corresponding units (linear/linear). Use “point-to-point” plotting for calculation of the standard curve by computer. The following plot is an example of a typical calibration curve. Please do not use this curve for the determination of antibody concentrations in patient samples.



If the extinction for a patient sample lies above the value of calibrator 1 (200 RU/ml), the result should be reported as ">200 RU/ml". It is recommended that the sample be re-tested at a dilution of e.g. 1:400. The result in RU/ml read from the calibration curve for this sample must then be multiplied by a factor of 4.

The upper limit of the normal range **(cut-off)** recommended by EUROIMMUN is 20 relative units (RU)/ml. EUROIMMUN recommends interpreting results as follows:

<20 RU/ml:	**negative**
≥20 RU/ml:	**positive**

For duplicate determinations the mean of the two values should be taken. If the two values deviate substantially from one another, EUROIMMUN recommends to retest the samples.

For diagnosis, the clinical picture of the patient always needs to be taken into account along with the serological findings.

Test characteristics

Calibration: As no international reference serum exists for antibodies against LKM-1, the calibration is performed in relative units (RU).

For every group of tests performed, the extinction values of the calibrators and the relative units and/or ratio determined for the positive and negative controls must lie within the limits stated for the relevant test kit lot. A quality control certificate containing these reference values is included. If the values specified for the controls are not achieved, the test results may be inaccurate and the test should be repeated.

The binding activity of the antibodies and the activity of the enzyme used are temperature-dependent. It is therefore recommended using a thermostat in all three incubation steps. The higher the room temperature during the incubation steps, the greater will be the extinction values. Corresponding variations apply also to the incubation times. However, the calibrators are subject to the same influences, with the result that such variations will be largely compensated in the calculation of the result.

Antigen: The reagent wells are coated with recombinant cytochrome P450 IID6 which constitutes the specific target antigen for antibodies against LKM1.

Linearity: The linearity of the Anti-LKM-1 ELISA (IgG) was determined by assaying 4 serial dilutions of different patient samples. The coefficient of determination R^2 for all sera was > 0.95. The Anti-LKM-1 ELISA (IgG) is linear at least in the tested concentration range (2 RU/ml to 194 RU/ml).

Detection limit: The lower detection limit is defined as the mean value of an analyte-free sample plus three times the standard deviation and is the smallest detectable antibody titer. The lower detection limit of the Anti-LKM-1 ELISA (IgG) is 1.4 RU/ml.

Cross reactivity: This ELISA showed no cross reactivity.

Interference: Haemolytic, lipaemic and icteric samples showed no influences on the result up to a concentration of 10 mg/ml for haemoglobin, 20 mg/ml for triglycerides and 0.4 mg/ml for bilirubin in this ELISA.

Reproducibility: The reproducibility of the test was investigated by determining the intra- and inter-assay coefficients of variation (CV) using 3 sera. The intra-assay CVs are based on 20 determinations and the inter-assay CVs on 4 determinations performed in 6 different test runs.

Intra-Assay Variation, n = 20		
Serum	**Mean value (RU/ml)**	**CV (%)**
1	55	3.0
2	96	2.7
3	158	2.3

Inter-Assay Variation, n = 4 x 6		
Serum	**Mean value (RU/ml)**	**CV (%)**
1	58	3.2
2	96	3.7
3	160	2.5

Specificity and sensitivity: 18 patient samples suffering from autoimmune hepatitis and 489 patient samples from a reference laboratory were investigated with the EUROIMMUN Anti-LKM-1 ELISA (IgG). The EUROIMMUN-IIFT (IgG) was used as a reference method. The ELISA has a specificity of 99.4% and a sensitivity of 100% with reference to the EUROIMMUN IIFT.

Serum panel (n = 507)		**IIFT (rat liver/rat kidney)**	
		positive	negative
Anti-LKM-1 ELISA	positive	27	3
	negative	0	477

A patient sample which reacted positive in ELISA and negative in IIFT belongs to a patient with characterised AIH.

Reference range: The levels of the anti-LKM-1 antibodies (IgG) were analysed with this EUROIMMUN ELISA in a panel of 200 healthy blood donors. With a cut-off of 20 RU/ml, 0.5% of the blood donors were anti-LKM-1 positive.

Clinical significance

In Western Europe the incidence of AIH is 1.9 cases per 100,000 in-habitants per year. Untreated, AIH soon develops into liver cirrhosis. However, if low-dose immunosuppressive therapy is started early enough and continued lifelong, patients have a normal life expectancy.

Circulating autoantibodies have come to play a significant role in the diagnosis of AIH. Antibodies against the following antigens are associated with AIH: soluble liver antigen/liver-pancreas antigen (SLA/LP), cell nuclei (ANA), nDNA, smooth muscles (SMA, the most important target antigen being F actin), liver-kidney microsomes (LKM-1, target antigen: cytochrome P450 IID6), liver cytosolic antigen type 1 (LC-1, target antigen: formiminotransferase cyclo-deaminase) and granulocytes (pANCA). Anti-mito-chondrial antibodies (AMA) are also investigated in this context to exclude the possibility of primary biliary cirrhosis (PBC). AIH is sometimes classified according to the antibody status, i.e., subtype I (ANA, SMA), subtype II (antibodies against LKM-1 and LC-1), or subtype III (antibodies against SLA/LP). However, this classification is probably neither clinically nor therapeutically or prognostically relevant, since 10 to 20% of patients with PBC develop secondary AIH (overlap). In these cases the same autoantibodies as in AIH are frequently detected.

Autoantibodies against LKM-1 (LKM-1, antigen: cytochrome P450 IID6) are present in 1% of adults with AIH. In children they are more common. Antibodies against LKM-1 are also found in 1 to 2% of patients with hepatitis C-positive serology.

The highest diagnostic accuray currently available for AIH is probably provided by the various EUROIMMUN enzyme immunoassays that detect autoantibodies against SLA/LP. Although SLA/LP autoantibodies have a prevalence of only 10 to 30% in AIH patients, the predictive value is nearly 100%. Every positive anti-SLA/LP result essentially indicates AIH (provided the relevant clinical symptoms are also present).

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HP Ag

Enzyme Immunoassay for the qualitative/quantitative determination of Helicobacter pylori Antigen in human stools

- for “in vitro” diagnostic use only -

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REF HPAG.CE.96
96 Tests

HP Ag

A. INTENDED USE
Enzyme Immunoassay (ELISA) for the one-step qualitative/quantitative determination of Helicobacter pylori Antigen (HP Ag) in human stools. The kit may be used for the follow-up of HP-infected patients and their pharmacological treatment. For "in vitro" diagnostic use only.

B. INTRODUCTION
Helicobacter pylori (Hp) is a Gram negative bacterium, firstly isolated in gastric mucosa by Marshall and Warren in 1983.
This bacterium is widely diffused in men, without limitations of sex and age; it has been found that infections can be transmitted directly by contact with contaminated biological fluids (saliva, stool, body secretions) and also from contaminated food and beverages.
H.pylori, and in particular some pathogenic strains (CagA +), is the etiological agent responsible of most of active infections and lesions of the gastric mucosa in man.
H.pylori infection moreover acts as cofactor in the development of tumoral pathologies of the gastric apparatus and it is suspected to be associated to some inflammatory pathologies of the genital female apparatus, evolving toward neoplastic transformation.
At the present time, the identification of Helicobacter pylori is mostly made with invasive histochemical techniques, with the determination of its urease activity on a isotopic substrate (breath test and mass analysis), with time-consuming bacteriological culture systems and with expensive molecular biology techniques (PCR).
ELISA for HP Ag have been only recently introduced as a specific, fast, non invasive (analysis of stools) and cheaper method of detection.

C. PRINCIPLE OF THE TEST
Stools from patients are used as a source of sample for the determination of HP antigen.
Microplates are coated with a cocktail of affinity purified mouse monoclonal antibodies directed to the most specific Helicobacter pylori antigens.
In the 1st incubation, the solid phase is treated with the sample, previously extracted from stools, and simultaneously with a mixture of monoclonal antibodies to Hp, conjugated with peroxidase (HRP).
After washing out all the other components of the sample, in the 2nd incubation the bound enzyme specifically present on the solid phase generates an optical signal that is proportional to the amount of H.pylori antigens present in the sample.

D. COMPONENTS
Code HPAG.CE.96 contains reagents to perform 96 tests.

1. Microplate MICROPLATE
8x12 microwell strips coated with anti HP Ag specific affinity purified mouse monoclonal antibodies and sealed into a bag with desiccant. Allow the microplate to reach room temperature before opening; reseal unused strips in the bag with desiccant and store at 4°C.

2. Calibration Set: CAL …
n° 1 set of 4 vials - Lyophilized calibrators. To be dissolved with EIA grade water. When dissolved, Calibrators have the following concentrations: **0 - 0.1 – 0.5 - 1.0 ug/ml HP Ag**
They contain fetal bovine serum, inactivated HP Ag, 10 mM phosphate buffer pH 7.4+/-0.1, 0.02% gentamicine sulphate and 0.045% ProClin 300 as preservatives.

Important Note: *Calibrators when dissolved are not stable. Proceed as described in the proper section for storage.*

3. Wash buffer concentrate WASHBUF 20X
1x60ml/bottle - 20x concentrated solution. Once diluted, the wash solution contains 10 mM phosphate buffer pH 7.0+/-0.2 and 0.05% Tween 20 and 0.045% ProClin 300

4. Enzyme Conjugate CONJ
1x16ml/vial - Ready to use component. It contains Horseradish Peroxidase (HRP) labeled mouse monoclonal antibodies to HP Ag , 10 mM Tris buffer pH 6.8+/-0.1, 2% BSA, 0.045% ProClin 300 and 0.02% gentamicine sulphate as preservatives.
The Enzyme Conjugate is color coded red.

5. Chromogen/Substrate SUBS TMB
1x25ml/vial - It contains a 50 mM citrate-phosphate buffered solution at pH 3.5-3.8, 0.03% tetra-methyl-benzidine (TMB) and 0.02% hydrogen peroxide (H_2O_2).
Note: To be stored protected from light as sensitive to strong illumination.

6. Specimen Diluent: DILSPE
2x60ml/vial - Buffered solution for the extraction of HP Ag from the specimen and preparation of the sample. It contains 10 mM Tris-HCl buffer pH 7.4+/-0.1, 2% BSA, 0.045% ProClin 300 and 0.02% gentamicine sulphate as preservatives.
The component is color coded blue.

7. Sulphuric Acid H_2SO_4 O.3 M
1x15ml/vial - It contains 0.3 M H2SO4 solution.
Attention: Irritant (H315, H319; P280, P302+P352, P332+P313, P305+P351+P338, P337+P313, P362+P363).

8. Plate sealing foils: n° 2

9. Package insert: n° 1

Upon request:

HP Ag Extraction kit n° 1
The kit contains all what is necessary to prepare n° 50 samples extracted from stools collected by patients.

E. MATERIALS REQUIRED BUT NOT PROVIDED
1. Calibrated variable volume Micropipettes ranging 1000 ul and 200 ul; disposable plastic tips.
2. EIA grade water (double distilled or deionised, charcoal treated to remove oxidizing chemicals used as disinfectants).
3. Timer with 60 minute range or higher.
4. Absorbent paper tissues.
5. Calibrated ELISA microplate thermostatic incubator (dry or wet), set at +37°C.
6. Calibrated ELISA microwell reader with 450nm (reading) and with 620-630nm (blanking) filters.
7. Calibrated ELISA microplate washer.
8. Vortex or similar mixing tools.
9. Disposable plastic micro-spoon stools collection container (available upon request from Dia.Pro s.r.l.)

F. WARNINGS AND PRECAUTIONS
1. The kit has to be used by skilled and properly trained technical personnel only, under the supervision of a medical doctor responsible of the laboratory.
2. All the personnel involved in performing the assay have to wear protective laboratory clothes, talc-free gloves and glasses. The use of any sharp (needles) or cutting (blades) devices should be avoided. All the personnel involved should be trained in biosafety procedures, as recommended by the Center for

Disease Control, Atlanta, U.S. and reported in the National Institute of Health's publication: "Biosafety in Microbiological and Biomedical Laboratories", ed. 1984.
3. The laboratory environment should be controlled so as to avoid contaminants such as dust or air-born microbial agents, when opening kit vials and microplates and when performing the test. Protect the Chromogen/Substrate from strong light and avoid vibration of the bench surface where the test is undertaken.
4. Upon receipt, store the kit at 2..8°C into a temperature controlled refrigerator or cold room.
5. Do not interchange components between different lots of the kits. It is recommended that components between two kits of the same lot should not be interchanged.
6. Check that the liquid components of the kit are clear and do not contain visible heavy particles or aggregates. If not, advise the laboratory supervisor to initiate the necessary procedures for kit replacement.
7. Avoid cross-contamination between samples by using disposable tips and changing them after each sample. Do not reuse disposable tips.
8. Avoid cross-contamination between kit components by using disposable tips and changing them between the use of each one. Do not reuse disposable tips.
9. Do not use the kit after the expiration date stated on the external container and internal (vials) labels.
10. Treat all specimens as potentially infective, according to national regulations and laws concerning biological sample handling and wasting.
11. The use of disposable plastic-ware is recommended in the preparation of the liquid components or in transferring components into automated workstations, in order to avoid cross contamination.
12. Wastes produced during the use of the kit have to be discarded in compliance with national directives and laws concerning laboratory waste of chemical and biological substances. In particular, liquid waste generated from the washing procedure, from residuals of controls and from samples has to be treated as potentially infective material and inactivated before waste. Suggested procedures of inactivation are treatment with a 10% final concentration of household bleach for 16-18 hrs or heat inactivation by autoclave at 121°C for 20 min..
13. Accidental spills from samples and operations have to be adsorbed with paper tissues soaked with household bleach and then with water. Tissues should then be discarded in proper containers designated for laboratory/hospital waste.
14. The Sulphuric Acid is an irritant. In case of spills, wash the surface with plenty of water
15. Other waste materials generated from the use of the kit (example: tips used for samples and controls, tools for the extraction of the sample from specimens, used microplates, etc.) should be handled as potentially infective and disposed according to national directives and laws concerning laboratory wastes.

G. SPECIMEN: COLLECTION, PREPARATION AND WARNINGS

1) It is recommended to collect fresh stools in the morning with the plastic collector provided on request together with the kit. Alternatively a conical bottomed disposable tube, provided by the laboratory to the patient, may be used.
2) The patient submitted to the test should not be under antibiotic or anti-bacterial treatments as this pharmaceutical therapy is known to affect H.pylori up to a certain extent, depending on the antibiotic used, giving origin to false interpretation.
3) The patient has to be asked to collect the specimen avoiding any possible contact with urine or water using the plastic spoon present in the stool collector and taking just the amount of specimen necessary to fill up the cavity of the spoon.
4) The patient is asked to deliver the specimen the same day to the laboratory. From the time of collection, the specimen can be stored in the laboratory up to 24 hr at 2..8°C or kept frozen at -20°C for longer time.
5) Specimens, and then samples derived from them, have to be clearly identified with codes or names in order to avoid misinterpretation of results. Bar code labeling and electronic reading is recommended when the number of samples on testing is pretty high.

***Important Note**: Degradation of HP antigen heavily occurs in stools after 24 hrs generating false negative results, even if the specimen is stored at 2..8°C.*

The next following operations are described and represented in figures in the Instructions for Use of the Stool Extraction Kit provided together with the kit.
Operate according to the following instructions:

1) Open the stool collection device and introduce the extraction brush deeply into the specimen. Rotate the brush 2-4 times in order to collect the right amount of biological material (about 0.2 gr).
2) Transfer the brush carefully into the test tube supplied in the kit and then add 1 ml Specimen Diluent. Keeping the brush inside the tube, mix vigorously on vortex for 1 min +/-10% in order to dissolve H.pylori into solution.
3) Discard the brush and insert the filtering piston, supplied with the kit, into the tube. Push gently the piston down into the tube in order to collect not more than 150-200 ul of the liquid phase of the suspension, volume enough to carry out the test.

***Important Notes**:*
***a)** Be careful not to apply a too strong manual pressure on the piston. The piston could break the tube and spills could be generated. If this should happen, use a paper towel soaked with an hospital disinfectant to clean up the contaminated surfaces.*

***b)** Avoid any addition of preservatives to samples, especially sodium azide as this chemical would affect the enzymatic activity of the conjugate, generating false negative results.*

H. PREPARATION OF COMPONENTS AND WARNINGS

A study conducted on an opened kit has not pointed out any relevant loss of activity up to 6 re-uses of the device and up to 3 months.

Microplates:
Allow the microplate to reach room temperature (about 1 hr) before opening the container. Check that the desiccant has not turned dark green, indicating a defect in storing.
In this case, call Dia.Pro's customer service.
Unused strips have to be placed back inside the aluminum pouch, with the desiccant supplied, firmly zipped and stored at +2°..8°C.
***Important Note**: After first opening, remaining strips are stable until the humidity indicator inside the desiccant bag turns from yellow to green.*

Calibration Set:
Add the volume of ELISA grade water reported in the label to the lyophilized powder of each Calibrator. Let fully dissolve the content and then gently mix on vortex.
***Important Note**: When dissolved, Calibrators are not stable. Store Calibrators frozen in aliquots at –20°C, carefully labeled with the content of HP Ag present in each of them.*

Wash buffer concentrate:
The concentrated solution has to be diluted 20x with ELISA grade water and mixed gently end-over-end before use. During

preparation avoid foaming as the presence of bubbles could impact on the efficiency of the washing cycles.
Important Note: *Once diluted, the wash solution is stable for 1 week at +2..8° C.*

Enzyme Conjugate:
Ready to use. Mix well on vortex before use.

Chromogen/Substrate:
Ready to use. Mix well on vortex before use.
Avoid contamination of the liquid with oxidizing chemicals, air-driven dust or microbes. Do not expose to strong light, oxidizing agents and metallic surfaces.
If this component has to be transferred use only plastic, and if possible, sterile disposable container.

Specimen Diluent:
Ready to use. Mix well on vortex before use.

Sulphuric Acid:
Ready to use. Mix well on vortex before use.
Attention: Irritant (H315, H319; P280, P302+P352, P332+P313, P305+P351+P338, P337+P313, P362+P363).

Legenda:

Warning **H statements:**
H315 – Causes skin irritation.
H319 – Causes serious eye irritation.

Precautionary **P statements:**
P280 – Wear protective gloves/protective clothing/eye protection/face protection.
P302 + P352 – IF ON SKIN: Wash with plenty of soap and water.
P332 + P313 – If skin irritation occurs: Get medical advice/attention.
P305 + P351 + P338 – IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P337 + P313 – If eye irritation persists: Get medical advice/attention.
P362 + P363 – Take off contaminated clothing and wash it before reuse.

I. INSTRUMENTS AND TOOLS USED IN COMBINATION WITH THE KIT

1. Micropipettes have to be calibrated to deliver the correct volume required by the assay and must be submitted to regular decontamination (70% ethanol, 10% solution of bleach, hospital grade disinfectants) of those parts that could accidentally come in contact with the sample or the components of the kit. They should also be regularly maintained in order to show a precision of 1% and a trueness of $\pm$2%.
2. The ELISA incubator has to be set at +37°C (tolerance of $\pm$0.5°C) and regularly checked to ensure the correct temperature is maintained. Both dry incubators and water baths are suitable for the incubations, provided that the instrument is validated for the incubation of ELISA tests.
3. The ELISA washer is extremely important to the overall performances of the assay. The washer must be carefully validated in advance, checked for the delivery of the right dispensation volume and regularly submitted to maintenance according to the manufacturer's instructions for use. In particular the washer, at the end of the daily workload, has to be extensively cleaned out of salts with deionized water. Before use, the washer has to be extensively primed with the diluted Washing Solution.
   The instrument weekly has to be submitted to decontamination according to its manual (NaOH 0.1 M decontamination suggested).
   5 washing cycles (aspiration + dispensation of 350ul/well of washing solution + 20 sec soaking = 1 cycle) are sufficient to ensure the assay with the declared performances. If soaking is not possible add one more cycle of washing.
   An incorrect washing cycle or salt-blocked needles are the major cause of false positive reactions. The washer has to be correctly optimized using the kit controls/calibrator and reference panels, before using the kit for routine laboratory tests.
   Important Note: *Due to the nature of the sample used and the possible presence of particles in the sample, be careful to control that the needles of the washer do not get blocked by the presence of stool bodies.*
4. Incubation times have a tolerance of $\pm$5%.
5. The ELISA microplate reader has to be equipped with a reading filter of 450nm and with a second filter of 620-630nm, mandatory for blanking purposes. Its standard performances should be (a) bandwidth $\leq$ 10 nm; (b) absorbance range from 0 to $\geq$ 2.0; (c) linearity to $\geq$ 2.0; repeatability $\geq$ 1%. Blanking is carried out on the well identified in the section "Internal Quality Control". The optical system of the reader has to be calibrated regularly to ensure that the correct optical density is measured. It should be regularly maintained according to the manufacturer 's instructions.
6. When using an ELISA automated workstation, all critical steps (dispensation, incubation, washing, reading, shaking, data handling) have to be carefully set, calibrated, controlled and regularly serviced in order to match the values reported in the sections "Internal Quality Control". The assay protocol has to be installed in the operating system of the unit and validated as for the washer and the reader. In addition, the liquid handling part of the station (dispensation and washing) has to be validated and correctly set. Particular attention must be paid to avoid carry over by the needles used for dispensing samples and for washing. This must be studied and controlled to minimize the possibility of contamination of adjacent wells due to strongly reactive samples, leading to false positive results. The use of ELISA automated work stations is recommended when the number of samples to be tested exceed 20-30 units per run.

Important Note: *Due to the nature of the sample used and the possible presence of particles in the sample, be careful to control that the needles of the workstation do not get blocked by the presence of stool bodies. We strongly suggest to use disposable sample tips in order to avoid any block or damage of fix probes.*

7. Dia.Pro's customer service offers support to the user in the setting and checking of instruments used in combination with the kit, in order to assure full compliance with the requirements described. Support is also provided for the installation of new instruments to be used with the kit.
8. Upon request, Dia.Pro srl offers a sample preparation device able to produce a particle free sample showing excellent performances in the assay. Please inquire.

L. PRE ASSAY CONTROLS AND OPERATIONS

1. Prepare the sample from stools as described in section G and represented in the Instructions for Use of the HP Ag Extraction Kit.
2. Check the expiration date of the kit printed on the external label of the kit box. Do not use if expired.
3. Check that the liquid components are not contaminated by naked-eye visible particles or aggregates. Check that the Chromogen/Substrate is colorless or pale blue by aspirating a small volume of it with a sterile transparent plastic pipette. Check that no breakage occurred in transportation and no spillage of liquid is present inside the box. Check that the aluminum pouch, containing the microplate, is not punctured or damaged.

4. Dilute all the content of the 20x concentrated Wash Solution as described above.
5. Dissolve the Calibrator Set as described above.
6. Allow all the other components to reach room temperature (about 1 hr) and then mix as described.
7. Set the ELISA incubator at +37°C and prepare the ELISA washer by priming with the diluted washing solution, according to the manufacturers instructions. Set the right number of washing cycles reported in the specific section.
8. Check that the ELISA reader has been turned on at least 20 minutes before reading.
9. If using an automated workstation, turn it on, check settings and be sure to use the right assay protocol.
10. Check that the micropipettes are set to the required volume.
11. Check that all the other equipment is available and ready to use.
12. In case of problems, do not proceed further with the test and advise the supervisor.

M. ASSAY PROCEDURE

The assay has to be carried out according to what reported below, taking care to maintain the same incubation time for all the samples in testing.
Two procedures are available: a quantitative method able to provide a quantification of HP Ag in the specimen and a qualitative method.

A. Quantitative Assay

1. Place the required number of strips in the plastic holder and carefully identify the wells for calibrators and samples. Leave A1+B1 wells empty for blanking purposes.

2. Pipette 100 µl Calibrators in duplicate into the calibration wells (see the example of dispensation reported below).

3. With the Pasteur pipette supplied aspirate the liquid filtered up into the inner chamber of the piston and dispense 2 drops (about 100 µl) of sample into the sample well. Check for the presence of samples in wells by naked eye (there is a marked color difference between empty and full wells) or by reading at 450/620nm. (samples show OD values higher than 0.100).

4. Dispense then 100 µl Enzymatic Conjugate in all wells, except for A1+B1, used for blanking operations.

Important note: *Be careful not to touch the inner surface of the well with the pipette tip when the conjugate is dispensed. Contamination might occur.*

5. Following addition of the conjugate, check that the color of the samples has turned from brown to pale reddish and incubate the microplate for **120 min at +37°C**.

Important notes: *Strips have to be sealed with the adhesive sealing foil only when the test is performed manually. Do not cover strips when using ELISA workstations.*

6. When the first incubation is over, wash the microwells as previously described (section I.3)

7. Pipette 200 µl Chromogen/Substrate into all the wells, A1+B1 included. Incubate the microplate protected from light at **room temperature (18-24°C) for 20 min.**

Important note: *Do not expose to strong direct light as a high background might be generated.*

8. Pipette 100 µl Sulphuric Acid into all the wells to stop the enzymatic reaction, using the same pipetting sequence as in step 6.

9. Measure the color intensity of the solution in each well, as described in section I.5 using a 450nm filter (reading) and a 620-630nm filter (background subtraction, mandatory)), blanking the instrument on A1 or B1 or both.

An example of dispensation scheme is reported below:

Microplate

	1	2	3	4	5	6
A	BLK	CAL4				
B	BLK	CAL4				
C	CAL1	S1				
D	CAL1	S2				
E	CAL2	S3				
F	CAL2	S4				
G	CAL3	S5				
H	CAL3	S6				

Legenda: BLK = Blank CAL = Calibrator S = Sample

B. Qualitative Assay

1. Place the required number of strips in the plastic holder and carefully identify the wells for calibrators and samples. Leave A1well empty for blanking purposes.

2. Pipette 100 µl Calibrator 1 in duplicate, 100 µl Calibrator 2 in duplicate, 100 µl Calibrator 4 in single and then 100 µl samples. Check for the presence of samples in wells as reported before.

3. Dispense 100 µl Enzymatic Conjugate in all wells, except for A1, used for blanking operations.

Important note: *Be careful not to touch the inner surface of the well with the pipette tip when the conjugate is dispensed. Contamination might occur.*

4. Following addition of the conjugate, check that the color of the samples has turned from brown to pale reddish and then incubate the microplate for **120 min at +37°C**.

Important notes: *Strips have to be sealed with the adhesive sealing foil only when the test is performed manually. Do not cover strips when using ELISA workstations.*

5. When the first incubation is over, wash the microwells as previously described (section I.3)

6. Pipette 200 µl Chromogen/Substrate into all the wells, A1 included. Incubate the microplate protected from light at **room temperature (18-24°C) for 20 min.**

Important note: *Do not expose to strong direct light as a high background might be generated.*

7. Pipette 100 µl Sulphuric Acid into all the wells to stop the enzymatic reaction, using the same pipetting sequence as in step 6.

8. Measure the color intensity of the solution in each well, as described in section I.5 using a 450nm filter (reading) and a 620-630nm filter (background subtraction, mandatory), blanking the instrument on A1.

An example of dispensation scheme is reported below:

Microplate

	1	2	3	4	5	6
A	BLK	S3				
B	CAL1	S4				
C	CAL1	S5				
D	CAL2	S6				
E	CAL2	S7				
F	CAL4	S8				
G	S1	S9				
H	S2	S10				

Legenda: BLK = Blank CAL = Calibrator S = Sample

Important notes:

1. *Ensure that no fingerprints or dust are present on the external bottom of the microwell before reading They could generate false positive results on reading*
2. *Reading should ideally be performed immediately after the addition of the acid solution but definitely no longer than 20 minutes afterwards. Some self-oxidation of the chromogen can occur leading to a higher background.*

N. ASSAY SCHEME

Operations	Procedure
Calibrators&samples	100 ul
Enzyme Conjugate	100 ul
1st incubation	**120 min**
Temperature	+37°C
Washing steps	n° 5 cycles with 20'' of soaking OR n° 6 cycles without soaking
Chromogen/Substrate	200ul
2nd incubation	**20 min**
Temperature	room
Sulphuric Acid	100 ul
Reading OD	450nm / 620-630nm

O. INTERNAL QUALITY CONTROL

A check is performed on the controls/calibrator any time the kit is used in order to verify whether the expected OD450nm/620-630nm or S/Co values have been matched in the analysis.
Ensure that:

Parameter	Requirements
Blank well	< 0.100 OD450nm value
CAL 0 ug/ml	< 0.200 mean OD450nm value after blanking
CAL 0.1 ug/ml	OD450nm > OD450nm CAL 0 ug/ml + 0.100
CAL 1 ug/ml	> 1.000 OD450nm value

If the results of the test match the requirements stated above, proceed to the next section. If they do not, do not proceed any further and perform the following checks:

Problem	Check
Blank well > 0.100 OD450nm	1. that the Chromogen/Substrate solution has not become contaminated during the assay
CAL 0 ug/ml > 0.200 OD450nm after blanking	1. that the washing procedure and the washer settings are as validated in the pre qualification study; 2. that the proper washing solution has been used and the washer has been primed with it before use; 3. that no mistake has been done in the assay procedure (dispensation of positive calibrators instead of the negative one); 4. that no contamination of the calibrator or of the wells where the calibrator was dispensed has occurred due to spills of positive samples or of the enzyme conjugate; 5. that micropipettes have not become contaminated with positive samples or with the enzyme conjugate 6. that the washer needles are not blocked or partially obstructed.
CAL 0.1 ug/ml OD450nm < Cal 0 ug/ml + 0.100	1. that the procedure has been correctly performed; 2. that no mistake has occurred during its distribution (ex.: dispensation of negative calibrator instead) 3. that the washing procedure and the washer settings are as validated in the pre qualification study; 4. that no external contamination of the calibrator has occurred.
CAL 1 ug/ml < 1.000 OD450nm	1. that the procedure has been correctly performed; 2. that no mistake has occurred during the distribution of the calibrator (dispensation of negative calibrator instead). 3. that the washing procedure and the washer settings are as validated in the pre qualification study; 4. that no external contamination of the calibrator has occurred.

If any of the above problems have occurred, report the problem to the supervisor for further actions.
Important note:
The analysis must be done proceeding as the reading step described in the section M, points A8 and B8.

P. CALCULATION OF RESULTS

Quantitative Assay:
Calculate the mean OD450nm//620-630nm value of the calibrators. Then draw a calibration curve possibly using a 4 parameters fitting curve system. Then calculate on the curve the concentration of HP antigen in the sample.

Qualitative Assay:
The test results are calculated by means of a cut-off value determined from the O450nm/ 620-630nm value of the CAL 0 ug/ml (CAL 0) and the OD450nm/620-630nm of the CAL 0.1 ug/ml (CAL 0.1) with the following formula:

Cut-Off = (CAL 0 + CAL 0.1) / 2

Important note*: When the calculation of results is performed by the operating system of an ELISA automated work station, ensure that the proper formulation is used to calculate the cut-off value and generate the correct interpretation of results.*

Q. INTERPRETATION OF RESULTS

In the quantitative assay, samples showing a concentration of H.pylori antigen higher than 0.05 ug/ml are considered positive.
For the qualitative assay, test results are interpreted as a ratio of the sample OD450nm/620-630nm (S) and the Cut-Off value (Co), mathematically S/Co, according to the following table:

S/Co	Interpretation
< 1.0	Negative
1.0 – 1.1	Equivocal
> 1.1	Positive

A negative result indicates that the patient is not infected by H.pylori.
Any patient showing an equivocal result should be retested on a second sample.
A positive result is indicative of HP infection and therefore the patient should be treated accordingly.

Important notes:

1. *Interpretation of results should be done under the supervision of the laboratory supervisor to reduce the risk of judgment errors and misinterpretations.*
2. *Any positive result should be confirmed first by repeating the test and then, if still positive, by an alternative method before a diagnosis of HP infection is confirmed.*
3. *When test results are transmitted from the laboratory to another department, attention must be paid to avoid erroneous data transfer.*
4. *Diagnosis of HP infection has to be taken and released to the patient by a suitably qualified medical doctor. This should be done taking also into account other diagnostic evidences of infection.*

An example of qualitative method is reported below. (data obtained proceeding as the the reading step described in the section M, point B8):

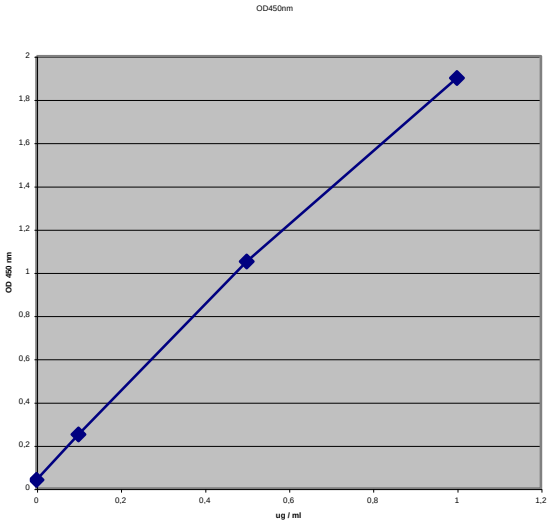
Note: The following data must not be used instead or real figures obtained by the user.

Cal 0 ug/ml: 0.040 –0.060 OD450nm
Mean Value: 0.050 OD450nm
Lower than 0.200 – Accepted

Cal 0.1 ug/ml: 0.210-0.230 OD450nm
Mean Value: 0.220 OD450nm
Higher than Cal 0 ug/ml + 0.100 – Accepted
Cut-Off = (CAL 0 + CAL 0.1) / 2 = 0.135
Calibrator 1 ug/ml: 2.000 OD450nm
OD450nm higher than 1.000 – Accepted

Sample 1: 0.028 OD450nm
Sample 2: 1.690 OD450nm
Sample 1 S/Co < 1.0 = negative
Sample 2 S/Co > 1.1 = positive

An Example of Calibration curve is reported below:



R. PERFORMANCE CHARACTERISTICS

Evaluation of Performances was conducted by testing negative and positive samples in an external clinical site and in the own laboratories as well.

1. Limit of detection

The limit of detection of the assay was calculated by examining serial dilution of HP antigen in Sample Diluent.
Results show that the analytical sensitivity of the assay is better than **0.05 ug/ml** when the limit of dilution is considered mean OD450nm/620-630nm CAL 0 ug/ml + 5 SD.

2. Diagnostic performances:

The diagnostic performances of the kit were studied in some external trials against (a) the “breath test”, considered by the medical literature the Gold Standard for HP Ag determination and (b) against a commercial CE-marked ELISA, as well.
In the studies were the breath test was used as reference stools were collected from patients the same day of the breath test.
In the study carried out with a commercial ELISA as reference the same samples were extracted with the specific devise supplied in combination with kits.
In the studies conducted in reference to the **breath test** the following mean values were obtained from two centers, one in Italy and one in Spain:

- Sensitivity: 98%
- Specificity: 96%

No confirmation by ELISA was possible on discrepant samples.

In the studies conducted in reference to a CE-marked commercial **ELISA**, similarly based on monoclonal antibodies to H.pylori, the following mean values were obtained:

- Sensitivity: 100%
- Specificity: 93%

The discrepant samples (7% “false positive”) turned to be positive in the breath test, revealing a better overall performance of our kit respect to the commercial reference one, when compared on the gold standard method.

Due to the highest specificity shown by the monoclonal antibodies used in the kit no cross-reaction was observed with Campilobacter species.

3. Precision:

The variability shown in the tables did not result in sample misclassification. CV values ranging 4-8%, depending on OD450nm/620-630nm values were observed.

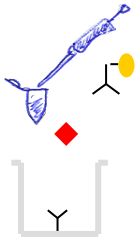
S. LIMITATIONS

False negative results were obtained from samples extracted from specimens stored for more than 1 day at 2..8°C. This finding is explain by the fact that the devise detects “live” H.pylori as confirmed by the results of excellent comparison to the breath test.

False positive results were mostly obtained from samples of liquid stools, difficult to handle and extract.

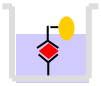
ASSAY GRAPHICAL SCHEME

Add 100 µl Calibrators, samples and conjugate to the plate and then incubate for 120 min at +37°C



Wash as described in the proper section

Add 200 µl Chromogen/Substrate and incubate 20 min at r.t.



Add 100 µl Sulphuric Acid

Read the plate at 450nm (reading) and at 620-630nm (blanking)

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All the IVD Products manufactured by the company are under the control of a certified Quality Management System in compliance with ISO 13485 rule. Each lot is submitted to a quality control and released into the market only if conforming with the EC technical specifications and acceptance criteria.

Manufacturer:
Dia.Pro Diagnostic Bioprobes Srl
Via G. Carducci n° 27 – Sesto San Giovanni (MI) – Italy

CE

Monobind Inc.
Lake Forest, CA 92630, USA

AccuBind
ELISA Microwells

Alpha-Fetoprotein (AFP) Test System
Product Code: 1925-300

1.0 INTRODUCTION

Intended Use: The Quantitative Determination of Alpha-Fetoprotein (AFP) Concentration in Human Serum by a Microplate Enzyme Immunoassay, Colorimetric

2.0 SUMMARY AND EXPLANATION OF THE TEST

Alpha-Fetoprotein (AFP) is a glycoprotein with a molecular weight of 70 kDA. AFP is normally produced during fetal development by the hepatocytes, yolk sac and, to a lesser extent, the gastrointestinal tract. Serum concentrations reach a peak level of up to 10 mg/ml at twelve weeks of gestation.[1] This peak level gradually decreases to less than 25 ng/ml after one year of postpartum. Thereafter, the levels reduce further to less than 10 ng/ml.

Elevated levels of AFP are found in patients with primary heptatoma and yolk sac-derived germ tumors. AFP is the most useful marker for the diagnosis and management of hepatocellular carcinoma.[2] AFP is also elevated in pregnant women. Presence of abnormally high AFP concentrations in pregnant women provides a risk marker for Down syndrome.[3]

In this method, AFP calibrator, patient specimen or control is first added to a streptavidin coated well. Biotinylated and enzyme labeled monoclonal antibodies (directed against distinct and different epitopes of AFP) are added and the reactants mixed. Reaction between the various AFP antibodies and native AFP forms a sandwich complex that binds with the streptavidin coated to the well. After the completion of the required incubation period, the enzyme-AFP antibody bound conjugate is separated from the unbound enzyme-AFP conjugate by aspiration or decantation. The activity of the enzyme present on the surface of the well is quantitated by reaction with a suitable substrate to produce color.

The employment of several serum references of known alpha-fetoprotein (AFP) levels permits the construction of a dose response curve of activity and concentration. From comparison to the dose response curve, an unknown specimen's activity can be correlated with AFP concentration.

3.0 PRINCIPLE

Immunoenzymometric assay (TYPE 3):
The essential reagents required for an immunoenzymometric assay include high affinity and specificity antibodies (enzyme and immobilized), with different and distinct epitope recognition, **in excess,** and native antigen. In this procedure, the immobilization takes place during the assay at the surface of a microplate well through the interaction of streptavidin coated on the well and exogenously added biotinylated monoclonal anti-AFP antibody.

Upon mixing monoclonal biotinylated antibody, the enzyme-labeled antibody and a serum containing the native antigen, reaction results between the native antigen and the antibodies, without competition or steric hindrance, to form a soluble sandwich complex. The interaction is illustrated by the following equation:

$$^{Enz}Ab + Ag_{AFP} + {}^{Btn}Ab_{(m)} \underset{k_{-a}}{\overset{k_a}{\rightleftharpoons}} {}^{Enz}Ab - Ag_{AFP} - {}^{Btn}Ab_{(m)}$$

$^{Btn}Ab_{(m)}$ = Biotinylated Monoclonal Antibody (Excess Quantity)
Ag_{AFP} = Native Antigen (Variable Quantity)
^{Enz}Ab = Enzyme labeled Antibody (Excess Quantity)
$^{Enz}Ab - Ag_{AFP} - {}^{Btn}Ab_{(m)}$ = Antigen-Antibodies Sandwich Complex
k_a = Rate Constant of Association
k_{-a} = Rate Constant of Dissociation

Simultaneously, the complex is deposited to the well through the high affinity reaction of streptavidin and biotinylated antibody. This interaction is illustrated below:
$^{Enz}Ab - Ag_{AFP} - {}^{Btn}Ab_{(m)} + Streptavidin_{C.W.} \Rightarrow$ Immobilized complex
$Streptavidin_{C.W.}$ = Streptavidin immobilized on well
Immobilized complex = sandwich complex bound to the well

After equilibrium is attained, the antibody-bound fraction is separated from unbound antigen by decantation or aspiration. The enzyme activity in the antibody-bound fraction is directly proportional to the native antigen concentration. By utilizing several different serum references of known antigen values, a dose response curve can be generated from which the antigen concentration of an unknown can be ascertained.

4.0 REAGENTS

Materials Provided:

A. AFP Calibrators – 1 ml/vial – Icons A-F
Six (6) vials of references AFP antigen at levels of 0 (**A**), 5 (**B**), 25 (**C**), 50 (**D**), 250 (**E**) and 500 (**F**)ng/ml. Store at 2-8°C. A preservative has been added.
Note: The calibrators, human serum based, were calibrated using a reference preparation, which was assayed against the WHO 1st IRP # 72/225.

B. AFP Enzyme Reagent – 13ml/vial – Icon Ⓔ
One (1) vial containing enzyme labeled antibody, biotinylated monoclonal mouse IgG in buffer, dye, and preservative. Store at 2-8°C.

C. Streptavidin Coated Microplate – 96 wells – Icon ⇓
One 96-well microplate coated with streptavidin and packaged in an aluminum bag with a drying agent. Store at 2-8°C.

D. Wash Solution Concentrate – 20ml/vial – Icon
One (1) vial containing a surfactant in buffered saline. A preservative has been added. Store at 2-8°C.

E. Substrate A – 7ml/vial – Icon S^A
One (1) vial containing tetramethylbenzidine (TMB) in buffer. Store at 2-8°C.

F. Substrate B – 7ml/vial – Icon S^B
One (1) vial containing hydrogen peroxide (H_2O_2) in buffer. Store at 2-8°C.

G. Stop Solution – 8ml/vial – Icon STOP
One (1) vial containing a strong acid (1N HCl). Store at 2-8°C.

H. Product Instructions.

Note 1: Do not use reagents beyond the kit expiration date.
Note 2: Opened reagents are stable for sixty (60) days when stored at 2-8°C. **Kit and component stability are identified on the label.**
Note 3: Above reagents are for a single 96-well microplate.

4.1 Required But Not Provided:
1. Pipette(s) capable of delivering 0.025 & 0.050ml (25 & 50µl) volumes with a precision of better than 1.5%.
2. Dispenser(s) for repetitive deliveries of 0.100 & 0.350ml (100 & 350µl) volumes with a precision of better than 1.5%.
3. Microplate washers or a squeeze bottle (optional).
4. Microplate Reader with 450nm and 620nm wavelength absorbance capability.
5. Absorbent Paper for blotting the microplate wells.
6. Plastic wrap or microplate cover for incubation steps.
7. Vacuum aspirator (optional) for wash steps.
8. Timer.
9. Quality control materials

5.0 PRECAUTIONS

For In Vitro Diagnostic Use
Not for Internal or External Use in Humans or Animals

All products that contain human serum have been found to be non-reactive for Hepatitis B Surface Antigen, HIV 1&2 and HCV Antibodies by FDA licensed reagents. Since no known test can offer complete assurance that infectious agents are absent, all human serum products should be handled as potentially hazardous and capable of transmitting disease. Good laboratory procedures for handling blood products can be found in the Center for Disease Control / National Institute of Health, "Biosafety in Microbiological and Biomedical Laboratories," 2nd Edition, 1988, HHS Publication No. (CDC) 88-8395.

Safe Disposal of kit components must be according to local regulatory and statutory requirement.

6.0 SPECIMEN COLLECTION AND PREPARATION

The specimens shall be blood, serum in type and the usual precautions in the collection of venipuncture samples should be observed. For accurate comparison to established normal values, a fasting morning serum sample should be obtained. The blood should be collected in a plain redtop venipuncture tube without additives or anti-coagulants. Allow the blood to clot. Centrifuge the specimen to separate the serum from the cells.

In patients receiving therapy with high biotin doses (i.e. >5mg/day), no sample should be taken until at least 8 hours after the last biotin administration, preferably overnight to ensure fasting sample.

Samples may be refrigerated at 2-8°C for a maximum period of five (5) days. If the specimen(s) cannot be assayed within this time, the sample(s) may be stored at temperatures of -20 °C for up to 30 days. Avoid use of contaminated devices. Avoid repetitive freezing and thawing. When assayed in duplicate, 0.050ml (50µl) of the specimen is required.

7.0 QUALITY CONTROL

Each laboratory should assay controls at levels in the low, normal and elevated range for monitoring assay performance. These controls should be treated as unknowns and values determined in every test procedure performed. Quality control charts should be maintained to follow the performance of the supplied reagents. Pertinent statistical methods should be employed to ascertain trends. Significant deviation from established performance can indicate unnoticed change in experimental conditions or degradation of kit reagents. Fresh reagents should be used to determine the reason for the variations.

8.0 REAGENT PREPARATION

1. **Wash Buffer**
Dilute contents of wash concentrate to 1000ml with distilled or deionized water in a suitable storage container. Store diluted buffer at 2-30°C for up to 60 days.
2. **Working Substrate Solution** - Stable for one (1) year
Pour the contents of the amber vial labeled Solution 'A' into the clear vial labeled Solution 'B'. Place the yellow cap on the clear vial for easy identification. Mix and label accordingly. Store at 2 - 8°C.

Note 1: Do not use the working substrate if it looks blue.
Note 2: Do not use reagents that are contaminated or have bacteria growth.

9.0 TEST PROCEDURE

Before proceeding with the assay, bring all reagents, serum reference calibrators and controls to room temperature (20-27°C).
*****Test Procedure should be performed by a skilled individual or trained professional*****

1. Format the microplates' wells for each serum reference calibrator, control and patient specimen to be assayed in duplicate. **Replace any unused microwell strips back into the aluminum bag, seal and store at 2-8°C.**
2. Pipette 0.025ml (25µl) of the appropriate serum reference calibrator, control or specimen into the assigned well.
3. Add 0.100ml (100µl) of the AFP Enzyme Reagent to each well. **It is very important to dispense all reagents close to the bottom of the coated well.**
4. Mix **(See Note)** the microplate for 20-30 seconds until homogenous.
5. Swirl the microplate gently for 20-30 seconds to mix and cover.
6. Incubate 60 minutes at room temperature.
7. Discard the contents of the microplate by decantation or aspiration. If decanting, tap and blot the plate dry with absorbent paper.
8. Add 0.350ml (350µl) of wash buffer (see Reagent Preparation Section), decant (tap and blot) or aspirate. Repeat two (2) additional times for a total of three (3) washes. **An automatic or manual plate washer can be used. Follow the manufacturer's instruction for proper usage. If a squeeze bottle is employed, fill each well by depressing the container (avoiding air bubbles) to dispense the wash. Decant the wash and repeat two (2) additional times.**
9. Add 0.100ml (100µl) of working substrate solution to all wells (see Reagent Preparation Section). **Always add reagents in the same order to minimize reaction time differences between wells.**
DO NOT SHAKE THE PLATE AFTER SUBSTRATE ADDITION
10. Incubate at room temperature for fifteen (15) minutes.
11. Add 0.050ml (50µl) of stop solution to each well and mix gently for 15-20 seconds. **Always add reagents in the same order to minimize reaction time differences between wells.**
12. Read the absorbance in each well at 450nm (using a reference wavelength of 620-630nm to minimize well imperfections) in a microplate reader. **The results should be read within thirty (30) minutes of adding the stop solution.**

Note: Cycle (start and stop) mixing (4 cycles) for 5-8 seconds/cycle is more efficient than one continuous (20-30 seconds) cycle to achieve homogeneity. A plate mixer can be used to perform the mixing cycle.

10.0 CALCULATION OF RESULTS

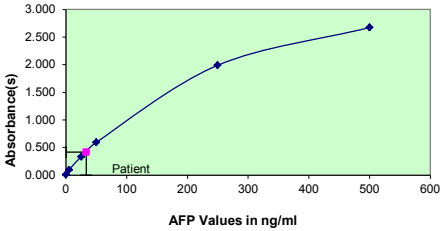
A dose response curve is used to ascertain the concentration of AFP in unknown specimens.
1. Record the absorbance obtained from the printout of the microplate reader as outlined in Example 1.
2. Plot the absorbance for each duplicate serum reference versus the corresponding AFP concentration in ng/ml on linear graph paper (do not average the duplicates of the serum references before plotting).
3. Draw the best-fit curve through the plotted points.
4. To determine the concentration of AFP for an unknown, locate the average absorbance of the duplicates for each unknown on the vertical axis of the graph, find the intersecting point on the curve, and read the concentration (in ng/ml) from the horizontal axis of the graph (the duplicates of the unknown may be averaged as indicated). In the following example, the average absorbance (0.420) intersects the dose response curve at 33.2 ng/ml AFP concentration (See Figure 1).

Note: Computer data reduction software designed for ELISA assays may also be used for the data reduction. If such software is utilized, the validation of the software should be ascertained.

EXAMPLE 1

Sample I.D.	Well Number	Abs (A)	Mean Abs (B)	Value (ng/ml)
Cal A	A1	0.012	0.011	0
	B1	0.011		
Cal B	C1	0.100	0.098	5
	D1	0.097		
Cal C	E1	0.336	0.335	25
	F1	0.333		
Cal D	G1	0.612	0.594	50
	H1	0.577		
Cal E	A2	2.005	1.990	250
	B2	1.975		
Cal F	C2	2.664	2.672	500
	D2	2.680		
Patient	E2	0.427	0.420	33.2
	F2	0.413		

Figure 1



*The data presented in Example 1 and Figure 1 is for illustration only and **should not** be used in lieu of a dose response curve prepared with each assay.

11.0 QC PARAMETERS

In order for the assay results to be considered valid the following criteria should be met:

1. The absorbance (OD) of calibrator F should be ≥ 1.3.
2. The absorbance (OD) of calibrator A should be ≤ 0.035.
3. Four out of six quality control pools should be within the established ranges.

12.0 RISK ANALYSIS

The MSDS and Risk Analysis Form for this product are available on request from Monobind Inc.

12.1 Assay Performance

1. It is important that the time of reaction in each well is held constant to achieve reproducible results.
2. Pipetting of samples should not extend beyond ten (10) minutes to avoid assay drift.
3. Highly lipemic, hemolyzed or grossly contaminated specimen(s) should not be used.
4. If more than one (1) plate is used, it is recommended to repeat the dose response curve.
5. The addition of substrate solution initiates a kinetic reaction, which is terminated by the addition of the stop solution. Therefore, the substrate and stop solution should be added in the same sequence to eliminate any time-deviation during reaction.
6. Plate readers measure vertically. Do not touch the bottom of the wells.
7. Failure to remove adhering solution adequately in the aspiration or decantation wash step(s) may result in poor replication and spurious results.
8. Use components from the same lot. No intermixing of reagents from different batches.
9. Patient specimens with AFP concentrations above 500 ng/ml may be diluted (for example 1/10 or higher) with normal male serum (AFP < 10 ng/ml) and re-assayed. The sample's concentration is obtained by multiplying the result by the dilution factor (x10).
10. Accurate and precise pipetting, as well as following the exact time and temperature requirements prescribed are essential. Any deviation from Monobind's IFU may yield inaccurate results.
11. All applicable national standards, regulations and laws, including, but not limited to, good laboratory procedures, must be strictly followed to ensure compliance and proper device usage.
12. It is important to calibrate all the equipment e.g. Pipettes, Readers, Washers and/or the automated instruments used with this device, and to perform routine preventative maintenance.
13. Risk Analysis- as required by CE Mark IVD Directive 98/79/EC - for this and other devices, made by Monobind, can be requested via email from Monobind@monobind.com.

12.2 Interpretation

1. ***Measurements and interpretation of results must be performed by a skilled individual or trained professional.***
2. Laboratory results alone are only one aspect for determining patient care and should not be the sole basis for therapy, particularly if the results conflict with other determinants.
3. The reagents for the test system procedures have been formulated to eliminate maximal interference; however, potential interaction between rare serum specimens and test reagents can cause erroneous results. Heterophilic antibodies often cause these interactions and have been known to be problems for all kinds of immunoassays (Boscato LM, Stuart MC. "Heterophilic antibodies: a problem for all immunoassays" *Clin.Chem.* 1988:3427-33). For diagnostic purposes, the results from this assay should be used in combination with clinical examination, patient history and all other clinical findings.
4. For valid test results, adequate controls and other parameters must be within the listed ranges and assay requirements.
5. If test kits are altered, such as by mixing parts of different kits, which could produce false test results, or if results are incorrectly interpreted, Monobind shall have no liability.
6. If computer controlled data reduction is used to interpret the results of the test, it is imperative that the predicted values for the calibrators fall within 10% of the assigned concentrations.
7. AFP has a low clinical sensitivity and specificity as a tumor marker. Clinically an elevated **AFP value alone is not of diagnostic value as a test for cancer** and should only be used in conjunction with other clinical manifestations (observations) and diagnostic parameters. AFP levels are known to be elevated in a number of benign diseases and conditions including pregnancy and non-malignant liver diseases such as hepatitis and cirrhosis.

13.0 EXPECTED RANGE OF VALUES

Approximately 97-98% of the normal healthy population has AFP levels less than 8.5ng/ml.[4] In high-risk patients, AFP values between 100-350 ng/ml suggest hepatocellular carcinoma. Concentrations over 350 ng/ml usually indicate the disease.

TABLE I
Expected Values for the AFP AccuBind® ELISA Test System

Male and Female	<8.5ng/ml (97-98%)

Values for AFP for a normal, healthy population and pregnant women, during gestation cycle, are given in Table 2. The values depicted below represent limited in house studies in concordance with published literature.[8,9,10]

TABLE 2
Median Values during Gestation.

Gestation (Week)	AFP (ng/ml)
15	40.14
16	42.91
17	52.34
18	61.50
19	75.57
20	83.31
21	90.46

It is important to keep in mind that establishment of a range of values, which can be expected to be found by a given method for a population of "normal" persons, is dependent upon a multiplicity of factors: the specificity of the method, the population tested and the precision of the method in the hands of the analyst. For these reasons, each laboratory should depend upon the range of expected values established by the Manufacturer only until an in-house range can be determined by the analysts using the method with a population indigenous to the area in which the laboratory is located.

14.0 PERFORMANCE CHARACTERISTICS

14.1 Precision

The within and between assay precision of the AFP AccuBind® ELISA test system were determined by analyses on three different levels of control sera. The number, mean value, standard deviation and coefficient of variation for each of these control sera are presented in Table 3 and Table 4.

TABLE 3
Within Assay Precision (Values in ng/ml)

Sample	N	X	σ	C.V.
Level 1	24	14.71	0.67	4.6
Level 2	24	71.89	2.68	3.7
Level 3	24	148.62	7.24	4.9

TABLE 4
Between Assay Precision* (Values in ng/ml)

Sample	N	X	σ	C.V.
Level 1	30	16.20	1.41	8.7
Level 2	30	88.26	7.47	8.5
Level 3	30	188.43	11.92	6.3

*As measured in thirty experiments in duplicate.

14.2 Sensitivity

The AFP AccuBind® ELISA Test System has a sensitivity of 0.01 ng. This is equivalent to a sample containing 0.44 ng/ml AFP concentration. The sensitivity (detection limit) was ascertained by determining the variability of the '0 ng/ml' calibrator and using the 2σ (95% certainty) statistic to calculate the minimum dose.

14.3 Accuracy

The AFP AccuBind® ELISA Test System was compared with a reference method. Biological specimens with concentrations ranging from 1.0 to 41 ng/ml were assayed. The total number of such specimens was 42. The least square regression equation and the correlation coefficient were computed for the AFP procedure in comparison with the reference method. The data obtained is displayed in Table 5.

TABLE 5

Method	Mean	Least Square Regression Analysis	Correlation Coefficient
This Method (Y)	5.27	y = 0.746(x) + 1.0007	0.973
Reference (X)	5.72		

Only slight amounts of bias between the AFP AccuBind® ELSIA Test System and the reference method are indicated by the closeness of the mean values. The least square regression equation and correlation coefficient indicates excellent method agreement.

14.4 Specificity

No interference was detected with the performance of AFP AccuBind® ELISA Test System upon addition of massive amounts of the following substances to a human serum pool.

SUBSTANCE	Cross Reactivity	Concentration
Acetylsalicylic Acid	ND	100 µg/ml
Amethopterin	ND	100 µg/ml
Ascorbic Acid	ND	100 µg/ml
Atropine	ND	100 µg/ml
Caffeine	ND	100 µg/ml
CEA	ND	10 µg/ml
PSA	ND	1.0 µg/ml
CA-125	ND	10,000 U/ml
hCG	ND	1000 IU/ml
hLH	ND	10 IU/ml
hTSH	ND	100 mIU/ml
hPRL	ND	100 µg/ml

14.5 Linearity & Hook Effect:

Three different lot preparations of the AFP AccuBind® ELISA test system reagents were used to assess the linearity and hook effect. Massive concentrations of AFP (> 100,000 ng/ml) were used for linear dilutions in pooled human patient sera.

The test showed no hook effect up to concentrations of 10,000 ng/ml and a with a dose recovery of 86.1 to 113.6%.

15.0 REFERENCES

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Effective Date: 2021-Sep-23 **Rev. 8** **DCO: 1509**
MP1925 **Product Code: 1925-300**

Size		96(A)	192(B)
Reagent (fill)	A)	1ml set	1ml set
	B)	1 (13ml)	2 (13ml)
	C)	1 plate	2 plates
	D)	1 (20ml)	1 (20ml)
	E)	1 (7ml)	2 (7ml)
	F)	1 (7ml)	2 (7ml)
	G)	1 (8ml)	2 (8ml)

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IVD 2° 8° CE
EC REP **CEpartner4U,** Esdoornlaan 13 3951 DBMaarn, The Neatherlands **www.cepartner4u.eu**

Please visit our website to learn more about our products and services.

Glossary of Symbols
(EN 980/ISO 15223)

IVD In Vitro - Diagnostic Medical Device	2° 8° Temperature Limitation Storage Condition (2-8° C)	Consult Instructions for Use
REF Catalogue Number	Σ Contains Sufficient Test for Σ	LOT Batch Code
Used By (Expiration Day)	Date of Manufacturer	Manufacturer
EC REP Authorized Rep in European Country	CE European Conformity	

NovaTec Immundiagnostica GmbH

NovaLisa®

Chlamydia pneumoniae IgG

ELISA

CE 0483

Only for in-vitro diagnostic use

Product Number: CHLG0510 (96 Determinations)

ENGLISH

1. INTRODUCTION

Chlamydiae are no motile, Gram negative and obligatory intracellular growing bacteria which form characteristic inclusions within the cytoplasm of parasitized cells. They are easily visible in the light microscope. Three different Chlamydia species pathogenic for humans are known: Chlamydia trachomatis, Chlamydia pneumoniae and Chlamydia psittaci, and one species only pathogenic for animals (C. pecorum). Chlamydia trachomatis is the most prevalent agent of sexually transmitted diseases worldwide (400-500 million cases) and the number of infections is constantly growing. Pregnant women infected with C. trachomatis may transmit these bacteria during childbirth, causing conjunctivitis or pneumonia in newborns. Untreated cases of chlamydial infection can lead to chronic salpingitis, possibly resulting in ectopic pregnancy or infertility. In males, C. trachomatis is a major cause of non-gonococcal urethritis. A severe problem in Chlamydia infections is the frequent asymptomatic insidious course which may result in the initiation of chronic diseases. In many instances primary infections are not recognized and only the sequelae caused by ascended, persisting agents are diagnosed..

Species	Disease	Symptoms (e.g.)	Transmission route
Chlamydia trachomatis	Trachoma	Bilateral conjunctivitis with foreign body sensation, tearing eyes, and discharge of serous secretions from the eye.	Sexually transmitted. Can be passed from an infected mother to her baby during childbirth.
	Lymphogranuloma venereum (LGV)	Genital lesion; swelling of the inguinal and femoral lymph nodes; formation of suppurating buboes.	
	Urogenital infections	**In men:** Inflammation of the urethra (urethritis), inflammation of the prostate (prostatitis) and the epididymis (epididymitis). Sterility (infertility). **In women:** Inflammation of the urethra (urethritis), Bartholin's glands, inflammation of the cervix (cervicitis), uterine mucous membrane (endometritis) and fallopian tubes (salpingitis) and vaginal discharge. Sterility (Infertility)	
C. pneumoniae	Respiratory diseases (e.g. pneumonia, bronchitis) discussed: endocarditis, coronary heart diseases	Fever, cough (usually productive)	Man to Man Aerogenic, droplet infection
C. psittaci	Ornithosis (Psittacosis)	hepatosplenomegaly; unproductive cough, fever and severe headache	Inhalation of feces from infected birds; contact with infected avian viscera

Infection or presence of pathogen may be identified by:

- Microscopy
- PCR
- Serology: Detection of antigens by ELISA
  Detection of antibodies by ELISA

2. INTENDED USE

The Chlamydia pneumoniae IgG ELISA is intended for the qualitative determination of IgG class antibodies against Chlamydia pneumoniae in human serum or plasma (citrate, heparin).

3. PRINCIPLE OF THE ASSAY

The qualitative immunoenzymatic determination of specific antibodies is based on the ELISA (Enzyme-linked Immunosorbent Assay) technique.
Microtiterplates are coated with specific antigens to bind corresponding antibodies of the sample. After washing the wells to remove all unbound sample material a horseradish peroxidase (HRP) labelled conjugate is added. This conjugate binds to the captured antibodies. In a second washing step unbound conjugate is removed. The immune complex formed by the bound conjugate is visualized by adding Tetramethylbenzidine (TMB) substrate which gives a blue reaction product.
The intensity of this product is proportional to the amount of specific antibodies in the sample. Sulphuric acid is added to stop the reaction. This produces a yellow endpoint colour. Absorbance at 450/620 nm is read using an ELISA Microtiterplate reader.

4. MATERIALS

4.1. Reagents supplied

- **Microtiterplate:** 12 break-apart 8-well snap-off strips coated with Chlamydia pneumoniae antigens; in resealable aluminium foil.
- **IgG Sample Dilution Buffer:** 1 bottle containing 100 mL of phosphate buffer (10 mM) for sample dilution; pH 7.2 ± 0.2; coloured yellow; ready to use; white ca; ≤ 0.0015% (v/v) CMIT/ MIT (3:1).
- **Stop Solution:** 1 bottle containing 15 mL sulphuric acid, 0.2 mol/L; ready to use; red cap.
- **Washing Buffer (20x conc.):** 1 bottle containing 50 mL of a 20-fold concentrated phosphate buffer (0.2 M), pH 7.2 ± 0.2, for washing the wells; white cap.
- **Conjugate:** 1 bottle containing 20 mL of peroxidase labelled antibody to human IgG in phosphate buffer (10 mM); coloured blue; ready to use; black cap.
- **TMB Substrate Solution:** 1 bottle containing 15 mL 3,3',5,5'-tetramethylbenzidine (TMB), < 0.1 %; ready to use; yellow cap.
- **Positive Control:** 1 vial containing 2 mL control; coloured yellow; ready to use; red cap; ≤ 0.02% (v/v) MIT.
- **Cut-off Control:** 1 vial containing 3 mL control; coloured yellow; ready to use; green cap; ≤ 0.02% (v/v) MIT.
- **Negative Control:** 1 vial containing 2 mL control; coloured yellow; ready to use; blue cap; ≤ 0.0015% (v/v) CMIT/ MIT (3:1).

For hazard and precautionary statements see 12.1
For potential hazardous substances please check the safety data sheet.

4.2. Materials supplied

- 1 Cover foil
- 1 Instruction for use (IFU)
- 1 Plate layout

4.3. Materials and Equipment needed

- ELISA Microtiterplate reader, equipped for the measurement of absorbance at 450/620 nm
- Incubator 37 °C
- Manual or automatic equipment for rinsing Microtiterplates
- Pipettes to deliver volumes between 10 and 1000 µL
- Vortex tube mixer
- Distilled water
- Disposable tubes

5. STABILITY AND STORAGE

Store the kit at 2...8 °C. The opened reagents are stable up to the expiry date stated on the label when stored at 2...8 °C.

6. REAGENT PREPARATION

It is very important to bring all reagents and samples to room temperature (20…25 °C) and mix them before starting the test run!

6.1. Microtiterplate

The break-apart snap-off strips are coated with Chlamydia pneumoniae antigens. Immediately after removal of the strips, the remaining strips should be resealed in the aluminium foil along with the desiccant supplied and stored at 2...8 °C.

6.2. Washing Buffer (20x conc.)

Dilute Washing Buffer 1 + 19; e. g. 10 mL Washing Buffer + 190 mL distilled water. The diluted buffer is stable for 5 days at room temperature (20…25 °C). In case crystals appear in the concentrate, warm up the solution to 37°C e.g. in a water bath. Mix well before dilution.

6.3. TMB Substrate Solution

The reagent is ready to use and has to be stored at 2...8 °C, away from the light. The solution should be colourless or could have a slight blue tinge. If the substrate turns into blue, it may have become contaminated and should be thrown away.

7. SAMPLE COLLECTION AND PREPARATION

Use human serum or plasma (citrate, heparin) samples with this assay. If the assay is performed within 5 days after sample collection, the samples should be kept at 2...8 °C; otherwise they should be aliquoted and stored deep-frozen (-70…-20 °C). If samples are stored frozen, mix thawed samples well before testing. Avoid repeated freezing and thawing.
Heat inactivation of samples is not recommended.

7.1. Sample Dilution

Before assaying, all samples should be diluted 1+100 with IgG Sample Dilution Buffer. Dispense 10 µL sample and 1 mL IgG Sample Dilution Buffer into tubes to obtain a 1+100 dilution and thoroughly mix with a Vortex.

8. ASSAY PROCEDURE

Please read the instruction for use carefully **before** performing the assay. Result reliability depends on strict adherence to the instruction for use as described. The following test procedure is only validated for manual procedure. If performing the test on ELISA automatic systems we recommend increasing the washing steps from three up to five and the volume of Washing Buffer from 300 µL to 350 µL to avoid washing effects. Pay attention to chapter 12. Prior to commencing the assay, the distribution and identification plan for all samples and standards/controls (duplicates recommended) should be carefully established on the plate layout supplied in the kit. Select the required number of microtiter strips or wells and insert them into the holder.

Perform all assay steps in the order given and without any delays.

A clean, disposable tip should be used for dispensing each standard/control and sample.

Adjust the incubator to 37 ± 1 °C.

1. Dispense 100 µL standards/controls and diluted samples into their respective wells. Leave well A1 for the Substrate Blank.
2. Cover wells with the foil supplied in the kit.
3. **Incubate for 1 hour ± 5 min at 37 ± 1 °C.**
4. When incubation has been completed, remove the foil, aspirate the content of the wells and wash each well three times with 300 µL of Washing Buffer. Avoid overflows from the reaction wells. The interval between washing and aspiration should be > 5 sec. At the end carefully remove remaining fluid by tapping strips on tissue paper prior to the next step!

   Note: Washing is important! Insufficient washing results in poor precision and false results.
5. Dispense 100 µL Conjugate into all wells except for the Substrate Blank well A1.
6. **Incubate for 30 min at room temperature (20...25 °C).** Do not expose to direct sunlight.
7. Repeat step 4.
8. Dispense 100 µL TMB Substrate Solution into all wells.
9. **Incubate for exactly 15 min at room temperature (20...25 °C) in the dark.** A blue colour occurs due to an enzymatic reaction.
10. Dispense 100 µL Stop Solution into all wells in the same order and at the same rate as for the TMB Substrate Solution, thereby a colour change from blue to yellow occurs.
11. Measure the absorbance at 450/620 nm within 30 min after addition of the Stop Solution.

8.1. Measurement

Adjust the ELISA Microtiterplate reader **to zero** using the **Substrate Blank**.

If - due to technical reasons - the ELISA Microtiterplate reader cannot be adjusted to zero using the Substrate Blank, subtract its absorbance value from all other absorbance values measured in order to obtain reliable results!

Measure the absorbance of all wells at **450 nm** and record the absorbance values for each standard/control and sample in the plate layout.

Bichromatic measurement using a reference wavelength of 620 nm is recommended.

Where applicable calculate the mean absorbance values of all duplicates.

9. RESULTS

9.1. Run Validation Criteria

In order for an assay run to be considered valid, these Instructions for Use have to be strictly followed and the following criteria must be met:

- **Substrate Blank**: Absorbance value **< 0.100**
- **Negative Control**: Absorbance value **< 0.200 and < Cut-off**
- **Cut-off Control**: Absorbance value **0.150 – 1.300**
- **Positive Control**: Absorbance value **> Cut-off**

If these criteria are not met, the test is not valid and must be repeated.

9.2. Calculation of Results

The Cut-off is the mean absorbance value of the Cut-off Control determinations.

Example: Absorbance value Cut-off Control 0.44 + absorbance value Cut-off control 0.42 = 0.86 / 2 = 0.43
Cut-off = 0.43

9.2.1. Results in Units [NTU]

$$\frac{\text{Sample (mean) absorbance value} \times 10}{\text{Cut-off}} = \text{[NovaTec Units = NTU]}$$

Example: $\frac{1.591 \times 10}{0.43} = 37$ NTU (Units)

9.3. Interpretation of Results

Cut-off	10 NTU	-
Positive	> 11 NTU	Antibodies against the pathogen are present. There has been a contact with the antigen (pathogen resp. vaccine).
Equivocal	9 – 11 NTU	Antibodies against the pathogen could not be detected clearly. It is recommended to repeat the test with a fresh sample in 2 to 4 weeks. If the result is equivocal again the sample is judged as **negative**.
Negative	< 9 NTU	The sample contains no antibodies against the pathogen. A previous contact with the antigen (pathogen resp. vaccine) is unlikely.
Diagnosis of an infectious disease should not be established on the basis of a single test result. A precise diagnosis should take into consideration clinical history, symptomatology as well as serological data. In immunocompromised patients and newborns serological data only have restricted value.		

9.3.1. Antibody Isotypes and State of Infection

Serology	Significance
IgM	Characteristic of the primary antibody response High IgM titer with low IgG titer: → suggests a current or very recent infection Rare: → persisting IgM
IgG	Characteristic of the secondary antibody response May persist for several years High IgG titer with low IgM titer: → may indicate a past infection
IgA	Produced in mucosal linings throughout the body (⇒ protective barrier) Usually produced early in the course of the infection

10. SPECIFIC PERFORMANCE CHARACTERISTICS

The results refer to the groups of samples investigated; these are not guaranteed specifications.

For further information about the specific performance characteristics please contact NovaTec Immundiagnostica GmbH.

10.1. Precision

Intraassay	**n**	**Mean (E)**	**CV (%)**
#1	24	0,376	11,46
#2	24	0,887	7,69
#3	24	1,105	8,01
Interassay	**n**	**Mean (NTU)**	**CV (%)**
#1	12	26,31	3,37
#2	12	25,00	5,09
#3	12	5,66	9,75

10.2. Diagnostic Specificity

The diagnostic specificity is defined as the probability of the assay of scoring negative in the absence of the specific analyte.
It is 95.12% (95% confidence interval: 83.47% - 99.4%).

10.3. Diagnostic Sensitivity

The diagnostic sensitivity is defined as the probability of the assay of scoring positive in the presence of the specific analyte.
It is 91.67% ((95% confidence interval: 81.61% - 97.24%).

10.4. Interferences

Interferences with hemolytic, lipemic or icteric samples are not observed up to a concentration of 10 mg/mL hemoglobin, 5 mg/mL triglycerides and 0.5 mg/mL bilirubin.

10.5. Cross Reactivity

Investigation of a sample panel with antibody activities to potentially cross-reacting parameters did not reveal evidence of false-positive results due to cross-reactions.
A cross reaction with Chlamydia trachomatis cannot be excluded with sera containing antibodies to LPS and MOMP.

11. LIMITATIONS OF THE PROCEDURE

Bacterial contamination or repeated freeze-thaw cycles of the sample may affect the absorbance values.

12. PRECAUTIONS AND WARNINGS

- The test procedure, the information, the precautions and warnings in the instructions for use have to be strictly followed. The use of the testkits with analyzers and similar equipment has to be validated. Any change in design, composition and test procedure as well as for any use in combination with other products not approved by the manufacturer is not authorized; the user himself is responsible for such changes. The manufacturer is not liable for false results and incidents for these reasons. The manufacturer is not liable for any results by visual analysis of the patient samples.
- Only for in-vitro diagnostic use.
- All materials of human or animal origin should be regarded and handled as potentially infectious.
- All components of human origin used for the production of these reagents have been tested for anti-HIV antibodies, anti-HCV antibodies and HBsAg and have been found to be non-reactive.
- Do not interchange reagents or Microtiterplates of different production lots.
- No reagents of other manufacturers should be used along with reagents of this test kit.
- Do not use reagents after expiry date stated on the label.
- Use only clean pipette tips, dispensers, and lab ware.
- Do not interchange screw caps of reagent vials to avoid cross-contamination.
- Close reagent vials tightly immediately after use to avoid evaporation and microbial contamination.
- After first opening and subsequent storage check conjugate and standard/control vials for microbial contamination prior to further use.
- To avoid cross-contamination and falsely elevated results pipette patient samples and dispense reagents without splashing accurately into the wells.
- The ELISA is only designed for qualified personnel following the standards of good laboratory practice (GLP).
- For further internal quality control each laboratory should additionally use known samples.

12.1. Safety note for reagents containing hazardous substances

Reagents may contain CMIT/MIT (3:1) or MIT (refer to 4.1)
Therefore, the following hazard and precautionary statements apply.

Warning

H317	May cause an allergic skin reaction.
P261	Avoid breathing spray
P280	Wear protective gloves/ protective clothing.
P302+P352	IF ON SKIN: Wash with plenty of soap and water.
P333+P313	If skin irritation or rash occurs: Get medical advice/ attention.
P362+P364	Take off contaminated and Wash it before reuse.

Further information can be found in the safety data sheet.

12.2. Disposal Considerations

Residues of chemicals and preparations are generally considered as hazardous waste. The disposal of this kind of waste is regulated through national and regional laws and regulations. Contact your local authorities or waste management companies which will give advice on how to dispose hazardous waste.

13. ORDERING INFORMATION

Prod. No.: CHLG0510 Chlamydia pneumoniae IgG ELISA (96 Determinations)

DEUTSCH

1. EINLEITUNG

Chlamydien sind unbewegliche, gramnegative, obligat intrazelluläre Bakterien, die charakteristische Einschlüsse im Cytoplasma der parasitierten Zelle bilden. Im Lichtmikroskop sind sie gut zu erkennen. Man unterscheidet drei für den Menschen pathogene Spezies: Chlamydia trachomatis, Chlamydia pneumoniae und Chlamydia psittaci. Chlamydia pecorum ist die einzige für Tiere pathogen Art. Chlamydia trachomatis ist die häufigste Ursache sexuell übertragener Erkrankungen weltweit (400-500 Millionen Fälle). Schwangere Frauen mit C. trachomatis können unter der Geburt das Neugeborene infizieren. Es kommt zu Konjunktivitiden oder Pneumonien. Unbehandelte Fälle von Chlamydien Infektionen können zu chronischer Salpingitis mit resultierenden ektopen Schwangerschaften oder Sterilität führen. Bei Männern ist Chlamydia trachomatis häufigste Ursache der nicht ghonorrhoischen Urethritiden. Ein ernstes Problem der Chlamydien Infektionen ist der häufig asymptomatische Verlauf, der letztlich jedoch in einer chronischen Erkrankung resultieren kann. In vielen Fällen wird die Primärinfektion nicht rechtzeitig erkannt und erst die Folgeschäden diagnostiziert.

Spezies	**Erkrankung**	**Symptome (z.B.)**	**Infektionsweg**
Chlamydia trachomatis	Trachom	Beidseitige Konjunktivitis mit Fremdkörpergefühl, tränenden Augen und Ausfluss von serösem Sekret aus dem Auge.	Kontakt-Schmierinfektion (Geschlechtsverkehr).
	Lymphogranuloma venereum	Genitale Läsion; Schwellung der Leisten- und Femorallymphknoten; Entzündung des Lymphknotens.	Übertragung von der Mutter auf das Kind bei der Geburt.
	Urogenitalinfektionen	**Beim Mann**: Entzündungen der Harnröhre (Urethritis), Entzündungen der Prostata (Prostatitis) und der Nebenhoden (Epididymitis). Sterilität (Unfruchtbarkeit). **Bei der Frau**: Entzündung der Harnröhre (Urethritis), der Bartholinschen Drüsen, Entzündungen des Gebärmutterhalses (Zervizitis), der Gebärmutterschleimhaut (Endometritis) und der Eileiter (Salpingitis) und vaginaler Ausfluss. Sterilität (Unfruchtbarkeit)	
C. pneumoniae	Erkrankungen der Atemwege (z.B. Bronchitis, Pneumonie). In Diskussion: Endokarditis, koronare Herzkrankheiten	Husten (in der Regel produktiv), Fieber	Mensch zu Mensch Aerogen; Tröpfcheninfektion
C. psittaci	Ornithose (psittacose)	Hepatosplenomegalie; unproduktiver Husten, Fieber und starke Kopfschmerzen	Inhalation von Fäkalien infizierter Vögel; Kontakt mit infizierten Vogelarten

Nachweis des Erregers bzw. der Infektion durch:

- Mikroskopie
- PCR
- Serologie: Nachweis der Antigene mittels ELISA
  Nachweis der Antikörper mittels IF, EIA, ELISA

2. VERWENDUNGSZWECK

Der Chlamydia pneumoniae IgG ELISA ist für den qualitativen Nachweis spezifischer IgG-Antikörper gegen Chlamydia pneumoniae in humanem Serum oder Plasma (Citrat, Heparin) bestimmt.

3. TESTPRINZIP

Die qualitative immunenzymatische Bestimmung von spezifischen Antikörpern beruht auf der ELISA (Enzyme-linked Immunosorbent Assay) Technik.
Die Mikrotiterplatten sind mit spezifischen Antigenen beschichtet, an welche die korrespondierenden Antikörper aus der Probe binden. Ungebundenes Probenmaterial wird durch Waschen entfernt. Anschließend erfolgt die Zugabe eines Meerettich-Peroxidase (HRP) Konjugates. Dieses Konjugat bindet an die an der Mikrotiterplatte gebundenen spezifischen Antikörper. In einem zweiten Waschschritt wird ungebundenes Konjugat entfernt. Die Immunkomplexe, die durch die Bindung des Konjugates entstanden sind, werden durch die Zugabe von Tetramethylbenzidin (TMB)-Substratlösung und eine resultierende Blaufärbung nachgewiesen.
Die Intensität des Reaktionsproduktes ist proportional zur Menge der spezifischen Antikörper in der Probe. Die Reaktion wird mit Schwefelsäure gestoppt, wodurch ein Farbumschlag von blau nach gelb erfolgt. Die Absorption wird bei 450/620 nm mit einem Mikrotiterplatten-Photometer gemessen.

4. MATERIALIEN

4.1. Mitgelieferte Reagenzien

- **Mikrotiterplatte:** 12 teilbare 8er-Streifen, beschichtet mit Chlamydia pneumoniae Antigenen; in wieder verschließbarem Aluminiumbeutel.
- **IgG-Probenverdünnungspuffer:** 1 Flasche mit 100 mL Phosphatpuffer (10 mM) zur Probenverdünnung; pH 7,2 ± 0,2; gelb gefärbt; gebrauchsfertig; weiße Verschlusskappe*;* ≤ 0,0015% (v/v) CMIT/ MIT (3:1).
- **Stopplösung:** 1 Flasche mit 15 mL Schwefelsäure, 0,2 mol/L; gebrauchsfertig; rote Verschlusskappe.
- **Waschpuffer (20x konz.):** 1 Flasche mit 50 mL eines 20-fach konzentrierten Phosphatpuffers (0,2 M), zum Waschen der Kavitäten; pH 7,2 ± 0,2; weiße Verschlusskappe.
- **Konjugat:** 1 Flasche mit 20 mL Peroxidase-konjugierten Antikörpern gegen humanes IgG in Phosphatpuffer (10 mM); blau gefärbt; gebrauchsfertig; schwarze Verschlusskappe.
- **TMB-Substratlösung:** 1 Flasche mit 15 mL 3,3`,5,5`-Tetramethylbenzidin (TMB), < 0,1 %; gebrauchsfertig; gelbe Verschlusskappe.
- **Positivkontrolle:** 1 Fläschchen mit 2 mL Kontrolle; gelb gefärbt; rote Verschlusskappe; gebrauchsfertig. ≤ 0,02% (v/v) MIT.
- **Cut-off Kontrolle:** 1 Fläschchen mit 3 mL Kontrolle; gelb gefärbt; grüne Verschlusskappe; gebrauchsfertig. ≤ 0,02% (v/v) MIT.
- **Negativkontrolle:** 1 Fläschchen mit 2 mL Kontrolle; gelb gefärbt; blaue Verschlusskappe; gebrauchsfertig; ≤ 0,0015% (v/v) CMIT/ MIT (3:1).

Für Gefahren- und Sicherheitshinweise siehe 12.1.
Für potenzielle Gefahrstoffe überprüfen Sie bitte das Sicherheitsdatenblatt.

4.2. Mitgeliefertes Zubehör

- 1 selbstklebende Abdeckfolie
- 1 Arbeitsanleitung
- 1 Plattenlayout

4.3. Erforderliche Materialien und Geräte

- Mikrotiterplatten-Photometer mit Filtern 450/620 nm
- Inkubator 37°C
- Manuelle oder automatische Waschvorrichtung für Mikrotiterplatten
- Mikropipetten (10 - 1000 µL)
- Vortex-Mischer
- Destilliertes Wasser
- Plastikröhrchen für den einmaligen Gebrauch

5. STABILITÄT UND LAGERUNG

Testkit bei 2...8 °C lagern. Die geöffneten Reagenzien sind bis zu den auf den Etiketten angegebenen Verfallsdaten verwendbar, wenn sie bei 2...8 °C gelagert werden.

6. VORBEREITUNG DER REAGENZIEN

Es ist sehr wichtig, alle Reagenzien und Proben vor ihrer Verwendung auf Raumtemperatur (20...25 °C) zu bringen und zu mischen!

6.1. Mikrotiterplatte

Die abbrechbaren Streifen sind mit Chlamydia pneumoniae Antigenen beschichtet. Nicht verbrauchte Vertiefungen im Aluminiumbeutel zusammen mit dem Trockenmittel sofort wieder verschließen und bei 2...8 °C lagern.

6.2. Waschpuffer (20x konz.)

Der Waschpuffer ist im Verhältnis 1 + 19 zu verdünnen; z.B. 10 mL Waschpuffer + 190 mL destilliertes Wasser.
Der verdünnte Puffer ist bei Raumtemperatur (20…25 °C) 5 Tage haltbar. Sollten Kristalle im Konzentrat auftreten, die Lösung z.B. in einem Wasserbad auf 37 °C erwärmen und vor dem Verdünnen gut mischen.

6.3. TMB-Substratlösung

Die gebrauchsfertige Lösung ist bei 2...8 °C vor Licht geschützt aufzubewahren. Die Lösung ist farblos, kann aber auch leicht hellblau sein. Sollte die TMB-Substratlösung blau sein, ist sie kontaminiert und kann nicht im Test verwendet werden.

7. ENTNAHME UND VORBEREITUNG DER PROBEN

Es sollten humane Serum- oder Plasmaproben (Citrat, Heparin) verwendet werden. Werden die Bestimmungen innerhalb von 5 Tagen nach Blutentnahme durchgeführt, können die Proben bei 2...8 °C aufbewahrt werden, sonst aliquotieren und tiefgefrieren (-70…-20 °C). Wieder aufgetaute Proben vor dem Verdünnen gut schütteln. Wiederholtes Tiefgefrieren und Auftauen vermeiden!
Hitzeinaktivierung der Proben wird nicht empfohlen.

7.1. Probenverdünnung

Proben vor Testbeginn im Verhältnis 1 + 100 mit IgG-Probenverdünnungspuffer verdünnen, z. B. 10 µL Probe und 1 mL IgG-Probenverdünnungspuffer in die entsprechenden Röhrchen pipettieren, um eine Verdünnung von 1 + 100 zu erhalten; gut mischen (Vortex).

8. TESTDURCHFÜHRUNG

Arbeitsanleitung **vor** Durchführung des Tests sorgfältig lesen. Für die Zuverlässigkeit der Ergebnisse ist es notwendig, die Arbeitsanleitung genau zu befolgen. Die folgende Testdurchführung ist für die manuelle Methode validiert. Beim Arbeiten mit ELISA Automaten empfehlen wir, um Wascheffekte auszuschließen, die Zahl der Waschschritte von drei auf bis zu fünf und das Volumen des Waschpuffers von 300 µL auf 350 µL zu erhöhen. Kapitel 12 beachten. Vor Testbeginn auf dem mitgelieferten Plattenlayout die Verteilung bzw. Position der Proben und der Standards/Kontrollen (Doppelbestimmung empfohlen) genau festlegen. Die benötigte Anzahl von Mikrotiterstreifen (Kavitäten) in den Streifenhalter einsetzen.

Den Test in der angegebenen Reihenfolge und ohne Verzögerung durchführen.

Für jeden Pipettierschritt der Standards/Kontrollen und Proben saubere Einmalspitzen verwenden.

Den Inkubator auf 37 ± 1 °C einstellen.

1. Je 100 µL Standards/Kontrollen und vorverdünnte Proben in die entsprechenden Vertiefungen pipettieren. Vertiefung A1 ist für den Substratleerwert vorgesehen.
2. Die Streifen mit der mitgelieferten Abdeckfolie bedecken.
3. **1 h ± 5 min bei 37 ± 1 °C inkubieren.**
4. Am Ende der Inkubationszeit Abdeckfolie entfernen und die Inkubationsflüssigkeit aus den Teststreifen absaugen. Anschließend dreimal mit 300 µL Waschpuffer waschen. Überfließen von Flüssigkeit aus den Vertiefungen vermeiden. Das Intervall zwischen Waschen und Absaugen sollte > 5 sec betragen. Nach dem Waschen die Teststreifen auf Fließpapier ausklopfen, um die restliche Flüssigkeit zu entfernen.

   Beachte: Der Waschvorgang ist wichtig, da unzureichendes Waschen zu schlechter Präzision und falschen Messergebnissen führt!
5. 100 µL Konjugat in alle Vertiefungen, mit Ausnahme der für die Berechnung des Leerwertes A1 vorgesehenen, pipettieren.
6. **30 min bei Raumtemperatur (20...25 °C) inkubieren.** Nicht dem direkten Sonnenlicht aussetzen.
7. Waschvorgang gemäß Punkt 4 wiederholen.
8. 100 µL TMB-Substratlösung in alle Vertiefungen pipettieren.
9. **Genau 15 min im Dunkeln bei Raumtemperatur (20...25 °C) inkubieren.** Bei enzymatischer Reaktion findet eine Blaufärbung statt.
10. In alle Vertiefungen 100 µL Stopplösung in der gleichen Reihenfolge und mit den gleichen Zeitintervallen wie bei Zugabe der TMB-Substratlösung pipettieren, dadurch erfolgt ein Farbwechsel von blau nach gelb.
11. Die Extinktion der Lösung in jeder Vertiefung bei 450/620 nm innerhalb von 30 min nach Zugabe der Stopplösung messen.

8.1. Messung

Mit Hilfe des Substratleerwertes den **Nullabgleich** des Mikrotiterplatten-Photometers vornehmen.

Falls diese Eichung aus technischen Gründen nicht möglich ist, muss nach der Messung der Extinktionswert des Substratleerwertes von allen anderen Extinktionswerten subtrahiert werden, um einwandfreie Ergebnisse zu erzielen!

Extinktion aller Kavitäten bei **450 nm** messen und die Messwerte der Standards/Kontrollen und Proben in das Plattenlayout eintragen.

Eine **bichromatische** Messung mit der Referenzwellenlänge 620 nm wird empfohlen.

Falls Doppel- oder Mehrfachbestimmungen durchgeführt wurden, den **Mittelwert der Extinktionswerte** berechnen.

9. BERECHNUNG DER ERGEBNISSE

9.1. Testgültigkeitskriterien

Damit ein Testlauf als valide betrachtet werden kann, muss diese Gebrauchsanweisung strikt befolgt werden, und die folgenden Kriterien müssen erfüllt sein:

- **Substrat-Leerwert**: Extinktionswert **< 0,100**
- **Negativkontrolle**: Extinktionswert **< 0,200 und < Cut-off**
- **Cut-off Kontrolle**: Extinktionswert **0,150 – 1,300**
- **Positivkontrolle**: Extinktionswert **> Cut-off**

Sind diese Kriterien nicht erfüllt, ist der Testlauf ungültig und muss wiederholt werden.

9.2. Messwertberechnung

Der Cut-off ergibt sich aus dem Mittelwert der gemessenen Extinktionen der Cut-off Kontrolle.

Beispiel: 0,44 OD Cut-off Kontrolle + 0,42 OD Cut-off Kontrolle = 0,86 : 2 = 0,43

Cut-off = 0,43

9.2.1. Ergebnisse in Einheiten [NTU]

$$\frac{\text{Mittlere Extinktion der Probe} \times 10}{\text{Cut-off}} = \text{[NovaTec Einheiten = NTU]}$$

Beispiel: $\frac{1{,}591 \times 10}{0{,}43} = 37$ NTU

9.3. Interpretation der Ergebnisse

Cut-off	10 NTU	-
Positiv	> 11 NTU	Es liegen Antikörper gegen den Erreger vor. Ein Kontakt mit dem Antigen (Erreger bzw. Impfstoff) hat stattgefunden.
Grenzwertig	9 – 11 NTU	Antikörper gegen den Erreger können nicht eindeutig nachgewiesen werden. Es wird empfohlen den Test nach 2 bis 4 Wochen mit einer frischen Patientenprobe zu wiederholen. Finden sich die Ergebnisse erneut im grenzwertigen Bereich, gilt die Probe als **negativ**.
Negativ	< 9 NTU	Es liegen keine Antikörper gegen den Erreger vor. Ein vorausgegangener Kontakt mit dem Antigen (Erreger bzw. Impfstoff) ist unwahrscheinlich.
Die Diagnose einer Infektionskrankheit darf nicht allein auf der Basis des Ergebnisses einer Bestimmung gestellt werden. Die anamnestischen Daten sowie die Symptomatologie des Patienten müssen zusätzlich zu den serologischen Ergebnissen in Betracht gezogen werden. Bei Immunsupprimierten und Neugeborenen besitzen die Ergebnisse serologischer Tests nur einen begrenzten Wert.		

9.3.1. Antikörper-Isotypen und Infektionsstatus

Serologie	Bedeutung
IgM	Typisch für Primärantwort Hoher IgM-Titer bei gleichzeitig niedrigem IgG-Titer: → Hinweis auf relativ frische Infektion Selten: → persistierendes IgM
IgG	Typisch für Sekundärantwort Können auch noch nach Jahren nachweisbar sein Hoher IgG-Titer bei gleichzeitig niedrigem IgM-Titer: → wahrscheinlich länger zurückliegende Infektion
IgA	Sezerniert in allen Schleimhäuten (⇒ Schutzbarriere) Meist früh im Verlauf einer Infektion gebildet

10. TESTMERKMALE

Die Ergebnisse beziehen sich auf die untersuchten Probenkollektive; es handelt sich nicht um garantierte Spezifikationen.

Für weitere Informationen zu den Testmerkmalen kontaktieren Sie bitte NovaTec Immundiagnostica GmbH.

10.1. Präzision

Intraassay	**n**	**Mittelwert (E)**	**Vk (%)**
#1	24	0,376	11,46
#2	24	0,887	7,69
#3	24	1,105	8,01
Interassay	**n**	**Mittelwert (NTU)**	**Vk (%)**
#1	12	26,31	3,37
#2	12	25,00	5,09
#3	12	5,66	9,75

10.2. Diagnostische Spezifität

Die diagnostische Spezifität ist definiert als die Wahrscheinlichkeit des Tests, ein negatives Ergebnis bei Fehlen des spezifischen Analyten zu liefern. Sie beträgt 95,12% (95% Konfidenzintervall: 83,47% - 99,4%).

10.3. Diagnostische Sensitivität

Die diagnostische Sensitivität ist definiert als die Wahrscheinlichkeit des Tests, ein positives Ergebnis bei Vorhandensein des spezifischen Analyten zu liefern. Sie ist 91,67% (95% Konfidenzintervall: 81,61% - 97,24%).

10.4. Interferenzen

Hämolytische, lipämische und ikterische Proben ergaben bis zu einer Konzentration von 10 mg/mL für Hämoglobin, von 5 mg/mL Triglyceride und von 0,5 mg/mL für Bilirubin keine Interferenzen im vorliegenden ELISA.

10.5. Kreuzreaktivität

Die Untersuchung eines Probenpanels mit Antikörperaktivitäten gegen potenziell kreuzreagierende Parameter ließ keine signifikanten Anzeichen von falsch-positiven Ergebnissen aufgrund von Kreuzreaktivitäten erkennen.
Eine Kreuzreaktion mit Chlamydia trachomatis kann nicht ausgeschlossen werden mit Seren, die Antikörper gegen LPS- und MOMP-Partikel enthalten.

11. GRENZEN DES VERFAHRENS

Kontamination der Proben durch Bakterien oder wiederholtes Einfrieren und Auftauen können zu einer Veränderung der Messwerte führen.

12. SICHERHEITSMASSNAHMEN UND WARNHINWEISE

- Die Testdurchführung, die Information, die Sicherheitsmaßnahmen und Warnhinweise in der Arbeitsanleitung sind strikt zu befolgen. Bei Anwendung des Testkits auf Diagnostika-Geräten ist die Testmethode zu validieren. Jede Änderung am Aussehen, der Zusammensetzung und der Testdurchführung sowie jede Verwendung in Kombination mit anderen Produkten, die der Hersteller nicht autorisiert hat, ist nicht zulässig; der Anwender ist für solche Änderungen selbst verantwortlich. Der Hersteller haftet für falsche Ergebnisse und Vorkommnisse aus solchen Gründen nicht. Auch für falsche Ergebnisse aufgrund von visueller Auswertung wird keine Haftung übernommen.
- Nur für in-vitro-Diagnostik.
- Alle Materialien menschlichen oder tierischen Ursprungs sind als potentiell infektiös anzusehen und entsprechend zu behandeln.
- Alle verwendeten Bestandteile menschlichen Ursprungs sind auf Anti-HIV-AK, Anti-HCV-AK und HBsAg nicht-reaktiv getestet.
- Reagenzien und Mikrotiterplatten unterschiedlicher Chargen nicht untereinander austauschen.
- Keine Reagenzien anderer Hersteller zusammen mit den Reagenzien dieses Testkits verwenden.
- Nicht nach Ablauf des Verfallsdatums verwenden.
- Nur saubere Pipettenspitzen, Dispenser und Labormaterialien verwenden.
- Verschlusskappen der einzelnen Reagenzien nicht untereinander vertauschen, um Kreuzkontaminationen zu vermeiden.
- Flaschen sofort nach Gebrauch fest verschließen, um Verdunstung und mikrobielle Kontamination zu vermeiden.
- Nach dem ersten Öffnen Konjugat und Standards/Kontrollen vor weiterem Gebrauch auf mikrobielle Kontamination prüfen.
- Zur Vermeidung von Kreuzkontamination und falsch erhöhten Resultaten, Reagenzien sorgfältig in die Kavitäten pipettieren.
- Der ELISA ist nur für qualifiziertes Personal bestimmt, das den Standards der Guten Laborpraxis (GLP) folgt.
- Zur weiteren internen Qualitätskontrolle sollte jedes Labor zusätzlich bekannte Proben verwenden.

12.1. Sicherheitshinweis für Reagenzien, die Gefahrstoffe enthalten

Die Reagenzien können CMIT/MIT (3:1) oder MIT enthalten (siehe 4.1)
Daher gelten die folgenden Gefahren- und Sicherheitshinweise.

Achtung

H317	Kann allergische Hautreaktionen verursachen.
P261	Einatmen von Aerosol vermeiden.
P280	Schutzhandschuhe/ Schutzkleidung tragen.
P302+P352	BEI BERÜHRUNG MIT DER HAUT: Mit viel Seife und Wasser waschen.
P333+P313	Bei Hautreizung oder -ausschlag: Ärztlichen Rat einholen/ ärztliche Hilfe hinzuziehen.
P362+P364	Kontaminierte Kleidung ausziehen und vor erneutem Tragen waschen

Weitere Informationen können dem Sicherheitsdatenblatt entnommen werden.

12.2. Entsorgungshinweise

Chemikalien und Zubereitungen sind in der Regel Sonderabfälle. Deren Beseitigung unterliegt den nationalen abfallrechtlichen Gesetzen und Verordnungen. Die zuständige Behörde informiert über die Entsorgung von Sonderabfällen.

13. BESTELLINFORMATIONEN

Produktnummer: CHLG0510 Chlamydia pneumoniae IgG ELISA (96 Bestimmungen)

FRANÇAIS

1. INTRODUCTION

Chlamydiae sont des bactéries à développement intracellulaire, immobiles et Gram-négatives, qui forment des inclusions caractéristiques dans le cytoplasme des cellules parasitées. Elles sont facilement visibles dans le photo-microscope. Trois espèces différentes de Chlamydia pathogènes pour les humains sont connues : Chlamydia trachomatis, Chlamydia pneumoniae et Chlamydia psittaci, ainsi qu'une espèce pathogène seulement pour des animaux (C. pecorum). Chlamydia trachomatis est l'agent le plus répandu des maladies transmises sexuellement dans le monde entier (400-500 millions de cas) et le nombre d'infections augmente constamment. Pendant l'accouchement, Chlamydia trachomatis entraîne la conjonctivite ou la pneumonie dans les nouveaux-nés. Les cas non traités de l'infection par Chlamydia peuvent mener aux salpingites chroniques, qui peuvent résulter en grossesse ectopique ou l'infertilité. Dans les mâles, Chlamydia trachomatis est une cause importante de l'urétrite non-gonococcique.

Un problème grave dans les infections de Chlamydia est le cours de la maladie, fréquemment insidieux et asymptomatique, qui peut avoir comme conséquence le déclenchement des maladies chroniques. Dans beaucoup de cas, des infections primaires ne sont pas identifiées et seulement les conséquences provoquées par des agents augmentés et persistants sont diagnostiquées.

Espèce	**La maladie**	**Symptômes**	**Modes de transmission**
C. trachomatis	Trachome Lymphogranuloma venereum (LGV) Infections urogénitales	conjonctivite bilatérale avec sensation de corps étranger, les yeux déchirer, et l'évacuation des sécrétions séreuses de l'œil. Lésion génitale; gonflement des ganglions lymphatiques inguinaux et du fémur; formation de bubons purulents. **Chez les hommes:** Inflammation de l'urètre (urétrite), inflammation de la prostate (prostatite) et l'épididyme (épididymite). Stérilité (infertilité). **Chez les femmes:** Inflammation de l'urètre (urétrite), les glandes de Bartholin, inflammation du col (cervicite), de l'utérus muqueuse (endométrite) et les trompes de Fallope (salpingite) et pertes vaginales. Stérilité (infertilité)	Transmission directe ou sexuelle. Transmis de la mère à son bébé perdant l'accouchement.
C. pneumoniae	Maladies respiratoires (p. ex. Bronchite, pneumonie) Discuté: endocardite, maladies coronaires	Fièvre, toux (en regel productive)	D'homme à homme Aérogène, infection gouttelette
C. psittaci	Ornithose (Psittacose)	hépatosplénomégalie; toux improductive, la fièvre et des maux de tête sévères	Inhalation des résidus des oiseaux infectés; contact avec les viscères aviens infectés

L'infection ou la présence d'un agent pathogène peut être identifiée par :

- Microscopie
- PCR
- Sérologie: Détection des antigènes par ELISA
  Détection des anticorps par ELISA

2. INDICATION D'UTILISATION

La trousse Chlamydia pneumoniae IgG ELISA est prévue pour la détection qualitative des anticorps IgG anti-Chlamydia pneumoniae dans le sérum humain ou plasma (citrate, héparine).

3. PRINCIPE DU TEST

La détermination immunoenzymatique qualitative des anticorps spécifiques est basée sur le la technique ELISA (du anglais, Enzyme-Linked Immunosorbent Assay).
Plaques de Microtitrage sont recouvertes d'antigènes spécifiques pour lier les anticorps correspondants de l'échantillon. Après le lavage des puits pour éliminer l'échantillon détaché, le conjugué peroxydase de raifort (HRP) est ajouté. Ce conjugué se lie aux anticorps capturés. Dans une deuxième étape de lavage, le conjugué non lié est éliminé. Le complexe immun formé par le conjugué lié est visualisé par l'addition tétraméthylbenzidine (TMB) qui donne un produit de réaction bleu.
L'intensité de ce produit est proportionnelle à la quantité d'anticorps spécifiques dans l'échantillon. L'acide sulfurique est ajouté pour arrêter la réaction. Cela produit un changement du bleu au jaune. L'absorbance à 450/620 nm est lue en utilisant un photomètre de Plaque de Microtitrage ELISA.

4. MATERIEL

4.1. Réactifs fournis

- **Plaque de Microtitrage:** 12 barrettes de 8 puits sécables revêtus d'antigène d'Chlamydia pneumoniae; en sachets d'aluminium refermables.
- **Tampon de Dilution d'Échantillon IgG:** 1 flacon contenant 100 mL de tampon phosphaté (10 mM) pour la dilution de l'échantillon; pH 7,2 ± 0,2; prêt à l'emploi; couleur jaune; bouchon blanc; ≤ 0,0015% (v/v) CMIT/ MIT (3:1).
- **Solution d'arrêt:** 1 flacon contenant 15 mL d'acide sulfurique, 0,2 mol/L; prêt à l'emploi; bouchonrouge.
- **Tampon de Lavage (concentré x 20):** 1 flacon contenant 50 mL d'un tampon phosphaté (0,2 M) concentré 20 fois (pH 7,2 ± 0,2) pour laver les puits; bouchon blanc.
- **Conjugué:** 1 flacon contenant 20 mL d'anticorps IgG anti-humaines conjuguées à de la peroxydase du raifort dans le tampon phosphaté (10 mM); prêt à l'emploi; couleur bleue, bouchon noir.
- **Solution de Substrat TMB:** 1 flacon contenant 15 mL de 3,3',5,5'-tétraméthylbenzidine (TMB), < 0,1 %; prêt à l'emploi; bouchon jaune.
- **Contrôle Positif:** 1 flacon contenant 2 mL contrôle; prêt à l'emploi; couleur jaune; bouchon rouge; ≤ 0,02% (v/v) MIT.
- **Contrôle Cut-off:** 1 flacon contenant 3 mL contrôle; prêt à l'emploi; couleur jaune; bouchon vert; ≤ 0,02% (v/v) MIT.
- **Contrôle Négatif:** 1 flacon contenant 2 mL contrôle; prêt à l'emploi; couleur jaune; bouchon bleu; ≤ 0,0015% (v/v) CMIT/ MIT (3:1).

Pour les mentions de danger et les conseils de prudence voir chapitre 12.1
Pour les substances potentiellement dangereuses s'il vous plaît vérifiez la fiche de données de sécurité.

4.2. Matériel fourni

- 1 couvercle autocollante
- 1 instructions d'utilisation
- 1 présentation de la plaque

4.3. Matériel et équipement requis

- Photomètre de Plaque de Microtitrage ELISA, pour mesurer l'absorbance à 450/620 nm
- Incubateur 37 °C
- Laveur manuel ou automatique pour le lavage des Plaques de Microtitrage
- Pipettes pour utilisation entre 10 et 1000 µL
- Mélangeur Vortex
- Eau distillée
- Tubes jetables

5. STABILITE ET CONSERVATION

Conserver le kit à 2...8°C. Les réactifs ouverts sont stables jusqu'à la date de péremption indiquée sur l'étiquette lorsqu'il est conservé à 2...8° C.

6. PREPARATION DES REACTIFS

Il est très important porter tous les réactifs et échantillons à température ambiante (20 ... 25 ° C) et les mélanger avant de commencer le test!

6.1. Plaque de Microtitrage

Les barrettes sécables sont revêtues d'antigène d'Chlamydia pneumoniae. Immédiatement après avoir prélevé les barrettes nécessaires, les barrette restantes doivent être scellés le vide dans de feuille d'aluminium avec le sac de silicium (le déshydratant) fourni et emmagasiner à 2...8°C.

6.2. Tampon de Lavage (conc. x 20)

Diluer le Tampon de Lavage 1+19; par exemple 10 mL du Tampon de Lavage + 190 mL d'eau distillée. Le Tampon de Lavage diluée est stable pendant 5 jours à la température ambiante (20…25 °C). Cas apparaissent des cristaux dans le concentré, chauffer la solution à 37 ° C par exemple dans un bain-marie mélangez bien avant dilution.

6.3. Solution de Substrat TMB

La solution est prête à utiliser et doit être emmagasiné à 2...8 °C, à l'abri de la lumière. La solution doit être incolore ou pourrait avoir une légère couleur bleu clair. Si le substrat devient bleu, il peut avoir été contaminé et ne peut pas être utilisé dans le test.

7. PRELEVEMENT ET PREPARATION DES ECHANTILLONS

Utiliser des échantillons humains de sérum ou plasma (citrate, héparine) pour ce test. Si le test est réalisé dans les 5 jours après le prélèvement, les échantillons doivent être conservés à 2...8 °C; autrement ils doivent être aliquotés et conservés surgelés (-70…-20 °C). Si les échantillons sont conservés congelés, bien mélanger les échantillons décongelés avant le test. Éviter les cycles répétés de congélation et décongélation.
L'inactivation par la chaleur des échantillons n'est pas recommandée.

7.1. Dilution de l'échantillon

Avant du test, tous les échantillons doivent être dilués 1 + 100 avec Tampon de Dilution d'Échantillon IgG. Diluer 10 µL d'échantillon avec 1 mL I Tampon de Dilution d'Échantillon IgG dans des tubes pour obtenir une dilution 1 + 100 et mélanger soigneusement sur un Vortex.

8. PROCEDE DE TEST

Lire attentivement les instructions d'utilisation **avant de** réaliser le test. La fiabilité des résultats dépend du suivi strict d'utilisation comme décrit. La technique de test suivante a été validée uniquement pour une procédure manuelle. Si le test doit être effectué sur un systèmes automatiques pour ELISA, nous conseillons d'augmenter le nombre d'étapes de lavage de trois à cinq et le volume du Tampon de Lavage de 300 à 350 µL. Faites attention au chapitre 12. Avant de commencer le test, le plan de distribution et d'identification de tous les échantillons et les étalons/contrôles (il est recommandé déterminer en double) doivent être soigneusement établi sur la feuille présentation de la plaque prévue dans le conseil de kit. Sélectionner le nombre de barrettes ou de puits nécessaires et les placer sur le support.

Réaliser toutes les étapes du test dans l'ordre donné et sans délai.

Un embout de pipette propre et jetable doit être utilisé pour distribuer chaque étalon/contrôle et échantillon.

Régler l'incubateur à 37 ± 1 °C.

1. Pipeter 100 µL de étalons/contrôles et d'échantillons dilués dans leurs puits respectifs. Garder le puits A1 pour le blanc substrat.
2. Couvrir les puits avec le couvercle, fourni dans le kit.
3. **Incuber pendant 1 heure ± 5 minutes à 37 ± 1 °C.**
4. A la fin de l'incubation, enlever le couvercle, aspirer le contenu des puits et laver chaque puits trois fois avec 300 µL du Tampon de Lavage. Éviter les débordements des puits de réaction. L'intervalle entre le cycle de lavage et l'aspiration doit être > 5 sec. À la fin, enlever soigneusement le liquide restant en tapotant les barrettes sur du papier absorbant avant la prochaine étape.

   Note: L'étape de lavage est très importante! Un lavage insuffisant peut conduire à une précision faible et de faux résultats !
5. Pipeter 100 µL du conjugué dans tous les puits sauf le puits Blanc A1.
6. **Incuber pendant 30 minutes à température ambiante (20…25°C).** N'exposer pas à la lumière directe du soleil.
7. Répéter l'étape numéro 4.
8. Pipeter 100 µL de la Solution de Substrat TMB dans tous les puits.
9. **Incuber pendant exactement 15 minutes à température ambiante (20…25°C) dans l'obscurité.** Une couleur bleue se produit en raison d'une réaction enzymatique.
10. Pipeter 100 µL de la solution d'arrêt dans tous les puits dans le même ordre et à la même vitesse que pour la Solution de Substrat TMB, ainsi, il y a un changement du bleu au jaune.
11. Mesurer l'absorbance à 450/620 nm dans les 30 minutes après l'addition de la solution d'arrêt.

8.1. Mesure

Réglez le Photomètre de Plaque de Microtitrages ELISA **à zéro** en utilisant **le Blanc substrat.**
Si - pour des raisons techniques - le Photomètre de Plaque de Microtitrages ELISA ne peut pas être ajusté à zéro en utilisant le Blanc substrat, la valeur d'absorbance de cette doit être soustraire la valeur d'absorbance de toutes les autres valeurs d'absorbance mesurées afin d'obtenir des résultats fiables!

Mesurer l'absorbance de tous les puits **à 450 nm** et enregistrer les valeurs d'absorbance pour chaque étalon/contrôle et échantillon dans la présentation de la plaque.

Il est recommandé d'effectuer la mesure **dichromatique** utilisant 620 nm comme longueur d'onde de référence.
Si doubles déterminations ont été effectuées, calculer **les valeurs moyennes d'absorbance.**

9. RESULTATS

9.1. Critères de validation

Pour qu'une série d'analyses soit considérée comme valide, ces instructions d'utilisation doivent être strictement suivies, et les critères suivants doivent être respectés :

- **Blanc Substrat**: Valeur d'absorbance **< 0,100**
- **Contrôle négatif**: Valeur d'absorbance **< 0,200 et < Cut-off**
- **Contrôle Cut-off**: Valeur d'absorbance **0,150 – 1,300**
- **Contrôle positif**: Valeur d'absorbance **> Contrôle Cut-off**

Lorsque ces critères ne sont pas remplis, le test n'est pas valide et doit être recommencé.

9.2. Calcul des résultats

La valeur seuil correspond à la moyenne des valeurs d'absorbance du Contrôle Cut-off.

Exemple: 0,44 DO Contrôle Cut-off + 0,42 DO Contrôle Cut-off = 0,86 : 2 = 0,43
Cut-off = 0,43

9.2.1. Résultats en unités [NTU]

$$\frac{\text{Valeur (moyenne) d'absorbance de l' échantillon} \times 10}{\text{Cut-off}} = \text{[unités NovaTec = NTU]}$$

Exemple: $\frac{1{,}591 \times 10}{0{,}43} = 37$ NTU

9.3. Interprétation des résultats

Cut-off	10 NTU	-
Positif	> 11 NTU	Les anticorps dirigés contre l'agent pathogène sont présents. Il ya eu un contact avec l'antigène (pathogène resp. vaccin).
Zone grise	9 – 11 NTU	Les anticorps dirigés contre l'agent pathogène ne pouvaient pas être détectés clairement. Il est recommandé de répéter le test avec un échantillon frais dans 2 à 4 semaines. Si le résultat est encore dans la zone grise l'échantillon est jugé **négatif**.
Negatif	< 9 NTU	L'échantillon ne contient pas d'anticorps contre l'agent pathogène. Un contact préalable avec l'antigène (pathogène resp. vaccin) est peu probable.
Le diagnostic d'une maladie infectieuse ne devrait pas être établi sur la base du résultat d'une seule analyse. Un diagnostic précis devrait prendre en considération l'histoire clinique, la symptomatologie ainsi que les données sérologiques. Les données sérologiques sont de valeur limité dans le cas des patients immunodéprimés et des nouveaux-nés.		

9.3.1. Isotypes d'anticorps et l'Etat de l'infection

Sérologie	Signification
IgM	Caractéristique de la réponse primaire du anticorps Titre élevé d'IgM avec une faible titre d'IgG : → suggère une infection très récente ou aigüe Rare: → persistante IgM
IgG	Caractéristique de la réponse secondaire du anticorps Peut persister pendant plusieurs années Des titres élevés d'IgG à faible titre d'IgM: → peuvent indiquer une infection ancienne
IgA	Ils sont produits au niveau des muqueuses dans tout le corps (⇒ barrière de protection) Habituellement ils sont produits en début d'infection

10. PERFORMANCES DU TEST

Ces résultats s'appuient sur les groupes d'échantillons étudiés; il n'agit pas de caractéristiques techniques garanties.

Pour plus d'informations sur les performances du test s'il vous plaît contactez NovaTec Immundiagnostica GmbH.

10.1. Précision

Intra-essai	n	moyenne (E)	CV (%)
#1	24	0,376	11,46
#2	24	0,887	7,69
#3	24	1,105	8,01
Inter-essai	**n**	**moyenne (NTU)**	**CV (%)**
#1	12	26,31	3,37
#2	12	25,00	5,09
#3	12	5,66	9,75

10.2. Spécificité diagnostique

La spécificité diagnostique est définie comme la probabilité d'obtenir un résultat négatif en l'absence d'un analyte spécifique. Elle est 95,12% (95% Intervalle de confiance: 83,47% - 99,4%).

10.3. Sensibilité diagnostique

La sensibilité diagnostique est définie comme la probabilité d'obtenir un résultat positif en présence d'un analyte spécifique. Elle est 91,67% (95% Intervalle de confiance: 81,61% - 97,24%).

10.4. Interférences

Des échantillons hémolytiques ou lipémiques ou ictériques n'ont pas montré d'interférences, avec des concentrations jusqu'à 10 mg/mL de hémoglobine, 5 mg/mL de triglycérides et 0,5 mg/mL de bilirubine.

10.5. Réaction croisée

L'étude d'un panel d'échantillons avec des anticorps dirigés contre différents paramètres interférents n'a pas révélé de preuves significatives de résultats faussement positifs dus à des réactions croisées.
Des réactions croisées avec Chlamydia trachomatis ne peut pas être exclue avec des sérums contenant des anticorps anti-LPS et MOMP.

11. LIMITES DE LA TECHNIQUE

Une contamination bactérienne ou des cycles de congélation/décongélation répétés de l´échantillon peuvent affecter les valeurs d'absorption.

12. PRECAUTIONS ET AVERTISSEMENTS

- La procédure de test, l'information, les précautions et mises en garde de la notice d'emploi, doivent être suivies de façon stricte. L'utilisation de ces trousses avec des automates ou dispositifs similaires doit être validée. Aucun changement de la conception, composition et procédure de test, ainsi que l'utilisation avec d'autres produits non approuvés par le fabricant, ne sont pas autorisés; seul l'utilisateur est responsable de tels changements. Le fabricant n'est pas responsable des faux résultats et des incidents dus à ces motifs. Le fabricant n'est pas responsable des résultats fournis par analyse visuelle des échantillons des patients.
- Uniquement pour diagnostic in vitro.
- Tous les matériaux d'origine humaine ou animale doivent être considérés et traités comme étant potentiellement infectieux.
- Tous les composants d'origine humaine utilisés pour la fabrication de ces réactifs ont été analysés et ont été trouvés non réactifs en Ag HBs, en anticorps anti-VHI 1 et 2 et en anticorps anti-VHC.
- Ne pas échanger les réactifs ou les Plaque de Microtitrage provenant de différents lots de production.
- Ne pas utiliser de réactifs provenant d'autres fabricants avec les réactifs de cette trousse.
- Ne pas utiliser les réactifs après la date de péremption indiquée sur l'étiquette.
- Utiliser seulement des embouts de pipette, des distributeurs et du matériel de laboratoire propres.
- Ne pas échanger les bouchons des flacons, pour éviter la contamination croisée.
- Fermer soigneusement les flacons après utilisation pour éviter l'évaporation et la contamination microbienne.
- Avant une nouvelle utilisation, vérifier les flacons de conjugué et de étalon/contrôle, déjà utilisés, pour exclure une contamination microbienne.
- Pour éviter la contamination croisée et des résultats faussement élevés, introduire les échantillons de patients et les réactifs exactement au fond des puits sans éclabousser.
- L'ELISA est uniquement conçu pour le personnel qualifié suivant les normes de bonnes pratiques de laboratoire (Good Laboratory Practice, GLP).
- Pour un contrôle de qualité interne plus poussé, chaque laboratoire doit en outre utiliser des échantillons connus.

12.1. Note de sécurité pour les réactifs contenant des substances dangereuses

Les réactifs peuvent contenir du CMIT/MIT (3 :1) ou du MIT (voir chapitre 4.1)
Par conséquent, les mentions de danger et les conseils de prudence suivants s'appliquent.

Attenttion	H317	Peut provoquer une allergie cutanée.
	P261	Éviter de respirer les aérosols.
	P280	Porter des gants de protection/ des vêtements de protection.
	P302+P352	EN CAS DE CONTACT AVEC LA PEAU: Laver abondamment savon à l'eau.
	P333+P313	En cas d'irritation ou d'éruption cutanée: consulter un médecin.
	P362+P364	Enlever les vêtements contaminés et les laver avant réutilisation.

De plus amples informations peuvent être trouvées dans la fiche de données de sécurité.

12.2. Elimination des déchets

Les résidus des produits chimiques et des préparations sont considérés en général comme des déchets dangereux. L'élimination de ce type de déchet est réglementée par des lois et réglementations nationales et régionales. Contacter les autorités compétentes ou les sociétés de gestion des déchets pour obtenir des renseignements sur l'élimination des déchets dangereux.

13. INFORMATION POUR LES COMMANDES

Référence: CHLG0510 Chlamydia pneumoniae IgG ELISA (96 déterminations)

ITALIANO

1. INTRODUZIONE

Le Chlamydie sono batteri immobili Gram negativi e parassiti intracellulari obbligati. Gli involucri esterni risultano privi della componente peptidoglicanica della parete cellulare. Hanno un ciclo vitale dimorfico: il corpo elementare è la forma infettante e molto piccola. È relativamente inerte dal punto di vista metabolico e sopravvive in ambiente extracellulare. Il corpo elementare viene introdotto in una cellula delle mucose per endocitosi, dove si trasforma in un corpo reticolare, che presenta una chiara organizzazione cellulare procariotica, è metabolicamente attivo e si moltiplica, per scissione binaria. Dopo poche ore questo subisce un processo di condensazione e disitratazione, ritrasformandosi in un corpo elementare che, liberato all'esterno per la morte della cellula parassitata, è pronto ad infettare altre cellule. Si distinguono tre specie patogene per l'uomo Chlamydia trachomatis, Chlamydia pneumoniae e Chlamydia psittaci.

La Chlamydia trachomatis può causare una malattia sessualmente trasmissibile. L'infezione da Clamidia è molto comune tra gli adulti giovani e tra gli adolescenti. Tuttavia molte persone non sanno di essere infette, poiché non hanno nessun sintomo. È la causa più frequente per malattie a trasmissione sessuale (400-500 millioni di casi in tutto il mondo). La Clamidia può essere trasmessa da madre a bambino durante la nascita. L'infezione da Clamidia nei neonati può causare una congiuntivite neonatale e polmonite. Donne infette possono trasmettere c. trachomatis al neonato durante il parto. Nelle donne, una infezione non curata può diffondersi nella zona pelvica e infettare l'utero, le tube di falloppio e le ovaie, portando alla malattia infiammatoria pelvica. Essa può causare danni permanenti agli organi riproduttivi della donna, può portare alla sterilità, dolore pelvico cronico e un rischio maggiore di gravidanza extrauterina. Negli uomini, una Clamidia non curata può avere un effetto deleterio sui testicoli, portando a gonfiori e dolore. Complicazioni collegate possono portare alla sterilità.

Specie	**Malattia**	**Sintomi**	**Via di trasmissione**
C. trachomatis	Trachoma Linfogranuloma venereo Infezioni del tratto urogenitale	Congiuntivite bilaterale con sensazione di corpo estraneo, gli occhi lacrimazione, e lo scarico delle secrezioni sierose dall'occhio. Lesione genitale; gonfiore dei linfonodi inguinali e femorali; formazione di bubboni suppuranti. Negli uomini: infiammazione dell'uretra (uretrite), infiammazione della prostata (prostatite) e l'epididimo (epididimite). Sterilità (sterilità). Nelle donne: infiammazione dell'uretra (uretrite), le ghiandole di Bartolini, infiammazione della cervice (cervicite), uterina mucose (endometrite) e tube di Falloppio (salpingite) e perdite vaginali. Sterilità (sterilità)	Trasmissione sessuale. Trasmissione da madre al suo bambino durante il parto.
C. pneumoniae	Malattie respiratorie (ad esempio polmonite, bronchite) in Discussione: endocardite, malattie coronariche	Febbre, tosse (in regel produttiva)	Trasmissione aerea; inalazione di goccioline infette.
C. psittaci	La psittacosi (ornitosi)	Epatosplenomegalia; tosse non produttiva, febbre e forti mal di testa	L'inalazione di feci di volatili infetti; contatto con visceri aviari infetta

L'infezione o la presenza di un agente patogeno può essere identificata da:

- Microscopia
- PCR
- Sierologia: p.es.: Rilevamento di antigeni per ELISA
  Rilevamento di anticorpi per ELISA

2. USO PREVISTO

Il Chlamydia pneumoniae IgG ELISA è un kit per la determinazione qualitativa degli anticorpi specifici della classe IgG per Chlamydia pneumoniae nel siero o plasma (citrato, eparina) umano.

3. PRINCIPIO DEL TEST

La determinazione immunoenzimatico qualitativa degli anticorpi specifici si basa sulla tecnica ELISA (d'inglese Enzyme-linked immunosorbent assay).
Piastre di Microtitolazione sono rivestite con antigeni specifici che si legano agli anticorpi presenti nel campione. Dopo aver lavato i pozzetti per rimuovere tutto il materiale campione non legato, il coniugato di perossidasi di rafano (HRP) è aggiunto. Questo coniugato si lega agli anticorpi catturati. In una seconda fase di lavaggio coniugato, non legato è rimosso. Il complesso immunitario formato dal coniugato legato sarà evidenziato aggiungendo tetrametilbenzidina (TMB) substrato che dà una colorazione blu.
L'intensità di questa colorazione è direttamente proporzionale alla quantità di anticorpi specifici presenti nel campione. Acido solforico è aggiunto per bloccare la reazione. Questo produce un cambiamento di colore dal blu al giallo. Assorbanza a 450/620 nm è letto utilizzando un fotometro di Piastre di Microtitolazione ELISA.

4. MATERIALI

4.1. Reagenti forniti

- **Piastre di Microtitolazione:** 12 strisce divisibili in 8 pozzetti, con adesi antigeni della Chlamydia pneumoniae; dentro una busta d'alluminio richiudibile.
- **Tampone di Diluizione del Campione IgG:** 1 flacone contenente 100 mL di tampone fosfato (10 mM) per diluire i campioni; pH 7,2 ± 0,2; colore giallo; pronto all'uso; tappo bianco; ≤ 0,0015% (v/v) CMIT/ MIT (3:1).
- **Soluzione Bloccante:** 1 flacone contenente 15 mL di acido solforico, 0,2 mol/L, pronto all'uso; tappo rosso.
- **Tampone di Lavaggio (20x conc.):** 1 flacone contenente 50 mL di un tampone fosfato concentrato 20 volte (0,2 M) per il lavaggio dei pozzetti; pH 7,2 ± 0,2; tappo bianco.
- **Coniugato:** 1 flacone contenente 20 mL di anticorpi IgG anti-umani, coniugati a perossidasi in tampone fosfato (10 mM); colore azzurro; pronto all'uso; tappo nero.
- **Soluzione Ssubstrato TMB:** 1 flacone contenente 15 mL di 3,3`,5,5`-Tetrametilbenzidina (TMB), < 0,1 %; pronto all'uso; tappo giallo.
- **Controllo Positivo:** 1 flacone da 2 mL controllo; colore giallo; tappo rosso; pronto all'uso; ≤ 0,02% (v/v) MIT.
- **Controllo Cut-off:** 1 flacone da 3 mL controllo; colore giallo; tappo verde; pronto all'uso; ≤ 0,02% (v/v) MIT.
- **Controllo Negativo:** 1 flacone da 2 mL controllo; colore giallo; tappo blu; pronto all'uso; ≤ 0,0015% (v/v) CMIT/ MIT (3:1).

Le indicazioni di pericolo e consigli di prudenza vedi capitolo 12.1.

Per le sostanze potenziali pericolose si prega di leggere la scheda di dati di sicurezza.

4.2. Accessori forniti

- 1 pellicola adesiva
- 1 istruzione per l'uso
- 1 schema della piastra

4.3. Materiali e attrezzature necessari

- Fotometro per Piastre di Microtitolazione con filtri da 450/620 nm
- Incubatrice 37°C
- Lavatore, manuale o automatico, di Piastre di Microtitolazione
- Micropipette per l'uso tra 10-1000 µL
- Vortex-Mixer
- Acqua distillata
- Provette monouso

5. MODALITÀ DI CONSERVAZIONE

Conservare il kit a 2...8 °C. I reagenti aperti sono stabili fino alla data di scadenza indicata sull'etichetta quando sono conservati a 2...8 °C.

6. PREPARAZIONE DEI REAGENTI

È molto importante, portare tutti i reagenti e campioni a temperatura ambiente (20…25 °C) e mescolare prima di iniziare il test.

6.1. Piastre di Microtitolazione

Le strisce divisibili sono rivestite con gli antigeni della Chlamydia pneumoniae. Immediatamente dopo la rimozione degli strisce necessarie, le strisce rimanenti devono essere sigillare nuovamente in un foglio di alluminio insieme con il sacchetto di gel di silice conservati a 2...8 °C.

6.2. Tampone di Lavaggio (20x conc.)

Diluire il Tampone di Lavaggio 1+19; per esempio: 10 mL del Tampone di Lavaggio + 190 mL di acqua distillata. Il Tampone di Lavaggio diluito è stabile per 5 giorni a temperatura ambiente (20…25 °C). Se cristalli appaiono nel concentrato, riscaldare la soluzione a 37 °C per esempio in un bagnomaria. Mescolare bene prima della diluizione.

6.3. Soluzione Substrato TMB

La soluzione sta pronta all'uso e deve essere conservata a 2...8 °C, al riparo dalla luce. La soluzione deve essere incolore o potrebbe avere un leggero colore blu chiaro. Se il substrato diventa blu, potrebbe essere stato contaminato e non può essere utilizzato nel test.

7. PRELIEVO E PREPARAZIONE DEI CAMPIONI

Per questo test si prega di usare campioni di siero o plasma (citrato, eparina) umano. Se il test è fatto entro 5 giorni dal prelievo i campioni possono essere conservati tra 2...8 °C. Altrimenti devono essere aliquotati e congelati tra (-70…-20 °C). Se i campioni sono conservati congelati, mescolare bene i campioni scongelati prima del test. Evitare cicli ripetuti di congelamento/scongelamento.
L'inattivazione dei campioni per mezzo del calore non è raccomandata.

7.1. Diluizione dei campioni

Prima del test, diluire i campioni 1+100 con Tampone di Diluizione del Campione IgG. Per esempio, pipettare nelle provette 10 µL di campione + 1 mL di Tampone di Diluizione del Campione IgG e mescolare bene (Vortex).

8. PROCEDIMENTO

Leggere bene le istruzioni per l'uso **prima** di iniziare il teste. L'affidabilità dei risultati dipende dalla stretta aderenza le istruzioni per l'uso di prova come descritto. La seguente procedura è stata validata per l'esecuzione manuale. Per un'esecuzione su strumentazione automatica si consiglia di incrementare il numero di lavaggi de 3 a 5 volte e il volume del Tampone di Lavaggio da 300 a 350 µL per evitare effetti di lavaggio. Prestare attenzione al capitolo 12. Stabilire innanzitutto il piano di distribuzione e identificazione dei campioni e standards/controlli (è raccomandato determinare in duplicato) sullo schema della piastra fornito con il kit. Inserire i pozzetti necessari nel supporto.

Eseguire il test nell'ordine stabilito dalle istruzioni, senza ritardi.

Sul pipettaggio utilizzare puntali nuovi e puliti per ogni campione e standard/controllo.

Regolare l'incubatore a 37 ± 1 °C.

1. Pipettare 100 µL di standard/controllo e di campione diluito nei relativi pozzetti. Usare il pozzetto A1 per il Bianco-substrato.
2. Coprire i pozzetti con la pellicola adesiva, fornita nel kit.
3. **Incubare 1 ora ± 5 min a 37 ± 1°C.**
4. Al termine dell'incubazione, togliere la pellicola e aspirare il liquido dai pozzetti. Successivamente lavare i pozzetti tre volte con 300 µL di Tampone di Lavaggio. Evitare che la soluzione trabocchi dai pozzetti. L'intervallo tra il lavaggio e l'aspirazione deve essere > 5 sec. Dopo il lavaggio picchiettare delicatamente i pozzetti su una carta assorbente per togliere completamente il liquido, prima del passo successivo.

   Attenzione: Il lavaggio è una fase molto importante. Da lavaggio insufficiente risulta una bassa precisione e risultati falsi!
5. Pipettare 100 µL di Coniugato in tutti i pozzetti, escludendo quello con il Bianco-substrato (Blank) A1.
6. **Incubare per 30 min a temperatura ambiente (20...25 °C).** Non esporre a fonti di luce diretta.
7. Ripetere il lavaggio secondo punto 4.
8. Pipettare 100 µL di Soluzione Ssubstrato TMB in tutti i pozzetti.
9. **Incubare precisamente per 15 min a temperatura ambiente (20...25 °C) al buio.** Un colore blu verifica a causa della reazione enzimatica.
10. Pipettare 100 µL di Soluzione Bloccante in tutti i pozzetti, nello stesso ordine della Soluzione Ssubstrato TMB, in tal modo un cambiamento di colore dal blu al giallo si verifica.
11. Misurare l'assorbanza a 450/620 nm entro 30 min dopo l'aggiunta della Soluzione Bloccante.

8.1. Misurazione

Regolare il fotometro per le Piastre di Microtitolazione ELISA **a zero** usando il substrato-Bianco (Blank).

Se, per motivi tecnici, non è possibile regolare il fotometro per le Piastre di Microtitolazione a zero usando il Bianco-substrato, il valore de assorbanza de questo deve essere sottratto dai valori dell'assorbanza da tutti i valori delle altre assorbanze per ottenere risultati affidabili!

Misurare l'assorbanza di tutti i pozzetti a **450 nm** e inserire tutti i valori misurati nello schema della piastra.

È raccomandato fare le misurazioni delle onde **bichrome** (due colori). Utilizzando la lunghezza d'onda de 620 nm come misura di riferimento.

Dove sono state misurate in doppio, calcolare **la media delle assorbanze**.

9. RISULTATI

9.1. Validazione del test

Affinché un test possa essere considerato valido, le presenti Istruzioni per l'uso devono essere rigorosamente seguite e devono essere soddisfatti i seguenti criteri:

- **Substrato Bianco (Blank)**: Valore di assorbanza **< 0,100**
- **Controllo Negativo**: Valore di assorbanza **< 0,200 e < Cut-off**
- **Controllo Cut-off**: Valore di assorbanza **0,150 – 1,300**
- **Controllo Positivo**: Valore di assorbanza **> Cut-off**

Se non sono soddisfatti questi criteri, il test non è valido e deve essere ripetuto.

9.2. Calcolo dei risultati

Il Cut-off è la media dei valori di assorbanza dei Controlli Cut-off.

Esempio: Valore di assorbanza del Controllo Cut-off 0,44 + valore di assorbanza del Controllo Cut-off 0,42 = 0,86/2 = 0,43
Cut-off = 0,43

9.2.1. Risultati in unità [NTU]

$$\frac{\text{Assorbanza media del campione} \times 10}{\text{Cut-off}} = \text{[unità NovaTec = NTU]}$$

Esempio: $\frac{1{,}591 \times 10}{0{,}43} = 37$ NTU

9.3. Interpretazione dei risultati

Cut-off	10 NTU	-
Positivo	> 11 NTU	Anticorpi contro il patogeno sono presenti. C'è stato un contatto con l'antigene (patogeno resp. vaccino).
Zona grigia	9 – 11 NTU	Anticorpi contro il patogeno non è stato possibile rilevare chiaramente. Si consiglia di ripetere il test con un nuovo campione in 2-4 settimane. Se il risultato è nuovamente nella zona grigia il campione viene giudicato come **negativo**.
Negativo	< 9 NTU	Il campione non contiene anticorpi contro il patogeno. Un precedente contatto con l'antigene (patogeno resp. vaccino) è improbabile.
La diagnosi di una malattia infettiva non deve essere fatta soltanto sulla risultanza di un unico test. È importante considerare anche l'anamnesi ed i sintomi del paziente. I risultati del test da pazienti immunosoppressi e neonati hanno un valore limitato.		

9.3.1. Isotipi degli anticorpi e Stato dell'infezione

Sierologia	Significato
IgM	Caratteristica della risposta primaria dell'anticorpo Alto titolo IgM con basso titolo IgG: →suggerisce una infezione molto recente o acuta Raro: → IgM persistente
IgG	Caratteristica della risposta secondaria dell'anticorpo Può persistere per diversi anni Alto titolo IgG con basso titolo IgM: → può indicare un'infezione passata
IgA	Sono prodotte a livello delle mucose in tutto il corpo (⇒ barriera protettiva) Solitamente sono prodotte all'inizio dell'infezione

10. CARATTERISTICHE DEL TEST

I risultati si riferiscono al gruppo di campioni investigato; questi non sono specifiche garantite.

Per ulteriori informazioni su caratteristiche del test, si prega, di contattare NovaTec Immundiagnostica GmbH.

10.1. Precisione

Intradosaggio	n	Media (E)	CV (%)
#1	24	0,376	11,46
#2	24	0,887	7,69
#3	24	1,105	8,01
Interdosaggio	**n**	**Media (NTU)**	**CV (%)**
#1	12	26,31	3,37
#2	12	25,00	5,09
#3	12	5,66	9,75

10.2. Specificità diagnostica

La specificità diagnostica è la probabilità del test di fornire un risultato negativo in assenza di analita specifici. La specificità diagnostica è 95,12% (95% intervallo di confidenza: 83,47% - 99,4%).

10.3. Sensibilità diagnostica

La sensibilità diagnostica è la probabilità del test di fornire un risultato positivo alla presenza di analita specifici. La sensibilità diagnostica è pari a 91,67% (95% intervallo di confidenza: 81,61% - 97,24%).

10.4. Possibili interferenze

Campioni emolitici, lipidici et itterici contenenti fino a 10 mg/mL di emoglobina, 5 mg/mL di trigliceridi e 0,5 mg/mL di bilirubina non hanno presentato fenomeni d'interferenza nel presente test.

10.5. Reattività crociata

L'investigazione di un gruppo di campioni con attività di anticorpi contro parametri potenzialmente interferenti non ha rivelato alcuna evidenza rilevante di risultati falsamente positivi dovuto a reattività crociata.
Non è quindi possibile escludere una reazione crociata con Chlamidya trachomatis con sieri contenenti anticorpi per LPS e MOMP.

11. LIMITAZIONI

Una contaminazione da microorganismi o ripetuti cicli di congelamento-scongelamento possono alterare i valori delle assorbanze.

12. PRECAUZIONI E AVVERTENZE

- La procedura analitica, le informazioni, le precauzioni e le avvertenze contenute nelle istruzioni per l'uso devono essere seguite scrupolosamente. L'uso dei kit con analizzatori e attrezzature similari deve essere previamente convalidato. Qualunque cambiamento nello scopo, nel progetto, nella composizione o struttura e nella procedura analitica, così come qualunque uso dei kit in associazione ad altri prodotti non approvati dal produttore non è autorizzato; l'utilizzatore stesso è responsabile di questi eventuali cambiamenti. Il produttore non è responsabile per falsi risultati e incidenti che possano essere causati da queste ragioni. Il produttore non è responsabile per qualunque risultato ottenuto attraverso esame visivo dei campioni dei pazienti.
- Solo per uso diagnostico in-vitro.
- Tutti i materiali di origine umana o animale devono essere considerati potenzialmente contagiosi e infettivi.
- Tutti gli elementi di origine umana sono stati trovati non reattivi con Anti-HIV-Ab, Anti-HCV-Ab e HBsAg.
- Non scambiare reagenti e Piastre di Microtitolazione di lotti diversi.
- Non utilizzare reagenti d'altri produttori insieme con i reagenti di questo kit.
- Non usare dopo la data di scadenza.
- Utilizzare soltanto punte per pipette, distributori, e articoli da laboratorio puliti.
- Non scambiare i tappi dei flaconi, per evitare contaminazione crociata.
- Richiudere i flaconi immediatamente dopo l'uso per evitare la vaporizzazione e contaminazione.
- Una volta aperti e dopo relativo stoccaggio verificare i reagenti per una loro eventuale contaminazione prima dell'uso.
- Per evitare contaminazioni crociate e risultati erroneamente alti pipettare i campioni e reagenti con molta precisione nei pozzetti senza spruzzi.
- L'ELISA è progettato solo per il personale qualificato che segue le norme di buona pratica di laboratorio (Good Laboratory Practice, GLP).
- Per un ulteriore controllo di qualità interno ogni laboratorio dovrebbe inoltre utilizzare campioni noti.

12.1. Nota di sicurezza per i reagenti contenenti sostanze pericolose

I reagenti possono contenere CMIT/MIT (3:1) o MIT (vedi capitolo 4.1)
Pertanto, si applicano le seguenti indicazioni di pericolo e le consigli di prudenza.

Attenzione

H317	Può provocare una reazione allergica cutanea.
P261	Evitare di respirare gli aerosol.
P280	Indossare guanti/ indumenti protettivi.
P302+P352	IN CASO DI CONTATTO CON LA PELLE: lavare abbondantemente con sapone acqua.
P333+P313	In caso di irritazione o eruzione della pelle: consultare un medico.
P362+P364	Togliere tutti gli indumenti contaminati e lavarli prima di indossarli nuovamente.

Ulteriori informazioni sono disponibili nella scheda di dati di sicurezza.

12.2. Smaltimento

In genere tutte le sostanze chimiche sono considerati rifiuti pericolosi. Lo smaltimento è regolato da leggi nazionali. Per ulteriori informazioni contattare l'autorità locale.

13. INFORMAZIONI PER GLI ORDINI

Numero del prodotto: CHLG0510 Chlamydia pneumoniae IgG ELISA (96 determinazioni)

ESPAÑOL

1. INTRODUCCIÓN

Las Chlamydias son bacterias inmóviles, gram-negativas, de hábitat intracelular. En comparación con otras bacterias son muy pequeñas (la unidad mas pequeña es de 0,2 µm), tienen un ciclo de reproducción especial y se manifiestan en dos formas morfológicas diferentes, las partículas elementales e las partículas iniciales. Las ínfimas partículas elementales garantizan la supervivencia fuera de una célula y representan la forma contagiosa. La reproducción es posible solamente cuando las partículas elementales están fagocitadas de la célula huésped donde comienzan a dividirse como partículas iniciales dentro del fagosoma. La célula huésped muere dos o tres días después de la infección y libera partículas elementales que de nuevo infectan a otras células. Se distinguen tres especies patógenos para el hombre: Chlamydia trachomatis, Chlamydia pneumoniae y Chlamydia psittaci.

La C. trachomatis es la causa más frecuente de enfermedades de transmisión sexual en el mundo (400 a 500 millones casos). Pueden resultar neumonías o conjuntivitis en neonatos por la transmisión de la bacteria de la madre al niño durante el nacimiento. Los casos que no reciben tratamiento pueden conducir a una salpingitis crónica con embarazos ectópicos o infertilidad. En el hombre la C. trachomatis es la causa más frecuente de la infección uretral no gonorreica. A menudo el curso asintomático resulta un problema serio ya que puede derivar en una enfermedad crónica. En muchos casos sólo la secuela de la infección primaria es detectada.

Especies	Enfermedad	Síntomas	Vía de transmisión
Chlamydia trachomatis	Trachoma	Conjuntivitis bilateral con sensación de cuerpo extraño, lagrimeo de los ojos, y la descarga de secreciones serosas del ojo.	transmisión sexual. Puede transmitirse de una madre infectada a su bebé durante el parto.
	Lymphogranuloma venereum	Lesión genital; inflamación de los ganglios linfáticos inguinales y femorales; formación de bubones supurantes.	
	Infecciones del tracto urogenital	En los hombres: inflamación de la uretra (uretritis), inflamación de la próstata (prostatitis) y el epidídimo (epididimitis). Esterilidad (infertilidad). En mujeres: inflamación de la uretra (uretritis), las glándulas de Bartolino, inflamación del cuello uterino (cervicitis), membrana mucosa uterina (endometritis) y las trompas de Falopio (salpingitis) y flujo vaginal. Esterilidad (Infertilidad).	
C. pneumoniae	enfermedades respiratorias (por ejemplo, neumonía, bronquitis) discutido: endocarditis, enfermedades coronarias del corazón	Fiebre, tos (en regel productiva)	De hombre a hombre Aerogénica, infección por gotitas
C. psittaci	Psitacosis u ornitosis	hepatoesplenomegalia; tos no productiva, fiebre y dolor de cabeza severo	La inhalación de las heces de las aves infectadas; en contacto con las vísceras de las aves infectadas.

La infección o la presencia de un patógeno puede identificarse mediante:

- Microscopia
- PCR
- Serología: Detección de antigénos a través de ELISA
  Detección de anticuerpos a través de IF, EIA, ELISA

2. USO PREVISTO

El enzimoinmunoensayo Chlamydia pneumoniae IgG ELISA se utiliza para la determinación cualitativa de anticuerpos IgG específicos contra Chlamydia pneumoniae en suero o plasma (citrato, heparina) humano.

3. PRINCIPIO DEL ENSAYO

La determinación inmunoenzimática cualitativa de anticuerpos específicos se basa en la técnica ELISA (Enzyme-linked Immunosorbent Assay).
Las Placas de Microtitulación están recubiertas con antígenos específicos unen a los anticuerpos de la muestra. Después de lavar los pocillos para eliminar todo el material de muestra no unida, el conjugado de peroxidasa de rábano (HRP) se añade. Este conjugado se une a los anticuerpos capturados. En una segunda etapa de lavado se retira el conjugado no unido. El complejo inmune formado por el conjugado unido se visualiza añadiendo substrato tetrametilbencidina (TMB), que da un producto de reacción azul.
La intensidad de este producto es proporcional a la cantidad de anticuerpos específicos en la muestra. se añade ácido sulfúrico para detener la reacción. Esto produce un cambio de color de azul a amarillo. La extinción a 450/620 nm se mide con un fotómetro de Placa de Microtitulación ELISA.

4. MATERIALES

4.1. Reactivos suministrados

- **Placa de Microtitulación:** 12 tiras de 8 pocillos rompibles, recubiertos con antígenos de Chlamydia pneumoniae, en bolsa de aluminio.
- **Tampón de Dilución de Muestras IgG:** 1 botella de 100 mL de solución de tampón de fosfato (10 mM) para diluir la muestra; pH 7,2 ± 0,2; color amarillo; listo para ser utilizado; tapa blanca; ≤ 0,0015% (v/v) CMIT/ MIT (3:1).
- **Solución de Parada:** 1 botella de 15 mL de ácido sulfúrico, 0,2 mol/L, listo para ser utilizado; tapa roja.
- **Tampón de Lavado (20x conc.):** 1 botella de 50 mL de una solución de tampón de fosfato 20x concentrado (0,2 M) para lavar los pocillos; pH 7,2 ± 0,2; tapa blanca.
- **Conjugado:** 1 botella de 20 mL de conjugado de anticuerpos IgG anti-humano con peroxidasa en tampón de fosfato (10 mM); color azul; tapa negra; listo para ser utilizado.
- **Solución de Sustrato de TMB:** 1 botella de 15 mL 3,3',5,5'-tetrametilbenzindina (TMB), < 0,1 %; listo para ser utilizado; tapa amarilla.
- **Control Positivo:** 1 botella de 2 mL control; color amarillo; tapa roja; listo para ser utilizado; ≤ 0,02% (v/v) MIT.
- **Control Cut-off:** 1 botella de 3 mL control; color amarillo; tapa verde; listo para ser utilizado; ≤ 0,02% (v/v) MIT.
- **Control Negativo:** 1 botella de 2 mL control; color amarillo; tapa azul; listo para ser utilizado; ≤ 0,0015% (v/v) CMIT/ MIT (3:1).

Para indicaciones de peligro y consejos de prudencia consulte el cap. 12.1.
Para sustancias potencialmente peligrosas por favor revise la ficha de datos de seguridad.

4.2. Accesorios suministrados

- 1 lámina autoadhesiva
- 1 instrucciones de uso
- 1 esquema de la placa

4.3. Materiales e instrumentos necesarios

- Fotómetro de Placa de Microtitulación con filtros de 450/620 nm
- Incubadora 37 °C
- Dispositivo de lavado manual o automático de Placas de Microtitulación
- Micropipetas para uso de (10-1000 µL)
- Mezcladora Vortex
- Agua destilada
- Tubos de plástico desechables

5. ESTABILIDAD Y ALMACENAJE

Almacene el kit a 2...8 °C. Los reactivos abiertos son estables hasta la fecha de caducidad indicada en la etiqueta cuando se almacena a 2...8 °C.

6. PREPARACIÓN DE LOS REACTIVOS

Es muy importante llevar todos los reactivos y las muestras a temperatura ambiente (20...25 °C) y mezclarlos antes de ser utilizados!

6.1. Placa de Microtitulação

Las tiras rompibles están recubiertas con antígeno de Chlamydia pneumoniae. Inmediatamente después de la eliminación de las tiras, las tiras restantes deben sellarse de nuevo en el papel de aluminio junto con la bolsita di dióxio de silicio y almacenar a 2...8 °C.

6.2. Tampón de Lavado (20x conc.)

Diluir el Tampón de Lavado 1+19; por ejemplo 10 mL del Tampón de Lavado + 190 mL de agua destilada. El Tampón de Lavado diluido es estable durante 5 días a temperatura ambiente (20…25 °C). En caso de aparecer cristales en el concentrado, calentar la solución a 37 °C, por ejemplo, en un baño María. Mezclar bien antes de la dilución.

6.3. Solución de Sustrato de TMB

La solución está lista para su uso y debe almacenarse a 2...8 °C, protegida de la luz. La solución debe ser incolora o podría tener un color ligeramente azul claro. Si el sustrato se convierte en azul, es posible que haya sido contaminado y no puede ser utilizado en el ensayo.

7. TOMA Y PREPARACIÓN DE LAS MUESTRAS

Usar muestras de suero o plasma (citrato, heparina) humano. Si el ensayo se realiza dentro de 5 días después de la toma de sangre, las muestras pueden ser almacenadas de 2...8 °C, en caso contrario deben ser alicotadas y almacenadas congeladas (-70...-20 °C). Agitar bien las muestras descongeladas antes de diluirlas. Evitar congelaciones y descongelaciones repetidas.
No se recomienda la inactivación por calor de las muestras.

7.1. Dilución de las muestras

Antes del ensayo, las muestras tienen que estar diluidas en relación 1 + 100 con el Tampón de Dilución de Muestras IgG, p. e. 10 µL de la muestra con 1 mL de Tampón de Dilución de Muestras IgG, mezclar bien con la mezcladora Vortex.

8. PROCEDIMIENTO

Por favor, leer cuidadosamente las instrucciones de uso del ensayo **antes** de realizarlo. Para el buen funcionamiento de la técnica es necesario seguir las instrucciones. El siguiente procedimiento es válido solamente para el método manual. Si se realiza el ensayo en los sistemas automáticos de ELISA es aconsejable elevar el número de lavados de tres hasta cinco veces y el volumen de Tampón de Lavado de 300 µL a 350 µL para excluir efectos de lavado. Preste atención al capítulo 12. Antes de comenzar, especificar exactamente la repartición y posición de las muestras y de los estándares/controles (se recomienda determinar en duplicado) en el esquema de la placa suministrada. Usar la cantidad necesaria de tiras o pocillos e insertarlos en el soporte.

Realizar el ensayo en el orden indicado y sin retraso.

Para cada paso de pipeteado en los estándares/controles y en las muestras, usar siempre puntas de pipeta de un solo uso.

Graduar la incubadora a 37 ± 1°C.

1. Pipetear 100 µL de estándares/controles y muestras en los pocillos respectivos. Dejar el pocillo A1 para el blanco.
2. Recubrir las tiras con los autoadhesivos suministrados.
3. **Incubar 1 h ± 5 min a 37 ± 1°C.**
4. Después de la incubación, retirar el autoadhesivo, aspirar el líquido de la tira y lavarla tres veces con 300 µL del Tampón de Lavado. Evitar el rebosamiento de los pocillos. El intervalo entre lavado y aspiración debe ser > 5 segundos. Para sacar el líquido restante de las tiras, es conveniente sacudirlas sobre papel absorbente.

   Nota: El lavado es muy importante! Un mal lavado insuficiente provoca una baja precisión y resultados falsamente elevados!
5. Pipetar 100 µL de conjugado en cada pocillo con excepción del blanco substrato A1.
6. **Incubar 30 min a la temperatura ambiente (20…25 °C).** Evitar la luz solar directa.
7. Repetir el lavado como en el paso numero 4.
8. Pipetar 100 µL de Solución de Sustrato de TMB en todos los pocillos.
9. **Incubar exactamente 15 min en oscuridad a temperatura ambiente (20...25 °C).** Un color azul se produce en las muestras positivas debido a la reacción enzimática.
10. Pipetear en todos los pocillos 100 µL de la Solución de Parada en el mismo orden y mismo intervalo de tiempo como con el Solución de Sustrato de TMB, por lo tanto un cambio de color de azul a amarillo se produce.
11. Medir la extinción con 450/620 nm en un periodo de 30 min después de añadir la Solución de Parada.

8.1. Medición

Ajustar el fotómetro de Placa de Microtitulación ELISA al cero utilizando el Blanco.

Si por razones técnicas el fotómetro de Placa de Microtitulación de ELISA no se puede ajustar a cero utilizando el Blanco, el valor de la absorbancia de este debe ser sustraído de los demás valores de absorbancia medidos con el fin de obtener resultados fiables!

Medir la **extinción** de todos los pocillos con **450 nm** y anotar los resultados de los estándares/controles y de las muestras en el esquema de la placa.

Es aconsejable realizar la medición **bicromática** a una longitud de onda de referencia de 620 nm.

Si se efectuaron análisis en duplicado o múltiples, hay que calcular **el promedio de los valores de extinción** de los pocillos correspondientes.

9. CÁLCULO DE LOS RESULTADOS

9.1. Criterios de validez del ensayo

Para que un ensayo se considere válido, deben seguirse estrictamente las presentes instrucciones de uso y deben cumplirse los siguientes criterios:

- **Blanco:** valor de la extinción **< 0,100**
- **Control Negativo:** valor de la extinción **< 0,200 y < Cut-off**
- **Control Cut-off:** valor de la extinción **0,150 – 1,300**
- **Control Positivo:** valor de la extinción **> Cut-off**

Si estos criterios no se cumplen, la prueba no es váida y deberá repetirse.

9.2. Cálculo del valor de la medición

El Cut-off se obtiene de los valores de la extinción de los dos controles Cut-off.

Ejemplo: 0,42 OD Control Cut-off + 0,44 OD Control Cut-off = 0,86:2 = 0,43
Cut-off = 0,43

9.2.1. Resultados en unidades [NTU]

$$\frac{\text{Promedio valor de la extinción de la muestra} \times 10}{\text{Cut-off}} = \text{[NovaTec-unidades = NTU]}$$

Ejemplo: $\frac{1{,}591 \times 10}{0{,}43} = 37$ NTU

9.3. Interpretación de los resultados

Cut-off	10 NTU	-
Positivo	> 11 NTU	Los anticuerpos contra el patógeno están presentes. Ha producido un contacto con el antígeno (patógeno resp. vacuna).
Zona intermedia	9 – 11 NTU	Los anticuerpos contra el patógeno no se pudieron detectar claramente. Se recomienda repetir la prueba con una muestra fresca en 2 a 4 semanas. Si el resultado es de nuevo en la zona intermedia, la muestra se considera como **negativa**.
Negativo	< 9 NTU	La muestra no contiene anticuerpos contra el patógeno. Un contacto previo con el antígeno (patógeno resp. vacuna) es poco probable.
El diagnóstico de una infección no solamente se debe basar en el resultado del ensayo. Es necesario considerar la anamnésis y la sintomatología del paciente junto al resultado serológico. Estos resultados sólo tienen valor restringido en pacientes inmunodeprimidos o en neonatos.		

9.3.1. Isotipos de anticuerpo y Estado de la Infección

Serología	Significado
IgM	Característica de la respuesta primaria del anticuerpo Alto título de IgM con bajo título de IgG → sugieren una infección muy reciente o aguda Raras: → persistente IgM
IgG	Característica de la respuesta secundario del anticuerpo Pueden persistir por varios años El alto título de IgG con bajo título de IgM: → pueden indicar una infección pasada
IgA	Producida en el revestimiento mucoso en todo el cuerpo (⇒ Barrera Protectora) Usualmente producida tempranamente en el transcurso de la infección

10. CARACTERÍSTICAS DEL ENSAYO

Los resultados están basados en el grupo de pruebas investigado; no se trata de especificaciones garantizadas.

Para obtener más información sobre las características del ensayo, por favor, entre en contacto NovaTec Immundiagnostica GmbH.

10.1. Precisión

Intra ensayo	n	Promedio (E)	CV (%)
#1	24	0,376	11,46
#2	24	0,887	7,69
#3	24	1,105	8,01
Inter ensayo	**n**	**Promedio (NTU)**	**CV (%)**
#1	12	26,31	3,37
#2	12	25,00	5,09
#3	12	5,66	9,75

10.2. Especificad diagnóstica

La especificidad del ensayo se define como la probabilidad que tiene el ensayo de dar un resultado negativo en ausencia del analítico específico. Es 95,12% (95% Intervalo de confianza: 83,47% - 99,4%).

10.3. Sensibilidad de diagnóstico

La sensibilidad del ensayo se define como la probabilidad que tiene el ensayo de dar un resultado positivo en presencia del analítico específico. Es 91,67% (95% Intervalo de confianza: 81,61% - 97,24%).

10.4. Interferencias

Las muestras lipémicas, ictéricas e hemolíticas no mostraron interferencias con este equipo ELISA hasta una concentración de 5 mg/mL para triglicéridos, de 0,5 mg/mL para bilirrubina y de 10 mg/mL hemoglobina.

10.5. Reactividad cruzada

La investigación del panel de muestras con actividad de los anticuerpos en los parámetros con potencial de reacción cruzada no reveló ninguna evidencia significativa de resultados positivos falsos debido a reacciones cruzadas.
Una reacción cruzada con Chlamydia trachomatis no puede excluirse con sueros que contengan anticuerpos contra LPS y MOMP.

11. LIMITACIONES DEL ENSAYO

Una contaminación de las muestras con bacterias, o una congelación y descongelación repetida pueden producir cambios en los valores de la extinción.

12. PRECAUCIONES Y ADVERTENCIAS

- El procedimiento, la información, las precauciones y los avisos de las instrucciones de uso han de ser seguidas estrictamente. La utilización de equipos con analizadores y equipamiento similar tiene que ser validada. No se autorizan cambios en el diseño, composición y procedimiento, así como cualquier utilización en combinación con otros productos no aprobados por el fabricante; el usuario debe hacerse responsable de estos cambios. El fabricante no responderá ante falsos resultados e incidentes debidos a estas razones. El fabricante no responderá ante cualquier resultado por análisis visual de las muestras de los pacientes.
- Solo para diagnostico in vitro.
- Todos los materiales de origen humano o animal deberán ser considerados y tratados como potencialmente infecciosos.
- Todos los componentes de origen humano han sido examinados y resultaron no reactivos a anticuerpos contra el VIH, VHC y HbsAG.
- No intercambiar reactivos y Placa de Microtitulación de cargas diferentes.
- No usar reactivos de otro fabricante para este ensayo.
- No usar después de la fecha de caducidad.
- Sólo usar recambios de pipetas, dispensadores y materiales de laboratorio limpios.
- No intercambiar las tapas de los diferentes reactivos, para evitar la contaminación cruzada.
- Para evitar la evaporación y una contaminación microbiana, cierre inmediatamente las botellas después de usarlas.
- Después de abrirlas y posterior almacenaje, asegurarse de que no existe contaminación microbiana antes de seguir usándolas.
- Para evitar contaminaciones cruzadas y resultados erróneamente aumentados, Pipetear cuidadosamente las muestras y los reactivos en los pocillos sin salpicar.
- El ELISA sólo está diseñado para personal cualificado siguiendo las normas de buenas prácticas de laboratorio (Good Laboratory Practice,GLP).
- Para un mayor control de calidad interno, cada laboratorio deberá utilizar además muestras conocidas.

12.1. Nota de seguridad para los reactivos que contienen sustancias peligrosas

Los reactivos pueden contener CMIT/MIT (3:1) o MIT (consulte el cap. 4.1)
Por lo tanto, se aplican las indicaciones de peligro y consejos de prudencia.

Atención	H317	Puede provocar una reacción alérgica en la piel.
	P261	Evitar respirar el aerosol.
	P280	Llevar guantes/ prendas de protección.
	P302+P352	EN CASO DE CONTACTO CON LA PIEL: Lavar con abundante jabón agua.
	P333+P313	En caso de irritación o erupción cutánea: Consultar a un médico.
	P362+P364	Quitar las prendas contaminadas y lavarlas antes de volver a usarlas.

Se puede encontrar más información en la ficha de datos de seguridad.

12.2. Indicaciones para la eliminación de residuos

Por regla general, los productos químicos y las preparaciones son residuos peligrosos. Su eliminación esta sometida a las leyes y los decretos nacionales sobre la eliminación de residuos. Las autoridades informan sobre la eliminación de residuos peligroso.

13. INFORMACIONES PARA PEDIDOS

N° del producto: CHLG0510 Chlamydia pneumoniae IgG ELISA (96 determinaciones)

PORTUGUÊS

1. INTRODUÇÃO

As Chlamydiae são bactérias sem motilidade, Gram negativo e de crescimento intracelular obrigatório que formam inclusões características no citoplasma das células parasitadas. São facilmente visíveis ao microscópio óptico. São conhecidas três espécies diferentes de Chlamydia patogénicas para os humanos: Chlamydia trachomatis, Chlamydia pneumoniae e Chlamydia psittaci, e uma espécie apenas patogénica para os animais (C. pecorum). A Chlamydia trachomatis é o agente mais prevalente das doenças sexualmente transmissíveis em todo o mundo (400-500 milhões de casos) e o número de infecções está a crescer constantemente. As mulheres grávidas infectadas com C. trachomatis podem transmitir estas bactérias durante o parto, causando conjuntivite ou pneumonia nos recém-nascidos. Casos não tratados de infecção clamidial podem levar a salpingite crónica, resultando possivelmente em gravidez ectópica ou infertilidade. Nos indivíduos do sexo masculino, a C. trachomatis é uma das principais causas de uretrite não gonocócica. Um problema grave das infecções por Chlamydia é o curso insidioso frequentemente assintomático que pode resultar no início de doenças crónicas. Em muitos casos, as infecções primárias não são reconhecidas e apenas as sequelas causadas pela ascensão de agentes persistentes são diagnosticados.

Espécies	**Doença**	**Sintomas**	**Via de transmissão**
C. trachomatis	Tracoma Linfogranuloma venéreo (LGV) Infecções do trato urogenital	conjuntivite bilateral com sensação de corpo estranho, olhos lacrimejando e descarga de secreções serosas do olho. Lesão genital; inchaço dos linfonodos inguinais e femorais; formação de ínguas supurantes. **Nos homens:** a inflamação da uretra (uretrite), inflamação da próstata (prostatite) e epidídimo (epididimite). Esterilidade (infertilidade). **Nas mulheres:** a inflamação da uretra (uretrite), glândulas de Bartholin, inflamação do colo do útero (cervicite), inflamação membrana mucosa uterina (endometrite), das trompas de Falópio (salpingite) e corrimento vaginal. Esterilidade (infertilidade)	Sexualmente transmissível. Pode ser transmitida da mãe infectada para o bebê durante o parto.
C. pneumoniae	As doenças respiratórias (por exemplo, pneumonia, bronquite) discutiveis: endocardite, doenças coronárias	Febre, tosse (em produtivo regel)	Homem para Homem Aerogenic, gotícula infectadas
C. psittaci	Ornitose (Psitacose)	hepatoesplenomegalia; tosse improdutiva, febre e dor de cabeça severa	A inalação de fezes de aves infectadas; entre em contato com as vísceras das aves infectadas

Infecção ou presença de patógeno pode ser identificada por:

- Microscopia
- PCR
- Serologia: Detecção de antigénios por ELISA
  Detecção de anticorpos por IF, EIA, ELISA

2. UTILIZAÇÃO PRETENDIDA

O kit Chlamydia pneumoniae IgG ELISA destina-se à determinação qualitativa de anticorpos da classe IgG contra Chlamydia pneumoniae no soro ou plasma (citrato, heparina) humanos.

3. PRINCÍPIO DO ENSAIO

A determinação imunoenzimática qualitativa de anticorpos específicos é baseado na técnica de ELISA (do inglês Enzyme-linked Immunosorbent Assay).
As Placas de Microtitulação são revestidas com antigénios específicos que se ligam os anticorpos correspondentes da amostra. Após lavagem dos poços, para remover todo o material de amostra não ligada, um conjugado de peroxidase de rábano (HRP) é adicionado. Este conjugado se liga aos anticorpos capturados. Num segundo passo de lavagem o conjugado não ligado é removido. O complexo imune formado pelo conjugado ligado é visualizado por adição de substrato de tetrametilbenzidina (TMB), o que dá um produto de reacção azul.
A intensidade deste produto é proporcional à quantidade de anticorpos específicos da amostra. O ácido sulfúrico é adicionado para parar a reacção. Isso produz uma mudança de cor de azul para amarelo.
Absorvância a 450/620 nm é lida utilizando um fotômetro de Placa de Microtitulação ELISA.

4. MATERIAIS

4.1. Reagentes fornecidos

- **Placa de Microtitulação:** 12 tiras de 8 poços, destacáveis e quebráveis, revestidas com antigénio de Chlamydia pneumoniae, em bolsas de folha de alumínio com fecho.
- **Tampão de Diluição de Amostra IgG:** 1 frasco contendo 100 mL de tampão fosfato (10 mM) para diluição da amostra, pH 7,2 ± 0,2; de cor amarela; pronto a usar; tampa branca; ≤ 0,0015% (v/v) CMIT/ MIT (3:1).
- **Solução de Bloqueio:** 1 frasco contendo 15 mL ácido sulfúrico; 0,2 mol/L; pronto a usar; tampa vermelha.
- **Tampão de Lavagem (conc. 20x):** 1 frasco contendo 50 mL de um tampão fosfato (0,2 M); concentrado 20 vezes (pH 7,2 ± 0,2) para a lavagem dos poços; tampa branca.
- **Conjugado:** 1 frasco contendo 20 mL de anticorpo para IgG humana marcados com peroxidase no tampão fosfato (10 mM); de cor azul, pronto a usar; tampa preta.
- **Solução Substrato TMB:** 1 frasco contendo 15 mL de 3,3',5,5'-tetrametilbenzidina (TMB), < 0,1 %; pronto a usar; tampa amarela.
- **Controle Positivo:** 1 frasco contendo 2 mL controle; de cor amarela; pronto a usar; tampa vermelha. ≤ 0,02% (v/v) MIT.
- **Controle Cut-off:** 1 frasco contendo 3 mL controle; de cor amarela; pronto a usar; tampa verde. ≤ 0,02% (v/v) MIT.
- **Controle Negativo:** 1 frasco contendo 2 mL controle; de cor amarela; pronto a usar; tampa azul; ≤ 0,0015% (v/v) CMIT/ MIT (3:1).

Para advertências de perigo e recomendaçaões de prudência ver capitulo 12.1.
Para substâncias potencialmente perigosas verifique a ficha de dados de segurança.

4.2. Materiais fornecidos

- 1 Película de cobertura
- 1 Instruções de uso
- 1 Layout da placa

4.3. Materiais e Equipamento necessários

- Fotômetro de Placa de Microtitulação ELISA, equipado para a medição da absorvância a 450/620 nm
- Incubadora 37 °C
- Equipamento manual ou automático para a lavagem de Placa de Microtitulação
- Pipetas para dispensar volumes entre 10 e 1000 µL
- Agitador de tubos tipo Vortex
- Água destilada
- Tubos descartáveis

5. ESTABILIDADE E ARMAZENAMENTO

Armazene o kit a 2...8 °C. Os reagentes abertos são estáveis até o prazo de validade impresso no rótulo quando armazenado a 2...8 °C.

6. PREPARAÇÃO DOS REAGENTES

É muito importante deixar todos os reagentes e amostras estabilizar à temperatura ambiente (20...25 °C) misturá-los antes de iniciar o teste!

6.1. Placa de Microtitulação

As tiras quebraveis são revestidas com antigénio de Chlamydia pneumoniae. Imediatamente após a remoção das tiras necessarias, as tiras restantes devem ser lacradas de novo na folha de alumínio juntamente com o saquinho de silicio fornecido e armazenadar a 2...8 °C.

6.2. Tampão de Lavagem (conc. 20x)

Diluir o Tampão de Lavagem 1+19; por exemplo. 10 mL do Tampão de Lavagem + 190 mL de água destilada. O Tampão de Lavagem diluído é estavel durante 5 dias à temperatura ambiente (20…25 °C). Caso apareça cristais no concentrado, aquecer a solução a 37 °C por exemplo, em banho Maria. Misture bem antes da diluição.

6.3. Solução Substrato TMB

A solucao está pronta para uso e tem de ser armazenada à 2...8 °C, protegida da luz. A solução deve ser incolor ou poderia ter uma ligeira coloração azul claro. Se o substrato se transforma em azul, pode ter sido contaminado e não pode ser usado no teste.

7. COLHEITA E PREPARAÇÃO DAS AMOSTRAS

Usar com este ensaio amostras de soro ou plasma (citrato, heparina) humanos. Se o ensaio for realizado dentro de 5 dias após colheita da amostra, o espécime deve ser mantido a 2…8 °C; caso contrário devem ser alicotadas e armazenadas congeladas (-70...-20 °C). Se as amostras forem armazenadas congeladas, misturar bem as amostras descongeladas antes de testar. Evitar congelar e descongelar repetidamente.
Não é recomendada a inactivação por calor das amostras.

7.1. Diluição das amostras

Antes de testar todas as amostras devem ser diluídas 1 + 100 com Tampão de Diluição de Amostra IgG. Dispensar 10 µL de amostra e 1 mL de Tampão de Diluição de Amostra IgG em tubos para obter uma diluição 1 + 100 e misturar meticulosamente com um vortex.

8. PROCEDIMENTO DO ENSAIO

Por favor, ler atentamente as instruções de uso **antes** de realizar o teste. A fiabilidade dos resultados depende da adesão estrita ao as instruções de uso, conforme descritas. O procedimento de ensaio a seguir está validado apenas para o procedimento manual. Se o teste for realizado em sistemas automáticos para teste ELISA é recomendável aumentar os passos de lavagem de três até cinco e o volume da Tampão de Lavagem de 300 µL para 350 µL para evitar efeitos de lavagem. Preste atenção ao capítulo 12. Antes de iniciar o teste, o plano de distribuição e identificação de todas as amostras e calibradores/controles (é recomendado determinar em duplicidade) deve ser cuidadosamente estabelecido no Layout da placa fornecida no kit. Seleccionar o número necessário de tiras ou poços e inserir os mesmos no suporte.

Realizar todas as etapas do teste na ordem indicada e sem atrasos significativos.

Na pipetagem deve ser utilizada uma ponta limpa e descartável para dispensar cada controle e amostra.

Ajustar a incubadora para 37 ± 1 °C.

1. Dispensar 100 µL dos calibradores/controles e das amostras diluídas nos poços respectivos. Deixar o poço A1 vazio para o branco substrato.
2. Cobrir os poços com a película fornecida no kit.
3. **Incubar durante 1 hora ± 5 min a 37 ± 1 °C.**
4. Quando terminar a incubação, remover a película, aspirar o conteúdo dos poços e lavar cada poço três vezes com 300 µL de Tampão de Lavagem. Evitar que os poços de reacção transbordem. O intervalo entre a lavagem e a aspiração deve ser > 5 seg. No final, retirar cuidadosamente o fluido restante batendo delicadamente as tiras sobre papel absorvente, antes da próxima etapa!

   Nota: A lavagem é muito importante! Lavagem insuficiente resulta em baixa precisão e falsos resultados.
5. Dispensar 100 µL de Conjugado em todos os poços, excepto no poço do Branco substrato A1.
6. **Incubar durante 30 min à temperatura ambiente (20...25°C).** Não expor diretamente à luz solar.
7. Repetir a etapa 4.
8. Dispensar 100 µL de Solução Substrato TMB em todos os poços.
9. **Incubar durante exactamente 15 min à temperatura ambiente (20...25°C) e no escuro.** A cor azul devido a uma reacção enzimática.
10. Dispensar 100 µL de Solução de Bloqueio em todos os poços, pela mesma ordem e com a mesma velocidade a que foi dispensada a Solução Substrato TMB,desse modo uma mudança de cor de azul para amarelo ocorre.
11. Medir a absorvância a 450/620 nm dentro de 30 min após a adição da Solução de Bloqueio.

8.1. Medição

Ajustar o fotômetro para Placa de Microtitulação ELISA **a zero** usando o **Branco substrato .**

Se - devido à razões técnicas – o fotômetro para Placa de Microtitulação ELISA não puder ser ajustado a zero usando o Branco substrato, valor da absorvância deste deve ser subtraido de todos os outros valores de absorvância medidos de forma a obter resultados fiáveis!

Medir a absorvância de todos os poços a **450 nm** e registar os valores da absorvância para cada calibrador/controle e amostra no Layout da placa.

É recomendado fazer a medição **dicromática** usando como referência um comprimento de onda de 620 nm.

Se determinações duplas foram realizadas, calcular **os valores médios de absorvância**.

9. RESULTADOS

9.1. Critérios de validação do ensaio

Para que um ensaio seja considerado válido, estas Instruções de Uso devem ser rigorosamente seguidas, e os seguintes critérios devem ser cumpridos:

- **Branco substrato**: Valor de Absorvância **< 0,100**
- **Controle Negativo**: Valor de Absorvância **< 0,200 e < Cut-off**
- **Controle Cut-off**: Valor de Absorvância **0,150 – 1,300**
- **Controle Positivo**: Valor de Absorvância **> Cut-off**

Se estes critérios não forem cumpridos, o teste não é válido e deve ser repetido.

9.2. Cálculo dos Resultados

O Cut-off é o valor médio da absorvância das determinações do controle Cut-off.

Exemplo: Valor da absorvância do Controle Cut-off 0,42 + valor da absorvância do Controle Cut-off 0,44 = 0,86 : 2 = 0,43
Cut-off = 0,43

9.2.1. Resultados em Unidades [NTU]

$$\frac{\text{Valor da absorvância (média) da amostra x 10}}{\text{Cut-off}} = \text{[Unidades NovaTec = NTU]}$$

Exemplo: $\frac{1{,}591 \times 10}{0{,}43} = 37$ NTU

9.3. Interpretação dos Resultados

Cut-off	10 NTU	-
Positivo	> 11 NTU	Os anticorpos contra o agente patogênico estão presente. Houve um contacto com o antigénio (patógeno resp vacina).
Zona cinzenta	9 – 11 NTU	Os anticorpos contra o agente patogênico não puderam ser claramente detectados. Recomenda-se a repetir o teste com uma amostra fresca em 2 a 4 semanas. Se o resultado estiver novamente dentro da zona cinzenta, a amostra é julgada como **negativa**.
Negativo	< 9 NTU	A amostra não contém os anticorpos contra o agente patogênico. Um contato prévio com o antígeno (patógeno resp. vacina) é improvável.
O diagnóstico de uma doença infecciosa não deve ser estabelecido com base num único resultado do teste. Um diagnóstico preciso deve ter em consideração a história clínica, a sintomatologia bem como dados serológicos. Em pacientes imunossuprimidos e recém-nascidos os dados serológicos têm apenas valor restrito.		

9.3.1. Isotipos de anticorpos e Estado da Infecção

Sorologia	Significado
IgM	Característica da resposta primária do anticorpo Alto título de IgM com baixo título de IgG: → sugere uma infecção muito recente ou aguda Raros: → persistente IgM
IgG	Característica da resposta secundária do anticorpo Podem persistir por vários anos Alto título de IgG com baixo título de IgM: → pode indicar uma infecção passada
IgA	Eles são produzidos a nível das mucosas em todo o corpo (⇒ barreira protectora) Geralmente são producidas no inicio infecção

10. CARACTERÍSTICAS DE DESEMPENHO ESPECÍFICAS

Os resultados referem-se aos grupos de amostras investigados; estas não são especificações garantidas.

Para mais informações sobre as características de desempenho específicas, por favor, entre em contato NovaTec Immundiagnostica GmbH.

10.1. Precisão

Intra ensaio	**n**	**Média (E)**	**CV (%)**
#1	24	0,376	11,46
#2	24	0,887	7,69
#3	24	1,105	8,01
Inter ensaio	**n**	**Média (NTU)**	**CV (%)**
#1	12	26,31	3,37
#2	12	25,00	5,09
#3	12	5,66	9,75

10.2. Especificidade Diagnóstica

A especificidade diagnóstica é definida como a probabilidade do ensaio ser negativo na ausência do analito específico.
É de 95,12% (95% Intervalo de confiança: 83,47% - 99,4%).

10.3. Sensibilidade Diagnóstica

A sensibilidade diagnóstica é definida como a probabilidade do ensaio ser positivo na presença do analito específico.
É de 91,67% (95% Intervalo de confiança: 81,61% - 97,24%).

10.4. Interferências

Não são observadas interferências com amostras hemolisadas, lipémicas ou ictéricas até uma concentração de hemoglobina de 10 mg/mL, de triglicerídeos de 5 mg/mL e de bilirrubina de 0,5 mg/mL.

10.5. Reacção cruzada

A investigação do painel de amostras com atividades de anticorpos em parâmetros com potencial de reação cruzada não revelou nenhuma significante evidencia de resultados falso-positivos devido a reações cruzadas.
Não pode ser excluída uma reacção cruzada com Chlamydia trachomatis com soros contendo anticorpos para LPS e MOMP.

11. LIMITAÇÕES DO PROCEDIMENTO

Contaminação bacteriana ou a repetição de ciclos de congelação-descongelação do espécime podem afectar os valores da absorvância.

12. PRECAUÇÕES E AVISOS

- O procedimento do teste, as informações, as precauções e avisos nas instruções para utilização têm de ser rigorosamente seguidas. O uso de kits de teste com analisadores e equipamento similar tem de ser validado. Qualquer alteração no desenho, composição e procedimento do teste bem como qualquer utilização em combinação com outros produtos não aprovados pelo fabricante não estão autorizados; o próprio utilizador é responsável por tais alterações. O fabricante não é legalmente responsável por resultados falsos e incidentes originados por estes motivos. O fabricante não é legalmente responsável por quaisquer resultados obtidos por análise visual das amostras dos pacientes.
- Apenas para uso no diagnóstico in-vitro.
- Todos os materiais de origem humana ou animal devem ser considerados e tratados como potencialmente infectantes.
- Todos os componentes de origem humana usados para a produção destes reagentes foram testados para anticorpos anti-HIV, anticorpos anti-HCV e HBsAg e foram considerados não-reactivos.
- Não trocar e/ou juntar reagentes ou Placa de Microtitulação de lotes de produção diferentes.
- Nenhuns reagentes de outros fabricantes devem ser usados juntamente com reagentes deste kit de teste.
- Não usar reagentes após a data de validade indicada no rótulo.
- Usar apenas pontas de pipeta, dispensadores e material de laboratório limpos.
- Não trocar as tampas dos frascos dos reagentes para evitar contaminação cruzada.
- Fechar firmemente os frascos dos reagentes imediatamente após a utilização para evitar evaporação e contaminação microbiana.
- Após a primeira abertura e armazenamento subsequente verificar se existe contaminação microbiana dos frascos do conjugado e dos calibradores/controles antes de utiliza-los novamente.
- Para evitar contaminação-cruzada e resultados falsamente elevados, pipetar as amostras dos pacientes e dispensar o reagentes precisamente nos poços sem salpicar.
- O ELISA é projetado apenas para pessoal qualificado seguindo os padrões de boas práticas de laboratório (Good Laboratory Practice, GLP).
- Para um controle de qualidade interno adicional cada laboratório deve utilizar amostras conhecidas.

12.1. Nota de segurança para reagentes que contenham substâncias perigosas

Os reagentes podem conter CMIT/MIT (3:1) ou MIT (ver capitulo 4.1)
Portanto, as seguintes advertências de perigo e recomendações de prudência aplicam-se.

Atenção

H317	Pode provocar uma reacção alérgica cutânea.
P261	Evitar respirar os aerossóis.
P280	Usar luvas de protecção/ vestuário de protecção.
P302+P352	SE ENTRAR EM CONTACTO COM A PELE: lavar abundantemente com sabão água.
P333+P313	Em caso de irritação ou erupção cutânea: consulte um médico.
P362+P364	Retirar a roupa contaminada e lavá-la antes de a voltar a usar.

Mais informações podem ser encontradas na ficha de dados de segurança.

12.2. Considerações de Eliminação

Resíduos de químicos e preparações são geralmente considerados como resíduos perigosos. A eliminação deste tipo de resíduos está regulada por leis e normativas nacionais e regionais. Contactar as autoridades locais ou empresas de gestão de resíduos as quais podem aconselhar sobre como eliminar resíduos perigosos.

13. INFORMAÇÃO DE PEDIDO

Prod. No.:	CHLG0510	Chlamydia pneumoniae IgG ELISA (96 Determinações)

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ABBREVIATIONS / ABKÜRZUNGEN / ABRÉVIATIONS / ABBREVIAZIONI / ABREVIACIÓNES / ABREVIATURAS

CMIT	5-chloro-2-methyl-4-isothiazolin-3-one
MIT	2-methyl-2H-isothiazol-3-one

SYMBOLS KEY / SYMBOLSCHLÜSSEL / EXPLICATION DES SYMBOLES / LEGENDA / SIMBOLOS / TABELA DE SIMBOLOS

	Manufactured by / Hergestellt von / Fabriqué par / Prodotto da / Fabricado por / Fabricado por
IVD	In Vitro Diagnostic Medical Device / In Vitro Diagnosticum / Dispositif médical de diagnostic in vitro / Diagnostico in vitro / Producto para diagnóstico In vitro / Dispositivo Médico para Diagnóstico In Vitro
LOT	Lot Number / Chargenbezeichnung / Numéro de lot / Lotto / Número de lote / Número de lote
	Expiration Date / Verfallsdatum / Date de péremption / Scadenza / Fecha de caducidad / Data de Validade
	Storage Temperature / Lagertemperatur / Température de conservation / Temperatura di conservazione / Temperatura de almacenamiento / Temperatura de Armazenamento
CE	CE Mark / CE-Zeichen / Marquage CE / Marchio CE / Marca CE / Marca CE
REF	Catalogue Number / Katalog Nummer / Référence du catalogue / Numero di codice / Número de Catálogo / Número de Catálogo
	Consult Instructions for Use / Arbeitsanleitung beachten / Consulter la notice d'utilisation / Consultare le istruzioni per l'uso / Consulte las Instrucciones de Uso / Consultar as Instruções de Utilização
MTP	Microtiterplate / Mikrotiterplatte / Plaque de Microtitrage / Piastre di Microtitolazione / Placa de Microtitulación / Placa de Microtitulação
CONJ	Conjugate / Konjugat / Conjugué / Coniugato / Conjugado / Conjugado
CONTROL | -	Negative Control / Negativkontrolle / Contrôle négatif / Controllo Negativo / Control Negativo / Controle Negativo
CONTROL | +	Positive Control / Positivkontrolle /Contrôle Positif / Controllo Positivo / Control Positivo / Controle Positivo
CUT OFF	Cut-off Control / Cut-off Kontrolle / Contrôle Cut-off / Controllo Cut-off / Control Cut-off / Controle Cut-off
DIL | G	IgG Sample Dilution Buffer / IgG-Probenverdünnungspuffer / Tampon de Dilution d'Échantillon IgG / Tampone di Diluizione del Campione IgG / Tampón de Dilución de Muestras IgG / Tampão de Diluição de Amostra IgG
SOLN | STOP	Stop Solution / Stopplösung / Solution D'arrêt / Soluzione Bloccante / Solción Parada/ Solução de Bloqueio
SUB | TMB	TMB Substrate Solution / TMB-Substratlösung / Solution de Substrat TMB / Soluzione Ssubstrato TMB / Solución de Sustrato de TMB / Solução Substrato TMB
WASH | BUF | 20x	Washing Buffer 20x concentrated / Waschpuffer 20x konzentriert / Tampon de Lavage concentré 20 x / Tampone di Lavaggio concentrazione x20 / Tampón de Lavado concentrado x20 / Tampão de Lavagem concentrada 20x
	Contains sufficient for "n" tests / Ausreichend für "n" Tests / Contenu suffisant pour "n" tests / Contenuto sufficiente per "n" saggi / Contenido suficiente para "n" tests / Conteúdo suficiente para "n" testes

SUMMARY OF TEST PROCEDURE / KURZANLEITUNG TESTDURCHFÜHRUNG / RÉSUMÉ DE LA PROCEDURE DE TEST / SCHEMA DELLA PROCEDURA / RESUMEN DE LA TÉCNICA / RESUMO DO PROCEDIMENTO DE TESTE

SCHEME OF THE ASSAY

Chlamydia pneumoniae IgG ELISA

Test Preparation

Prepare reagents and samples as described. Establish the distribution and identification plan for all samples and standards/controls on the plate layout supplied in the kit. Select the required number of microtiter strips or wells and insert them into the holder.

Assay Procedure

	Substrate Blank (A1)	Negative Control	Cut-off Control	Positive Control	Sample (diluted 1+100)
Negative Control	-	100 µL	-	-	-
Cut-off Control	-	-	100 µL	-	-
Positive Control	-	-	-	100 µL	-
Sample (diluted 1+100)	-	-	-	-	100 µL
Cover wells with foil supplied in the kit **Incubate for 1 h at 37 ± 1 °C** Wash each well three times with 300 µL of Washing Buffer					
Conjugate	-	100 µL	100 µL	100 µL	100 µL
Incubate for 30 min at room temperature (20...25 °C) Do not expose to direct sunlight Wash each well three times with 300 µL of Washing Buffer					
TMB Substrate Solution	100 µL	100 µL	100 µL	100 µL	100 µL
Incubate for exactly 15 min at room temperature (20...25 °C) in the dark					
Stop Solution	100 µL	100 µL	100 µL	100 µL	100 µL
Photometric measurement at 450 nm (reference wavelength: 620 nm)					

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NovaTec Immundiagnostica GmbH

NovaLisa®

Chlamydia pneumoniae IgM

ELISA

CE 0483

Only for in-vitro diagnostic use

Product Number: CHLM0510 (96 Determinations)

ENGLISH

1. INTRODUCTION

Chlamydiae are no motile, Gram negative and obligatory intracellular growing bacteria which form characteristic inclusions within the cytoplasm of parasitized cells. They are easily visible in the light microscope. Three different Chlamydia species pathogenic for humans are known: Chlamydia trachomatis, Chlamydia pneumoniae and Chlamydia psittaci, and one species only pathogenic for animals (C. pecorum). Chlamydia trachomatis is the most prevalent agent of sexually transmitted diseases worldwide (400-500 million cases) and the number of infections is constantly growing. Pregnant women infected with C. trachomatis may transmit these bacteria during childbirth, causing conjunctivitis or pneumonia in newborns. Untreated cases of chlamydial infection can lead to chronic salpingitis, possibly resulting in ectopic pregnancy or infertility. In males, C. trachomatis is a major cause of non-gonococcal urethritis. A severe problem in Chlamydia infections is the frequent asymptomatic insidious course which may result in the initiation of chronic diseases. In many instances primary infections are not recognized and only the sequelae caused by ascended, persisting agents are diagnosed.

Species	**Disease**	**Symptoms (e.g.)**	**Transmission route**
Chlamydia trachomatis	Trachoma	Bilateral conjunctivitis with foreign body sensation, tearing eyes, and discharge of serous secretions from the eye.	Sexually transmitted. Can be passed from an infected mother to her baby during childbirth.
	Lymphogranuloma venereum (LGV)	Genital lesion; swelling of the inguinal and femoral lymph nodes; formation of suppurating buboes.	
	Urogenital infections	**In men:** Inflammation of the urethra (urethritis), inflammation of the prostate (prostatitis) and the epididymis (epididymitis). Sterility (infertility). **In women:** Inflammation of the urethra (urethritis), Bartholin's glands, inflammation of the cervix (cervicitis), uterine mucous membrane (endometritis) and fallopian tubes (salpingitis) and vaginal discharge. Sterility (Infertility)	
C. pneumoniae	Respiratory diseases (e.g. pneumonia, bronchitis) discussed: endocarditis, coronary heart diseases	Fever, cough (usually productive)	Man to Man Aerogenic, droplet infection
C. psittaci	Ornithosis (Psittacosis)	Hepatosplenomegaly; Unproductive cough, fever and severe headache	Inhalation of feces from infected birds; contact with infected avian viscera

Infection or presence of pathogen may be identified by:

- Microscopy
- PCR
- Serology: Detection of antigens e.g. by ELISA
  Detection of antibodies e.g. by ELISA

2. INTENDED USE

The Chlamydia pneumoniae IgM ELISA is intended for the qualitative determination of IgM class antibodies against Chlamydia pneumoniae in human serum or plasma (citrate, heparin).

3. PRINCIPLE OF THE ASSAY

The qualitative immunoenzymatic determination of specific antibodies is based on the ELISA (Enzyme-linked Immunosorbent Assay) technique.
Microtiterplates are coated with specific antigens to bind corresponding antibodies of the sample. After washing the wells to remove all unbound sample material a horseradish peroxidase (HRP) labelled conjugate is added. This conjugate binds to the captured antibodies. In a second washing step unbound conjugate is removed. The immune complex formed by the bound conjugate is visualized by adding Tetramethylbenzidine (TMB) substrate which gives a blue reaction product.
The intensity of this product is proportional to the amount of specific antibodies in the sample. Sulphuric acid is added to stop the reaction. This produces a yellow endpoint colour. Absorbance at 450/620 nm is read using an ELISA Microtiterplate reader.

4. MATERIALS

4.1. Reagents supplied

- **Microtiterplate:** 12 break-apart 8-well snap-off strips coated with Chlamydia pneumoniae antigens; in resealable aluminium foil.
- **IgM Sample Dilution Buffer:** 1 bottle containing 100 mL of phosphate buffer (10 mM) for sample dilution; pH 7.2 ± 0.2; anti-human IgG (RF Absorbent); coloured green; ready to use; white cap; ≤ 0.0015% (v/v) CMIT/ MIT (3:1).
- **Stop Solution:** 1 bottle containing 15 mL sulphuric acid, 0.2 mol/L; ready to use; red cap.
- **Washing Buffer (20x conc.):** 1 bottle containing 50 mL of a 20-fold concentrated phosphate buffer (0.2 M), pH 7.2 ± 0.2, for washing the wells; white cap.
- **Conjugate:** 1 bottle containing 20 mL of peroxidase labelled antibody to human IgM in phosphate buffer (10 mM); coloured red; ready to use; black cap.
- **TMB Substrate Solution:** 1 bottle containing 15 mL 3,3',5,5'-tetramethylbenzidine (TMB), < 0.1 %; ready to use; yellow cap.
- **Positive Control:** 1 vial containing 2 mL control; coloured yellow; ready to use; red cap; ≤ 0.02% (v/v) MIT.
- **Cut-off Control:** 1 vial containing 3 mL control; coloured yellow; ready to use; green cap; ≤ 0.02% (v/v) MIT.
- **Negative Control:** 1 vial containing 2 mL control; coloured yellow; ready to use; blue cap; ≤ 0.0015% (v/v) CMIT/ MIT (3:1).

For hazard and precautionary statements see 12.1
For potential hazardous substances please check the safety data sheet.

4.2. Materials supplied

- 1 Cover foil
- 1 Instruction for use (IFU)
- 1 Plate layout

4.3. Materials and Equipment needed

- ELISA Microtiterplate reader, equipped for the measurement of absorbance at 450/620 nm
- Incubator 37 °C
- Manual or automatic equipment for rinsing Microtiterplates
- Pipettes to deliver volumes between 10 and 1000 µL
- Vortex tube mixer
- Distilled water
- Disposable tubes

5. STABILITY AND STORAGE

Store the kit at 2...8 °C. The opened reagents are stable up to the expiry date stated on the label when stored at 2...8 °C.

6. REAGENT PREPARATION

It is very important to bring all reagents and samples to room temperature (20…25 °C) and mix them before starting the test run!

6.1. Microtiterplate

The break-apart snap-off strips are coated with Chlamydia pneumoniae antigens. Immediately after removal of the strips, the remaining strips should be resealed in the aluminium foil along with the desiccant supplied and stored at 2...8 °C.

6.2. Washing Buffer (20x conc.)

Dilute Washing Buffer 1 + 19; e. g. 10 mL Washing Buffer + 190 mL distilled water. The diluted buffer is stable for 5 days at room temperature (20…25 °C). In case crystals appear in the concentrate, warm up the solution to 37°C e.g. in a water bath. Mix well before dilution.

6.3. TMB Substrate Solution

The reagent is ready to use and has to be stored at 2...8 °C, away from the light. The solution should be colourless or could have a slight blue tinge. If the substrate turns into blue, it may have become contaminated and should be thrown away.

7. SAMPLE COLLECTION AND PREPARATION

Use human serum or plasma (citrate, heparin) samples with this assay. If the assay is performed within 5 days after sample collection, the samples should be kept at 2...8 °C; otherwise they should be aliquoted and stored deep-frozen (-70…-20 °C). If samples are stored frozen, mix thawed samples well before testing. Avoid repeated freezing and thawing.
Heat inactivation of samples is not recommended.

7.1. Sample Dilution

Before assaying, all samples should be diluted 1+100 with IgM Sample Dilution Buffer. Dispense 10 µL sample and 1 mL IgM Sample Dilution Buffer into tubes to obtain a 1+100 dilution and thoroughly mix with a Vortex.

8. ASSAY PROCEDURE

Please read the instruction for use carefully **before** performing the assay. Result reliability depends on strict adherence to the instruction for use as described. The following test procedure is only validated for manual procedure. If performing the test on ELISA automatic systems we recommend increasing the washing steps from three up to five and the volume of Washing Buffer from 300 µL to 350 µL to avoid washing effects. Pay attention to chapter 12. Prior to commencing the assay, the distribution and identification plan for all samples and standards/controls (duplicates recommended) should be carefully established on the plate layout supplied in the kit. Select the required number of microtiter strips or wells and insert them into the holder.

Perform all assay steps in the order given and without any delays.

A clean, disposable tip should be used for dispensing each standard/control and sample.

Adjust the incubator to 37 ± 1 °C.

1. Dispense 100 µL standards/controls and diluted samples into their respective wells. Leave well A1 for the Substrate Blank.
2. Cover wells with the foil supplied in the kit.
3. **Incubate for 1 hour ± 5 min at 37 ± 1 °C.**
4. When incubation has been completed, remove the foil, aspirate the content of the wells and wash each well three times with 300 µL of Washing Buffer. Avoid overflows from the reaction wells. The interval between washing and aspiration should be > 5 sec. At the end carefully remove remaining fluid by tapping strips on tissue paper prior to the next step!

   Note: Washing is important! Insufficient washing results in poor precision and false results.
5. Dispense 100 µL Conjugate into all wells except for the Substrate Blank well A1.
6. **Incubate for 30 min at room temperature (20...25 °C).** Do not expose to direct sunlight.
7. Repeat step 4.
8. Dispense 100 µL TMB Substrate Solution into all wells.
9. **Incubate for exactly 15 min at room temperature (20...25 °C) in the dark.** A blue colour occurs due to an enzymatic reaction.
10. Dispense 100 µL Stop Solution into all wells in the same order and at the same rate as for the TMB Substrate Solution, thereby a colour change from blue to yellow occurs.
11. Measure the absorbance at 450/620 nm within 30 min after addition of the Stop Solution.

8.1. Measurement

Adjust the ELISA Microtiterplate reader **to zero** using the **Substrate Blank**.

If - due to technical reasons - the ELISA Microtiterplate reader cannot be adjusted to zero using the Substrate Blank, subtract its absorbance value from all other absorbance values measured in order to obtain reliable results!

Measure the absorbance of all wells at **450 nm** and record the absorbance values for each standard/control and sample in the plate layout.

Bichromatic measurement using a reference wavelength of 620 nm is recommended.

Where applicable calculate the mean absorbance values of all duplicates.

9. RESULTS

9.1. Run Validation Criteria

In order for an assay run to be considered valid, these Instructions for Use have to be strictly followed and the following criteria must be met:

- **Substrate Blank**: Absorbance value **< 0.100**
- **Negative Control**: Absorbance value **< 0.200 and < Cut-off**
- **Cut-off Control**: Absorbance value **0.150 – 1.300**
- **Positive Control**: Absorbance value **> Cut-off**

If these criteria are not met, the test is not valid and must be repeated.

9.2. Calculation of Results

The Cut-off is the mean absorbance value of the Cut-off Control determinations.

Example: Absorbance value Cut-off Control 0.44 + absorbance value Cut-off control 0.42 = 0.86 / 2 = 0.43
Cut-off = 0.43

9.2.1. Results in Units [NTU]

$$\frac{\text{Sample (mean) absorbance value} \times 10}{\text{Cut-off}} = \text{[NovaTec Units = NTU]}$$

Example: $\frac{1.591 \times 10}{0.43} = 37$ NTU (Units)

9.3. Interpretation of Results

Cut-off	10 NTU	-
Positive	> 11 NTU	Antibodies against the pathogen are present. There has been a contact with the antigen (pathogen resp. vaccine).
Equivocal	9 – 11 NTU	Antibodies against the pathogen could not be detected clearly. It is recommended to repeat the test with a fresh sample in 2 to 4 weeks. If the result is equivocal again the sample is judged as **negative**.
Negative	< 9 NTU	The sample contains no antibodies against the pathogen. A previous contact with the antigen (pathogen resp. vaccine) is unlikely.
Diagnosis of an infectious disease should not be established on the basis of a single test result. A precise diagnosis should take into consideration clinical history, symptomatology as well as serological data. In immunocompromised patients and newborns serological data only have restricted value.		

9.3.1. Antibody Isotypes and State of Infection

Serology	Significance
IgM	Characteristic of the primary antibody response High IgM titer with low IgG titer: → suggests a current or very recent infection Rare: → persisting IgM
IgG	Characteristic of the secondary antibody response May persist for several years High IgG titer with low IgM titer: → may indicate a past infection
IgA	Produced in mucosal linings throughout the body (⇒ protective barrier) Usually produced early in the course of the infection

10. SPECIFIC PERFORMANCE CHARACTERISTICS

The results refer to the groups of samples investigated; these are not guaranteed specifications.

For further information about the specific performance characteristics please contact NovaTec Immundiagnostica GmbH.

10.1. Precision

Intraassay	**n**	**Mean (E)**	**CV (%)**
#1	24	0.470	4.00
#2	24	1.070	5.38
#3	24	1.846	2.96
Interassay	**n**	**Mean (NTU)**	**CV (%)**
#1	12	23.38	3.86
#2	12	33.19	7.44
#3	12	4.18	9.88

10.2. Diagnostic Specificity

The diagnostic specificity is defined as the probability of the assay of scoring negative in the absence of the specific analyte.

It is 99.26% (95% confidence interval: 95.97% - 99.98%).

10.3. Diagnostic Sensitivity

The diagnostic sensitivity is defined as the probability of the assay of scoring positive in the presence of the specific analyte.

It is 90.0% (95% confidence interval: 55.5% - 99.75%).

10.4. Interferences

Interferences with hemolytic, lipemic or icteric samples are not observed up to a concentration of 10 mg/mL hemoglobin, 5 mg/mL triglycerides and 0.5 mg/mL bilirubin.

10.5. Cross Reactivity

Investigation of a sample panel with antibody activities to potentially cross-reacting parameters did not reveal significant evidence of false-positive results due to cross-reactions.
A cross reaction with Chlamydia trachomatis cannot be excluded with sera containing antibodies to LPS and MOMP.

11. LIMITATIONS OF THE PROCEDURE

Bacterial contamination or repeated freeze-thaw cycles of the sample may affect the absorbance values.

12. PRECAUTIONS AND WARNINGS

- The test procedure, the information, the precautions and warnings in the instructions for use have to be strictly followed. The use of the testkits with analyzers and similar equipment has to be validated. Any change in design, composition and test procedure as well as for any use in combination with other products not approved by the manufacturer is not authorized; the user himself is responsible for such changes. The manufacturer is not liable for false results and incidents for these reasons. The manufacturer is not liable for any results by visual analysis of the patient samples.
- Only for in-vitro diagnostic use.
- All materials of human or animal origin should be regarded and handled as potentially infectious.
- All components of human origin used for the production of these reagents have been tested for anti-HIV antibodies, anti-HCV antibodies and HBsAg and have been found to be non-reactive.
- Do not interchange reagents or Microtiterplates of different production lots.
- No reagents of other manufacturers should be used along with reagents of this test kit.
- Do not use reagents after expiry date stated on the label.
- Use only clean pipette tips, dispensers, and lab ware.
- Do not interchange screw caps of reagent vials to avoid cross-contamination.
- Close reagent vials tightly immediately after use to avoid evaporation and microbial contamination.
- After first opening and subsequent storage check conjugate and standard/control vials for microbial contamination prior to further use.
- To avoid cross-contamination and falsely elevated results pipette patient samples and dispense reagents without splashing accurately into the wells.
- The ELISA is only designed for qualified personnel following the standards of good laboratory practice (GLP).
- For further internal quality control each laboratory should additionally use known samples.

12.1. Safety note for reagents containing hazardous substances

Reagents may contain CMIT/MIT (3:1) or MIT (refer to 4.1)
Therefore, the following hazard and precautionary statements apply.

Warning		
	H317	May cause an allergic skin reaction.
	P261	Avoid breathing spray
	P280	Wear protective gloves/ protective clothing.
	P302+P352	IF ON SKIN: Wash with plenty of soap and water.
	P333+P313	If skin irritation or rash occurs: Get medical advice/ attention.
	P362+P364	Take off contaminated and Wash it before reuse.

Further information can be found in the safety data sheet.

12.2. Disposal Considerations

Residues of chemicals and preparations are generally considered as hazardous waste. The disposal of this kind of waste is regulated through national and regional laws and regulations. Contact your local authorities or waste management companies which will give advice on how to dispose hazardous waste.

13. ORDERING INFORMATION

Prod. No.: CHLM0510 Chlamydia pneumoniae IgM ELISA (96 Determinations)

DEUTSCH

1. EINLEITUNG

Chlamydien sind unbewegliche, gramnegative, obligat intrazelluläre Bakterien, die charakteristische Einschlüsse im Cytoplasma der parasitierten Zelle bilden. Im Lichtmikroskop sind sie gut zu erkennen. Man unterscheidet drei für den Menschen pathogene Spezies: Chlamydia trachomatis, Chlamydia pneumoniae und Chlamydia psittaci. Chlamydia pecorum ist die einzige für Tiere pathogen Art. Chlamydia trachomatis ist die häufigste Ursache sexuell übertragener Erkrankungen weltweit (400-500 Millionen Fälle). Schwangere Frauen mit C. trachomatis können unter der Geburt das Neugeborene infizieren. Es kommt zu Konjunktivitiden oder Pneumonien. Unbehandelte Fälle von Chlamydien Infektionen können zu chronischer Salpingitis mit resultierenden ektopen Schwangerschaften oder Sterilität führen. Bei Männern ist Chlamydia trachomatis häufigste Ursache der nicht ghonorrhoischen Urethritiden. Ein ernstes Problem der Chlamydien Infektionen ist der häufig asymptomatische Verlauf, der letztlich jedoch in einer chronischen Erkrankung resultieren kann. In vielen Fällen wird die Primärinfektion nicht rechtzeitig erkannt und erst die Folgeschäden diagnostiziert.

Spezies	Erkrankung	Symptome (z.B.)	Infektionsweg
Chlamydia trachomatis	Trachoma	Beidseitige Konjunktivitis mit Fremdkörpergefühl, tränenden Augen und Ausfluss von serösem Sekret aus dem Auge.	Kontakt-Schmierinfektion (Geschlechtsverkehr). Übertragung von der Mutter auf das Kind bei der Geburt.
	Lymphogranuloma venereum	Genitale Läsion; Schwellung der Leisten- und Femorallymphknoten; Entzündung des Lymphknotens.	
	Urogenitalinfektionen	**Beim Mann**: Entzündungen der Harnröhre (Urethritis), Entzündungen der Prostata (Prostatitis) und der Nebenhoden (Epididymitis). Sterilität (Unfruchtbarkeit). **Bei der Frau**: Entzündung der Harnröhre (Urethritis), der Bartholinschen Drüsen, Entzündungen des Gebärmutterhalses (Zervizitis), der Gebärmutterschleimhaut (Endometritis) und der Eileiter (Salpingitis) und vaginaler Ausfluss. Sterilität (Unfruchtbarkeit)	
C. pneumoniae	Erkrankungen der Atemwege (z.B. Bronchitis, Pneumonie). In Diskussion: Endokarditis, koronare Herzkrankheiten	Husten (in der Regel produktiv), Fieber	Mensch zu Mensch Aerogen, Tröpfcheninfektion
C. psittaci	Ornithose (psittacose)	Hepatosplenomegalie; unproduktiver Husten, Fieber und starke Kopfschmerzen	Inhalation von Fäkalien infizierter Vögel; Kontakt mit infizierten Vogelarten

Nachweis des Erregers bzw. der Infektion durch:

- Mikroskopie
- PCR
- Serologie: Nachweis der Antigene mittels ELISA
  Nachweis der Antikörper mittels, ELISA

2. VERWENDUNGSZWECK

Der Chlamydia pneumoniae IgM ELISA ist für den qualitativen Nachweis spezifischer IgM-Antikörper gegen Chlamydia pneumoniae in humanem Serum oder Plasma (Citrat, Heparin) bestimmt.

3. TESTPRINZIP

Die qualitative immunenzymatische Bestimmung von spezifischen Antikörpern beruht auf der ELISA (Enzyme-linked Immunosorbent Assay) Technik.
Die Mikrotiterplatten sind mit spezifischen Antigenen beschichtet, an welche die korrespondierenden Antikörper aus der Probe binden. Ungebundenes Probenmaterial wird durch Waschen entfernt. Anschließend erfolgt die Zugabe eines Meerettich-Peroxidase (HRP) Konjugates. Dieses Konjugat bindet an die an der Mikrotiterplatte gebundenen spezifischen Antikörper. In einem zweiten Waschschritt wird ungebundenes Konjugat entfernt. Die Immunkomplexe, die durch die Bindung des Konjugates entstanden sind, werden durch die Zugabe von Tetramethylbenzidin (TMB)-Substratlösung und eine resultierende Blaufärbung nachgewiesen.
Die Intensität des Reaktionsproduktes ist proportional zur Menge der spezifischen Antikörper in der Probe. Die Reaktion wird mit Schwefelsäure gestoppt, wodurch ein Farbumschlag von blau nach gelb erfolgt. Die Absorption wird bei 450/620 nm mit einem Mikrotiterplatten-Photometer gemessen.

4. MATERIALIEN

4.1. Mitgelieferte Reagenzien

- **Mikrotiterplatte:** 12 teilbare 8er-Streifen, beschichtet mit Chlamydia pneumoniae Antigenen; in wieder verschließbarem Aluminiumbeutel.
- **IgM-Probenverdünnungspuffer:** 1 Flasche mit 100 mL Phosphatpuffer (10 mM) zur Probenverdünnung; pH 7,2 ± 0,2; anti-human IgG (RF-Absorbens); grün gefärbt; gebrauchsfertig; weiße Verschlusskappe*;* ≤ 0,0015% (v/v) CMIT/ MIT (3:1).
- **Stopplösung:** 1 Flasche mit 15 mL Schwefelsäure, 0,2 mol/L; gebrauchsfertig; rote Verschlusskappe.
- **Waschpuffer (20x konz.):** 1 Flasche mit 50 mL eines 20-fach konzentrierten Phosphatpuffers (0,2 M), zum Waschen der Kavitäten; pH 7,2 ± 0,2; weiße Verschlusskappe.
- **Konjugat:** 1 Flasche mit 20 mL Peroxidase-konjugierten Antikörpern gegen humanes IgM in Phosphatpuffer (10 mM); rot gefärbt; gebrauchsfertig; schwarze Verschlusskappe.
- **TMB-Substratlösung:** 1 Flasche mit 15 mL 3,3`,5,5`-Tetramethylbenzidin (TMB), < 0,1 %; gebrauchsfertig; gelbe Verschlusskappe.
- **Positivkontrolle:** 1 Fläschchen mit 2 mL Kontrolle; gelb gefärbt; rote Verschlusskappe; gebrauchsfertig; ≤ 0,02% (v/v) MIT.
- **Cut-off Kontrolle:** 1 Fläschchen mit 3 mL Kontrolle; gelb gefärbt; grüne Verschlusskappe; gebrauchsfertig; ≤ 0,02% (v/v) MIT.
- **Negativkontrolle:** 1 Fläschchen mit 2 mL Kontrolle; gelb gefärbt; blaue Verschlusskappe; gebrauchsfertig; ≤ 0,0015% (v/v) CMIT/ MIT (3:1).

Für Gefahren- und Sicherheitshinweise siehe 12.1.
Für potenzielle Gefahrstoffe überprüfen Sie bitte das Sicherheitsdatenblatt.

4.2. Mitgeliefertes Zubehör

- 1 selbstklebende Abdeckfolie
- 1 Arbeitsanleitung
- 1 Plattenlayout

4.3. Erforderliche Materialien und Geräte

- Mikrotiterplatten-Photometer mit Filtern 450/620 nm
- Inkubator 37 °C
- Manuelle oder automatische Waschvorrichtung für Mikrotiterplatten
- Mikropipetten (10 - 1000 µL)
- Vortex-Mischer
- Destilliertes Wasser
- Plastikröhrchen für den einmaligen Gebrauch

5. STABILITÄT UND LAGERUNG

Testkit bei 2...8 °C lagern. Die geöffneten Reagenzien sind bis zu den auf den Etiketten angegebenen Verfallsdaten verwendbar, wenn sie bei 2...8 °C gelagert werden.

6. VORBEREITUNG DER REAGENZIEN

Es ist sehr wichtig, alle Reagenzien und Proben vor ihrer Verwendung auf Raumtemperatur (20...25 °C) zu bringen und zu mischen!

6.1. Mikrotiterplatte

Die abbrechbaren Streifen sind mit Chlamydia pneumoniae Antigenen beschichtet. Nicht verbrauchte Vertiefungen im Aluminiumbeutel zusammen mit dem Trockenmittel sofort wieder verschließen und bei 2...8 °C lagern.

6.2. Waschpuffer (20x konz.)

Der Waschpuffer ist im Verhältnis 1 + 19 zu verdünnen; z.B. 10 mL Waschpuffer + 190 mL destilliertes Wasser.
Der verdünnte Puffer ist bei Raumtemperatur (20…25 °C) 5 Tage haltbar. Sollten Kristalle im Konzentrat auftreten, die Lösung z.B. in einem Wasserbad auf 37 °C erwärmen und vor dem Verdünnen gut mischen.

6.3. TMB-Substratlösung

Die gebrauchsfertige Lösung ist bei 2...8 °C vor Licht geschützt aufzubewahren. Die Lösung ist farblos, kann aber auch leicht hellblau sein. Sollte die TMB-Substratlösung blau sein, ist sie kontaminiert und kann nicht im Test verwendet werden.

7. ENTNAHME UND VORBEREITUNG DER PROBEN

Es sollten humane Serum- oder Plasmaproben (Citrat, Heparin) verwendet werden. Werden die Bestimmungen innerhalb von 5 Tagen nach Blutentnahme durchgeführt, können die Proben bei 2...8 °C aufbewahrt werden, sonst aliquotieren und tiefgefrieren (-70…-20 °C). Wieder aufgetaute Proben vor dem Verdünnen gut schütteln. Wiederholtes Tiefgefrieren und Auftauen vermeiden!
Hitzeinaktivierung der Proben wird nicht empfohlen.

7.1. Probenverdünnung

Proben vor Testbeginn im Verhältnis 1 + 100 mit IgM-Probenverdünnungspuffer verdünnen, z. B. 10 µL Probe und 1 mL IgM-Probenverdünnungspuffer in die entsprechenden Röhrchen pipettieren, um eine Verdünnung von 1 + 100 zu erhalten; gut mischen (Vortex).

8. TESTDURCHFÜHRUNG

Arbeitsanleitung **vor** Durchführung des Tests sorgfältig lesen. Für die Zuverlässigkeit der Ergebnisse ist es notwendig, die Arbeitsanleitung genau zu befolgen. Die folgende Testdurchführung ist für die manuelle Methode validiert. Beim Arbeiten mit ELISA Automaten empfehlen wir, um Wascheffekte auszuschließen, die Zahl der Waschschritte von drei auf bis zu fünf und das Volumen des Waschpuffers von 300 µL auf 350 µL zu erhöhen. Kapitel 12 beachten. Vor Testbeginn auf dem mitgelieferten Plattenlayout die Verteilung bzw. Position der Proben und der Standards/Kontrollen (Doppelbestimmung empfohlen) genau festlegen. Die benötigte Anzahl von Mikrotiterstreifen (Kavitäten) in den Streifenhalter einsetzen.

Den Test in der angegebenen Reihenfolge und ohne Verzögerung durchführen.

Für jeden Pipettierschritt der Standards/Kontrollen und Proben saubere Einmalspitzen verwenden.

Den Inkubator auf 37 ± 1 °C einstellen.

1. Je 100 µL Standards/Kontrollen und vorverdünnte Proben in die entsprechenden Vertiefungen pipettieren. Vertiefung A1 ist für den Substratleerwert vorgesehen.
2. Die Streifen mit der mitgelieferten Abdeckfolie bedecken.
3. **1 h ± 5 min bei 37 ± 1 °C inkubieren.**
4. Am Ende der Inkubationszeit Abdeckfolie entfernen und die Inkubationsflüssigkeit aus den Teststreifen absaugen. Anschließend dreimal mit 300 µL Waschpuffer waschen. Überfließen von Flüssigkeit aus den Vertiefungen vermeiden. Das Intervall zwischen Waschen und Absaugen sollte > 5 sec betragen. Nach dem Waschen die Teststreifen auf Fließpapier ausklopfen, um die restliche Flüssigkeit zu entfernen.

   Beachte: Der Waschvorgang ist wichtig, da unzureichendes Waschen zu schlechter Präzision und falschen Messergebnissen führt!
5. 100 µL Konjugat in alle Vertiefungen, mit Ausnahme der für die Berechnung des Leerwertes A1 vorgesehenen, pipettieren.
6. **30 min bei Raumtemperatur (20...25 °C) inkubieren.** Nicht dem direkten Sonnenlicht aussetzen.
7. Waschvorgang gemäß Punkt 4 wiederholen.
8. 100 µL TMB-Substratlösung in alle Vertiefungen pipettieren.
9. **Genau 15 min im Dunkeln bei Raumtemperatur (20...25 °C) inkubieren.** Bei enzymatischer Reaktion findet eine Blaufärbung statt.
10. In alle Vertiefungen 100 µL Stopplösung in der gleichen Reihenfolge und mit den gleichen Zeitintervallen wie bei Zugabe der TMB-Substratlösung pipettieren, dadurch erfolgt ein Farbwechsel von blau nach gelb.
11. Die Extinktion der Lösung in jeder Vertiefung bei 450/620 nm innerhalb von 30 min nach Zugabe der Stopplösung messen.

8.1. Messung

Mit Hilfe des Substratleerwertes den **Nullabgleich** des Mikrotiterplatten-Photometers vornehmen.

Falls diese Eichung aus technischen Gründen nicht möglich ist, muss nach der Messung der Extinktionswert des Substratleerwertes von allen anderen Extinktionswerten subtrahiert werden, um einwandfreie Ergebnisse zu erzielen!

Extinktion aller Kavitäten bei **450 nm** messen und die Messwerte der Standards/Kontrollen und Proben in das Plattenlayout eintragen.

Eine **bichromatische** Messung mit der Referenzwellenlänge 620 nm wird empfohlen.

Falls Doppel- oder Mehrfachbestimmungen durchgeführt wurden, den **Mittelwert der Extinktionswerte** berechnen.

9. BERECHNUNG DER ERGEBNISSE

9.1. Testgültigkeitskriterien

Damit ein Testlauf als valide betrachtet werden kann, muss diese Gebrauchsanweisung strikt befolgt werden, und die folgenden Kriterien müssen erfüllt sein:

- **Substrat-Leerwert**: Extinktionswert **< 0,100**
- **Negativkontrolle**: Extinktionswert **< 0,200 und < Cut-off**
- **Cut-off Kontrolle**: Extinktionswert **0,150 – 1,300**
- **Positivkontrolle**: Extinktionswert **> Cut-off**

Sind diese Kriterien nicht erfüllt, ist der Testlauf ungültig und muss wiederholt werden.

9.2. Messwertberechnung

Der Cut-off ergibt sich aus dem Mittelwert der gemessenen Extinktionen der Cut-off Kontrolle.

Beispiel: 0,44 OD Cut-off Kontrolle + 0,42 OD Cut-off Kontrolle = 0,86 : 2 = 0,43

Cut-off = 0,43

9.2.1. Ergebnisse in Einheiten [NTU]

$$\frac{\text{Mittlere Extinktion der Probe} \times 10}{\text{Cut-off}} = \text{[NovaTec Einheiten = NTU]}$$

Beispiel: $\frac{1{,}591 \times 10}{0{,}43} = 37$ NTU

9.3. Interpretation der Ergebnisse

Cut-off	10 NTU	-
Positiv	> 11 NTU	Es liegen Antikörper gegen den Erreger vor. Ein Kontakt mit dem Antigen (Erreger bzw. Impfstoff) hat stattgefunden.
Grenzwertig	9 – 11 NTU	Antikörper gegen den Erreger können nicht eindeutig nachgewiesen werden. Es wird empfohlen den Test nach 2 bis 4 Wochen mit einer frischen Patientenprobe zu wiederholen. Finden sich die Ergebnisse erneut im grenzwertigen Bereich, gilt die Probe als **negativ**.
Negativ	< 9 NTU	Es liegen keine Antikörper gegen den Erreger vor. Ein vorausgegangener Kontakt mit dem Antigen (Erreger bzw. Impfstoff) ist unwahrscheinlich.
Die Diagnose einer Infektionskrankheit darf nicht allein auf der Basis des Ergebnisses einer Bestimmung gestellt werden. Die anamnestischen Daten sowie die Symptomatologie des Patienten müssen zusätzlich zu den serologischen Ergebnissen in Betracht gezogen werden. Bei Immunsupprimierten und Neugeborenen besitzen die Ergebnisse serologischer Tests nur einen begrenzten Wert.		

9.3.1. Antikörper-Isotypen und Infektionsstatus

Serologie	Bedeutung
IgM	Typisch für Primärantwort Hoher IgM-Titer bei gleichzeitig niedrigem IgG-Titer: → Hinweis auf relativ frische Infektion Selten: → persistierendes IgM
IgG	Typisch für Sekundärantwort Können auch noch nach Jahren nachweisbar sein Hoher IgG-Titer bei gleichzeitig niedrigem IgM-Titer: → wahrscheinlich länger zurückliegende Infektion
IgA	Sezerniert in allen Schleimhäuten (⇒ Schutzbarriere) Meist früh im Verlauf einer Infektion gebildet

10. TESTMERKMALE

Die Ergebnisse beziehen sich auf die untersuchten Probenkollektive; es handelt sich nicht um garantierte Spezifikationen.

Für weitere Informationen zu den Testmerkmalen kontaktieren Sie bitte NovaTec Immundiagnostica GmbH.

10.1. Präzision

Intraassay	**n**	**Mittelwert (E)**	**Vk (%)**
#1	24	0,470	4,00
#2	24	1,070	5,38
#3	24	1,846	2,96
Interassay	**n**	**Mittelwert (NTU)**	**Vk (%)**
#1	12	23,38	3,86
#2	12	33,19	7,44
#3	12	4,18	9,88

10.2. Diagnostische Spezifität

Die diagnostische Spezifität ist definiert als die Wahrscheinlichkeit des Tests, ein negatives Ergebnis bei Fehlen des spezifischen Analyten zu liefern. Sie beträgt 99,26% (95% Konfidenzintervall: 95,97% - 99,98%).

10.3. Diagnostische Sensitivität

Die diagnostische Sensitivität ist definiert als die Wahrscheinlichkeit des Tests, ein positives Ergebnis bei Vorhandensein des spezifischen Analyten zu liefern. Sie ist 90,0% (95% Konfidenzintervall: 55,5% - 99,75%).

10.4. Interferenzen

Hämolytische, lipämische und ikterische Proben ergaben bis zu einer Konzentration von 10 mg/mL für Hämoglobin, von 5 mg/mL Triglyceride und von 0,5 mg/mL für Bilirubin keine Interferenzen im vorliegenden ELISA.

10.5. Kreuzreaktivität

Die Untersuchung eines Probenpanels mit Antikörperaktivitäten gegen potenziell kreuzreagierende Parameter ließ keine Anzeichen von falsch-positiven Ergebnissen aufgrund von Kreuzreaktivitäten erkennen.
Eine Kreuzreaktion mit Chlamydia trachomatis kann nicht ausgeschlossen werden mit Seren, die Antikörper gegen LPS- und MOMP-Partikel enthalten.

11. GRENZEN DES VERFAHRENS

Kontamination der Proben durch Bakterien oder wiederholtes Einfrieren und Auftauen können zu einer Veränderung der Messwerte führen.

12. SICHERHEITSMASSNAHMEN UND WARNHINWEISE

- Die Testdurchführung, die Information, die Sicherheitsmaßnahmen und Warnhinweise in der Arbeitsanleitung sind strikt zu befolgen. Bei Anwendung des Testkits auf Diagnostika-Geräten ist die Testmethode zu validieren. Jede Änderung am Aussehen, der Zusammensetzung und der Testdurchführung sowie jede Verwendung in Kombination mit anderen Produkten, die der Hersteller nicht autorisiert hat, ist nicht zulässig; der Anwender ist für solche Änderungen selbst verantwortlich. Der Hersteller haftet für falsche Ergebnisse und Vorkommnisse aus solchen Gründen nicht. Auch für falsche Ergebnisse aufgrund von visueller Auswertung wird keine Haftung übernommen.
- Nur für in-vitro-Diagnostik.
- Alle Materialien menschlichen oder tierischen Ursprungs sind als potentiell infektiös anzusehen und entsprechend zu behandeln.
- Alle verwendeten Bestandteile menschlichen Ursprungs sind auf Anti-HIV-AK, Anti-HCV-AK und HBsAg nicht-reaktiv getestet.
- Reagenzien und Mikrotiterplatten unterschiedlicher Chargen nicht untereinander austauschen.
- Keine Reagenzien anderer Hersteller zusammen mit den Reagenzien dieses Testkits verwenden.
- Nicht nach Ablauf des Verfallsdatums verwenden.
- Nur saubere Pipettenspitzen, Dispenser und Labormaterialien verwenden.
- Verschlusskappen der einzelnen Reagenzien nicht untereinander vertauschen, um Kreuzkontaminationen zu vermeiden.
- Flaschen sofort nach Gebrauch fest verschließen, um Verdunstung und mikrobielle Kontamination zu vermeiden.
- Nach dem ersten Öffnen Konjugat und Standards/Kontrollen vor weiterem Gebrauch auf mikrobielle Kontamination prüfen.
- Zur Vermeidung von Kreuzkontamination und falsch erhöhten Resultaten, Reagenzien sorgfältig in die Kavitäten pipettieren.
- Der ELISA ist nur für qualifiziertes Personal bestimmt, das den Standards der Guten Laborpraxis (GLP) folgt.
- Zur weiteren internen Qualitätskontrolle sollte jedes Labor zusätzlich bekannte Proben verwenden.

12.1. Sicherheitshinweis für Reagenzien, die Gefahrstoffe enthalten

Die Reagenzien können CMIT/MIT (3:1) oder MIT enthalten (siehe 4.1)
Daher gelten die folgenden Gefahren- und Sicherheitshinweise.

Achtung

H317	Kann allergische Hautreaktionen verursachen.
P261	Einatmen von Aerosol vermeiden.
P280	Schutzhandschuhe/ Schutzkleidung tragen.
P302+P352	BEI BERÜHRUNG MIT DER HAUT: Mit viel Seife und Wasser waschen.
P333+P313	Bei Hautreizung oder -ausschlag: Ärztlichen Rat einholen/ ärztliche Hilfe hinzuziehen.
P362+P364	Kontaminierte Kleidung ausziehen und vor erneutem Tragen waschen

Weitere Informationen können dem Sicherheitsdatenblatt entnommen werden.

12.2. Entsorgungshinweise

Chemikalien und Zubereitungen sind in der Regel Sonderabfälle. Deren Beseitigung unterliegt den nationalen abfallrechtlichen Gesetzen und Verordnungen. Die zuständige Behörde informiert über die Entsorgung von Sonderabfällen.

13. BESTELLINFORMATIONEN

Produktnummer: CHLM0510 Chlamydia pneumoniae IgM ELISA (96 Bestimmungen)

FRANÇAIS

1. INTRODUCTION

Chlamydiae sont des bactéries à développement intracellulaire, immobiles et Gram-négatives, qui forment des inclusions caractéristiques dans le cytoplasme des cellules parasitées. Elles sont facilement visibles dans le photo-microscope. Trois espèces différentes de Chlamydia pathogènes pour les humains sont connues : Chlamydia trachomatis, Chlamydia pneumoniae et Chlamydia psittaci, ainsi qu'une espèce pathogène seulement pour des animaux (C. pecorum). Chlamydia trachomatis est l'agent le plus répandu des maladies transmises sexuellement dans le monde entier (400-500 millions de cas) et le nombre d'infections augmente constamment. Pendant l'accouchement, Chlamydia trachomatis entraîne la conjonctivite ou la pneumonie dans les nouveaux-nés. Les cas non traités de l'infection par Chlamydia peuvent mener aux salpingites chroniques, qui peuvent résulter en grossesse ectopique ou l'infertilité. Dans les mâles, Chlamydia trachomatis est une cause importante de l'urétrite non-gonococcique.

Un problème grave dans les infections de Chlamydia est le cours de la maladie, fréquemment insidieux et asymptomatique, qui peut avoir comme conséquence le déclenchement des maladies chroniques. Dans beaucoup de cas, des infections primaires ne sont pas identifiées et seulement les conséquences provoquées par des agents augmentés et persistants sont diagnostiquées.

Espèce	La maladie	Symptômes (p.ex.)	Modes de transmission
C. trachomatis	Trachome Lymphogranuloma venereum (LGV) Infections urogénitales	Conjonctivite bilatérale avec sensation de corps étranger, les yeux déchirer, et l'évacuation des sécrétions séreuses de l'œil. Lésion génitale; gonflement des ganglions lymphatiques inguinaux et du fémur; formation de bubons purulents. **Chez les hommes:** Inflammation de l'urètre (urétrite), inflammation de la prostate (prostatite) et l'épididyme (épididymite). Stérilité (infertilité). **Chez les femmes:** Inflammation de l'urètre (urétrite), les glandes de Bartholin, inflammation du col (cervicite), de l'utérus muqueuse (endométrite) et les trompes de Fallope (salpingite) et pertes vaginales. Stérilité (infertilité)	Transmission directe ou sexuelle. Transmis de la mère à son bébé perdant l'accouchement.
C. pneumoniae	Maladies respiratoires (p. ex. Bronchite, pneumonie) Discuté: endocardite, maladies coronaires	Fièvre, toux (en regel productive)	D'homme à homme Aérogène, infection gouttelette
C. psittaci	Ornithose (Psittacose)	hépatosplénomégalie; toux improductive, la fièvre et des maux de tête sévères	Inhalation des résidus des oiseaux infectés; contact avec les viscères aviens infectés

L'infection ou la présence d'un agent pathogène peut être identifiée par:

- Microscopie
- PCR
- Sérologie: Détection des antigènes par ELISA
  Détection des anticorps par ELISA

2. INDICATION D'UTILISATION

La trousse Chlamydia pneumoniae IgM ELISA est prévue pour la détection qualitative des anticorps IgM anti-Chlamydia pneumoniae dans le sérum humain ou plasma (citrate, héparine).

3. PRINCIPE DU TEST

La détermination immunoenzymatique qualitative des anticorps spécifiques est basée sur le la technique ELISA (du anglais, Enzyme-Linked Immunosorbent Assay).
Plaque de Microtitrages sont recouvertes d'antigènes spécifiques pour lier les anticorps correspondants de l'échantillon. Après le lavage des puits pour éliminer l'échantillon détaché, le conjugué peroxydase de raifort (HRP) est ajouté. Ce conjugué se lie aux anticorps capturés. Dans une deuxième étape de lavage, le conjugué non lié est éliminé. Le complexe immun formé par le conjugué lié est visualisé par l'addition tétraméthylbenzidine (TMB) qui donne un produit de réaction bleu.
L'intensité de ce produit est proportionnelle à la quantité d'anticorps spécifiques dans l'échantillon. L'acide sulfurique est ajouté pour arrêter la réaction. Cela produit un changement du bleu au jaune. L'absorbance à 450/620 nm est lue en utilisant un Photomètre de Plaque de Microtitrage ELISA.

4. MATERIEL

4.1. Réactifs fournis

- **Plaque de Microtitrage:** 12 barrettes de 8 puits sécables revêtus d'antigène d'Chlamydia pneumoniae; en sachets d'aluminium refermables.
- **Tampon de Dilution d'Échantillon IgM:** 1 flacon contenant 100 mL de tampon phosphaté (10 mM) pour la dilution de l'échantillon; pH 7,2 ± 0,2; anti-humaine IgG (RF-Absorbant); prêt à l'emploi; couleur vert; bouchon blanc; ≤ 0,0015% (v/v) CMIT/ MIT (3:1).
- **Solution d'arrêt:** 1 flacon contenant 15 mL d'acide sulfurique, 0,2 mol/L; prêt à l'emploi; bouchon rouge.
- **Tampon de Lavage (concentré x 20):** 1 flacon contenant 50 mL d'un tampon phosphaté (0,2 M) concentré 20 fois (pH 7,2 ± 0,2) pour laver les puits; bouchon blanc.
- **Conjugué:** 1 flacon contenant 20 mL d'anticorps IgM anti-humaines conjuguées à de la peroxydase du raifort dans le tampon phosphaté (10 mM); prêt à l'emploi; couleur rouge, bouchon noir.
- **Solution de Substrat TMB:** 1 flacon contenant 15 mL de 3,3',5,5'-tétraméthylbenzidine (TMB), < 0,1 %; prêt à l'emploi; bouchon jaune.
- **Contrôle Positif:** 1 flacon contenant 2 mL contrôle; prêt à l'emploi; couleur jaune; bouchon rouge; ≤ 0,02% (v/v) MIT.
- **Contrôle Cut-off:** 1 flacon contenant 3 mL contrôle; prêt à l'emploi; couleur jaune; bouchon vert; ≤ 0,02% (v/v) MIT.
- **Contrôle Négatif:** 1 flacon contenant 2 mL contrôle; prêt à l'emploi; couleur jaune; bouchon bleu; ≤ 0,0015% (v/v) CMIT/ MIT (3:1).

Pour les mentions de danger et les conseils de prudence voir chapitre 12.1.
Pour les substances potentiellement dangereuses s'il vous plaît vérifiez la fiche de données de sécurité.

4.2. Matériel fourni

- 1 couvercle autocollante
- 1 instructions d'utilisation
- 1 présentation de la plaque

4.3. Matériel et équipement requis

- Photomètre de Plaques de Microtitrage ELISA, pour mesurer l'absorbance à 450/620 nm
- Incubateur 37 °C
- Laveur manuel ou automatique pour le lavage des Plaques de Microtitrage
- Pipettes pour utilisation entre 10 et 1000 µL
- Mélangeur Vortex
- Eau distillée
- Tubes jetables

5. STABILITE ET CONSERVATION

Conserver le kit à 2...8 °C. Les réactifs ouverts sont stables jusqu'à la date de péremption indiquée sur l'étiquette lorsqu'il est conservé à 2...8 °C .

6. PREPARATION DES REACTIFS

Il est très important porter tous les réactifs et échantillons à température ambiante (20... 25 °C) et les mélanger avant de commencer le test!

6.1. Plaque de Microtitrage

Les barrettes sécables sont revêtues d'antigène d'Chlamydia pneumoniae. Immédiatement après avoir prélevé les barrettes nécessaires, les barrette restantes doivent être scellés le vide dans de feuille d'aluminium avec le sac de silicium (le déshydratant) fourni et emmagasiner à 2...8 °C.

6.2. Tampon de Lavage (conc. x 20)

Diluer le Tampon de Lavage 1+19; par exemple 10 mL du Tampon de Lavage + 190 mL d'eau distillée. Le Tampon de Lavage diluée est stable pendant 5 jours à la température ambiante (20…25 °C). Cas apparaissent des cristaux dans le concentré, chauffer la solution à 37 °C par exemple dans un bain-marie mélangez bien avant dilution.

6.3. Solution de Substrat TMB

La solution est prête à utiliser et doit être emmagasiné à 2...8 °C, à l'abri de la lumière. La solution doit être incolore ou pourrait avoir une légère couleur bleu clair. Si le substrat devient bleu, il peut avoir été contaminé et ne peut pas être utilisé dans le test.

7. PRELEVEMENT ET PREPARATION DES ECHANTILLONS

Utiliser des échantillons humains de sérum ou plasma (citrate, héparine) pour ce test. Si le test est réalisé dans les 5 jours après le prélèvement, les échantillons doivent être conservés à 2...8 °C; autrement ils doivent être aliquotés et conservés surgelés (-70…-20 °C). Si les échantillons sont conservés congelés, bien mélanger les échantillons décongelés avant le Test. Éviter les cycles répétés de congélation et décongélation.
L'inactivation par la chaleur des échantillons n'est pas recommandée.

7.1. Dilution de l'échantillon

Avant du test, tous les échantillons doivent être dilués 1 + 100 avec Tampon de Dilution d'Échantillon IgM. Diluer 10 µL d'échantillon avec 1 mL de Tampon de Dilution d'Échantillon IgM dans des tubes pour obtenir une dilution 1 + 100 et mélanger soigneusement sur un Vortex.

8. PROCEDE DE TEST

Lire attentivement les instructions d'utilisation **avant de** réaliser le test. La fiabilité des résultats dépend du suivi strict d'utilisation comme décrit. La technique de test suivante a été validée uniquement pour une procédure manuelle. Si le test doit être effectué sur un systèmes automatiques pour ELISA, nous conseillons d'augmenter le nombre d'étapes de lavage de trois à cinq et le volume du Tampon de Lavage de 300 à 350 µL. Faites attention au chapitre 12. Avant de commencer le test, le plan de distribution et d'identification de tous les échantillons et les étalons/contrôles (il est recommandé déterminer en double) doivent être soigneusement établi sur la feuille présentation de la plaque prévue dans le conseil de kit. Sélectionner le nombre de barrettes ou de puits nécessaires et les placer sur le support.

Réaliser toutes les étapes du test dans l'ordre donné et sans délai.

Un embout de pipette propre et jetable doit être utilisé pour distribuer chaque étalon/contrôle et échantillon.

Régler l'incubateur à 37 ± 1 °C.

1. Pipeter 100 µL de étalons/contrôles et d'échantillons dilués dans leurs puits respectifs. Garder le puits A1 pour le blanc substrat.
2. Couvrir les puits avec le couvercle, fourni dans le kit.
3. **Incuber pendant 1 heure ± 5 minutes à 37 ± 1 °C.**
4. A la fin de l'incubation, enlever le couvercle, aspirer le contenu des puits et laver chaque puits trois fois avec 300 µL du Tampon de Lavage. Éviter les débordements des puits de réaction. L'intervalle entre le cycle de lavage et l'aspiration doit être > 5 sec. À la fin, enlever soigneusement le liquide restant en tapotant les barrettes sur du papier absorbant avant la prochaine étape.

   Note: L'étape de lavage est très importante! Un lavage insuffisant peut conduire à une précision faible et de faux résultats !
5. Pipeter 100 µL du conjugué dans tous les puits sauf le puits Blanc A1.
6. **Incuber pendant 30 minutes à température ambiante (20…25°C).** N'exposer pas à la lumière directe du soleil.
7. Répéter l'étape numéro 4.
8. Pipeter 100 µL de la Solution de Substrat TMB dans tous les puits.
9. **Incuber pendant exactement 15 minutes à température ambiante (20…25°C) dans l'obscurité.** Une couleur bleue se produit en raison d'une réaction enzymatique.
10. Pipeter 100 µL de la solution d'arrêt dans tous les puits dans le même ordre et à la même vitesse que pour la Solution de Substrat TMB, ainsi, il y a un changement du bleu au jaune.
11. Mesurer l'absorbance à 450/620 nm dans les 30 minutes après l'addition de la solution d'arrêt.

8.1. Mesure

Réglez le Photomètre de Plaque de Microtitrage ELISA **à zéro** en utilisant **le Blanc substrat.**
Si - pour des raisons techniques - le Photomètre de Plaque de Microtitrage ELISA ne peut pas être ajusté à zéro en utilisant le Blanc substrat, la valeur d'absorbance de cette doit être soustraire la valeur d'absorbance de toutes les autres valeurs d'absorbance mesurées afin d'obtenir des résultats fiables!

Mesurer l'absorbance de tous les puits **à 450 nm** et enregistrer les valeurs d'absorbance pour chaque étalon/contrôle et échantillon dans la présentation de la plaque.

Il est recommandé d'effectuer la mesure **dichromatique** utilisant 620 nm comme longueur d'onde de référence.
Si doubles déterminations ont été effectuées, calculer **les valeurs moyennes d'absorbance.**

9. RESULTATS

9.1. Critères de validation

Pour qu'une série d'analyses soit considérée comme valide, ces instructions d'utilisation doivent être strictement suivies, et les critères suivants doivent être respectés:

- **Blanc Substrat**: Valeur d'absorbance **< 0,100**
- **Contrôle Négatif**: Valeur d'absorbance **< 0,200 et < Cut-off**
- **Contrôle Cut-off**: Valeur d'absorbance **0,150 – 1,300**
- **Contrôle Positif**: Valeur d'absorbance **> Contrôle Cut-off**

Lorsque ces critères ne sont pas remplis, le test n'est pas valide et doit être recommencé.

9.2. Calcul des résultats

La valeur seuil correspond à la moyenne des valeurs d'absorbance du Contrôle Cut-off.

Exemple: 0,44 DO Contrôle Cut-off + 0,42 DO Contrôle Cut-off = 0,86 : 2 = 0,43
Cut-off = 0,43

9.2.1. Résultats en unités [NTU]

$$\frac{\text{Valeur (moyenne) d'absorbance de l' échantillon} \times 10}{\text{Cut-off}} = \text{[unités NovaTec = NTU]}$$

Exemple: $\frac{1{,}591 \times 10}{0{,}43} = 37$ NTU

9.3. Interprétation des résultats

Cut-off	10 NTU	-
Positif	> 11 NTU	Les anticorps dirigés contre l'agent pathogène sont présents. Il ya eu un contact avec l'antigène (pathogène resp. vaccin).
Zone grise	9 – 11 NTU	Les anticorps dirigés contre l'agent pathogène ne pouvaient pas être détectés clairement. Il est recommandé de répéter le test avec un échantillon frais dans 2 à 4 semaines. Si le résultat est encore dans la zone grise l'échantillon est jugé **négatif**.
Negatif	< 9 NTU	L'échantillon ne contient pas d'anticorps contre l'agent pathogène. Un contact préalable avec l'antigène (pathogène resp. vaccin) est peu probable.
Le diagnostic d'une maladie infectieuse ne devrait pas être établi sur la base du résultat d'une seule analyse. Un diagnostic précis devrait prendre en considération l'histoire clinique, la symptomatologie ainsi que les données sérologiques. Les données sérologiques sont de valeur limité dans le cas des patients immunodéprimés et des nouveaux-nés.		

9.3.1. Isotypes d'anticorps et l'Etat de l'infection

Sérologie	Signification
IgM	Caractéristique de la réponse primaire du anticorps Titre élevé d'IgM avec une faible titre d'IgG : → suggère une infection très récente ou aigüe Rare: → persistante IgM
IgG	Caractéristique de la réponse secondaire du anticorps Peut persister pendant plusieurs années Des titres élevés d'IgG à faible titre d'IgM: → peuvent indiquer une infection ancienne
IgA	Ils sont produits au niveau des muqueuses dans tout le corps (⇒ barrière de protection) Habituellement ils sont produits en début d'infection

10. PERFORMANCES DU TEST

Ces résultats s'appuient sur les groupes d'échantillons étudiés; il n'agit pas de caractéristiques techniques garanties.

Pour plus d'informations sur les performances du Test s'il vous plaît contactez NovaTec Immundiagnostica GmbH.

10.1. Précision

Intra-essai	**n**	**moyenne (E)**	**CV (%)**
#1	24	0,470	4,00
#2	24	1,070	5,38
#3	24	1,846	2,96
Inter-essai	**n**	**moyenne (NTU)**	**CV (%)**
#1	12	23,38	3,86
#2	12	33,19	7,44
#3	12	4,18	9,88

10.2. Spécificité diagnostique

La spécificité diagnostique est définie comme la probabilité d'obtenir un résultat négatif en l'absence d'un analyte spécifique. Elle est 99,26% (95% Intervalle de confiance: 95,97% - 99,98%).

10.3. Sensibilité diagnostique

La sensibilité diagnostique est définie comme la probabilité d'obtenir un résultat positif en présence d'un analyte spécifique. Elle est 90,0% (95% Intervalle de confiance: 55,5% - 99,75%).

10.4. Interférences

Des échantillons hémolytiques ou lipémiques ou ictériques n'ont pas montré d'interférences, avec des concentrations jusqu'à 10 mg/mL de hémoglobine, 5 mg/mL de triglycérides et 0,5 mg/mL de bilirubine.

10.5. Réaction croisée

L'étude d'un panel d'échantillons avec des anticorps dirigés contre différents paramètres interférents n'a pas révélé de preuves significatives de résultats faussement positifs dus à des réactions croisées. Des réactions croisées avec Chlamydia trachomatis ne peut pas être exclue avec des sérums contenant des anticorps anti-LPS et MOMP.

11. LIMITES DE LA TECHNIQUE

Une contamination bactérienne ou des cycles de congélation/décongélation répétés de l`échantillon peuvent affecter les valeurs d'absorption.

12. PRECAUTIONS ET AVERTISSEMENTS

- La procédure de test, l'information, les précautions et mises en garde de la notice d'emploi, doivent être suivies de façon stricte. L'utilisation de ces trousses avec des automates ou dispositifs similaires doit être validée. Aucun changement de la conception, composition et procédure de test, ainsi que l'utilisation avec d'autres produits non approuvés par le fabricant, ne sont pas autorisés; seul l'utilisateur est responsable de tels changements. Le fabricant n'est pas responsable des faux résultats et des incidents dus à ces motifs. Le fabricant n'est pas responsable des résultats fournis par analyse visuelle des échantillons des patients.
- Uniquement pour diagnostic in vitro.
- Tous les matériaux d'origine humaine ou animale doivent être considérés et traités comme étant potentiellement infectieux.
- Tous les composants d'origine humaine utilisés pour la fabrication de ces réactifs ont été analysés et ont été trouvés non réactifs en Ag HBs, en anticorps anti-VHI 1 et 2 et en anticorps anti-VHC.
- Ne pas échanger les réactifs ou les Plaque de Microtitrage provenant de différents lots de production.
- Ne pas utiliser de réactifs provenant d'autres fabricants avec les réactifs de cette trousse.
- Ne pas utiliser les réactifs après la date de péremption indiquée sur l'étiquette.
- Utiliser seulement des embouts de pipette, des distributeurs et du matériel de laboratoire propres.
- Ne pas échanger les bouchons des flacons, pour éviter la contamination croisée.
- Fermer soigneusement les flacons après utilisation pour éviter l'évaporation et la contamination microbienne.
- Avant une nouvelle utilisation, vérifier les flacons de conjugué et de étalon/contrôle, déjà utilisés, pour exclure une contamination microbienne.
- Pour éviter la contamination croisée et des résultats faussement élevés, introduire les échantillons de patients et les réactifs exactement au fond des puits sans éclabousser.
- L'ELISA est uniquement conçu pour le personnel qualifié suivant les normes de bonnes pratiques de laboratoire (Good Laboratory Practice, GLP).
- Pour un contrôle de qualité interne plus poussé, chaque laboratoire doit en outre utiliser des échantillons connus.

12.1. Note de sécurité pour les réactifs contenant des substances dangereuses

Les réactifs peuvent contenir du CMIT/MIT (3:1) ou du MIT (voir chapitre 4.1)
Par conséquent, les mentions de danger et les conseils de prudence suivants s'appliquent.

Attenttion

H317	Peut provoquer une allergie cutanée.
P261	Éviter de respirer les aérosols.
P280	Porter des gants de protection/ des vêtements de protection.
P302+P352	EN CAS DE CONTACT AVEC LA PEAU: Laver abondamment savon à l'eau.
P333+P313	En cas d'irritation ou d'éruption cutanée: consulter un médecin.
P362+P364	Enlever les vêtements contaminés et les laver avant réutilisation.

De plus amples informations peuvent être trouvées dans la fiche de données de sécurité.

12.2. Elimination des déchets

Les résidus des produits chimiques et des préparations sont considérés en général comme des déchets dangereux. L'élimination de ce type de déchet est réglementée par des lois et réglementations nationales et régionales. Contacter les autorités compétentes ou les sociétés de gestion des déchets pour obtenir des renseignements sur l'élimination des déchets dangereux.

13. INFORMATION POUR LES COMMANDES

Référence:	CHLM0510	Chlamydia pneumoniae IgM ELISA (96 déterminations)

ITALIANO

1. INTRODUZIONE

Le Chlamydie sono batteri immobili Gram negativi e parassiti intracellulari obbligati. Gli involucri esterni risultano privi della componente peptidoglicanica della parete cellulare. Hanno un ciclo vitale dimorfico: il corpo elementare è la forma infettante e molto piccola. È relativamente inerte dal punto di vista metabolico e sopravvive in ambiente extracellulare. Il corpo elementare viene introdotto in una cellula delle mucose per endocitosi, dove si trasforma in un corpo reticolare, che presenta una chiara organizzazione cellulare procariotica, è metabolicamente attivo e si moltiplica, per scissione binaria. Dopo poche ore questo subisce un processo di condensazione e disitratazione, ritrasformandosi in un corpo elementare che, liberato all'esterno per la morte della cellula parassitata, è pronto ad infettare altre cellule. Si distinguono tre specie patogene per l'uomo Chlamydia trachomatis, Chlamydia pneumoniae e Chlamydia psittaci.

La Chlamydia trachomatis può causare una malattia sessualmente trasmissibile. L'infezione da Clamidia è molto comune tra gli adulti giovani e tra gli adolescenti. Tuttavia molte persone non sanno di essere infette, poiché non hanno nessun sintomo. È la causa più frequente per malattie a trasmissione sessuale (400-500 millioni di casi in tutto il mondo). La Clamidia può essere trasmessa da madre a bambino durante la nascita. L'infezione da Clamidia nei neonati può causare una congiuntivite neonatale e polmonite. Donne infette possono trasmettere c. trachomatis al neonato durante il parto. Nelle donne, una infezione non curata può diffondersi nella zona pelvica e infettare l'utero, le tube di falloppio e le ovaie, portando alla malattia infiammatoria pelvica. Essa può causare danni permanenti agli organi riproduttivi della donna, può portare alla sterilità, dolore pelvico cronico e un rischio maggiore di gravidanza extrauterina. Negli uomini, una Clamidia non curata può avere un effetto deleterio sui testicoli, portando a gonfiori e dolore. Complicazioni collegate possono portare alla sterilità.

Specie	Malattia	Sintomi (p.es.)	Via di trasmissione
C. trachomatis	Trachoma	Congiuntivite bilaterale con sensazione di corpo estraneo, gli occhi lacrimazione, e lo scarico delle secrezioni sierose dall'occhio.	Trasmissione sessuale. Trasmissione da madre al suo bambino durante il parto.
	Linfogranuloma venereo	Lesione genitale; gonfiore dei linfonodi inguinali e femorali; formazione di bubboni suppuranti.	
	Infezioni del tratto urogenitale	Negli uomini: infiammazione dell'uretra (uretrite), infiammazione della prostata (prostatite) e l'epididimo (epididimite). Sterilità (sterilità). Nelle donne: infiammazione dell'uretra (uretrite), le ghiandole di Bartolini, infiammazione della cervice (cervicite), uterina mucose (endometrite) e tube di Falloppio (salpingite) e perdite vaginali. Sterilità (sterilità)	
C. pneumoniae	Malattie respiratorie (ad esempio polmonite, bronchite) in Discussione: endocardite, malattie coronariche	Febbre, tosse (di regola produttiva)	Trasmissione persona-persona, aerogena; inalazione di goccioline infette.
C. psittaci	La psittacosi (ornitosi)	Epatosplenomegalia; tosse non produttiva, febbre e forti mal di testa	L'inalazione di feci di volatili infetti; contatto con visceri aviari infetta.

L'infezione o la presenza di un agente patogeno può essere identificata da:

- Microscopia
- PCR
- Sierologia: p.es.: Rilevamento di antigeni per ELISA
  Rilevamento di anticorpi per ELISA

2. USO PREVISTO

Il Chlamydia pneumoniae IgM ELISA è un kit per la determinazione qualitativa degli anticorpi specifici della classe IgM per Chlamydia pneumoniae nel siero o plasma (citrato, eparina) umano.

3. PRINCIPIO DEL TEST

La determinazione immunoenzimatico qualitativa degli anticorpi specifici si basa sulla tecnica ELISA (d'inglese Enzyme-linked immunosorbent assay).
Piastre di Microtitolazione sono rivestite con antigeni specifici che si legano agli anticorpi presenti nel campione. Dopo aver lavato i pozzetti per rimuovere tutto il materiale campione non legato, il coniugato di perossidasi di rafano (HRP) è aggiunto. Questo coniugato si lega agli anticorpi catturati. In una seconda fase di lavaggio coniugato, non legato è rimosso. Il complesso immunitario formato dal coniugato legato sarà evidenziato aggiungendo tetrametilbenzidina (TMB) substrato che dà una colorazione blu.
L'intensità di questa colorazione è direttamente proporzionale alla quantità di anticorpi specifici presenti nel campione. Acido solforico è aggiunto per bloccare la reazione. Questo produce un cambiamento di colore dal blu al giallo. Assorbanza a 450/620 nm è letto utilizzando un fotometro di Piastre di Microtitolazione ELISA.

4. MATERIALI

4.1. Reagenti forniti

- **Piastre di Microtitolazione:** 12 strisce divisibili in 8 pozzetti, con adesi antigeni della Chlamydia pneumoniae; dentro una busta d'alluminio richiudibile.
- **Tampone di Diluizione del Campione IgM:** 1 flacone contenente 100 mL di tampone fosfato (10 mM) per diluire i campioni; pH 7,2 ± 0,2; anti-umane IgG (mezzo di assorbimento RF); colore verde; pronto all'uso; tappo bianco; ≤ 0,0015% (v/v) CMIT/ MIT (3:1).
- **Soluzione Bloccante:** 1 flacone contenente 15 mL di acido solforico, 0,2 mol/L, pronto all'uso; tappo rosso.
- **Tampone di Lavaggio (20x conc.):** 1 flacone contenente 50 mL di un tampone fosfato concentrato 20 volte (0,2 M) per il lavaggio dei pozzetti; pH 7,2 ± 0,2; tappo bianco.
- **Coniugato:** 1 flacone contenente 20 mL di anticorpi IgM anti-umani, coniugati a perossidasi in tampone fosfato (10 mM); colore rosso; pronto all'uso; tappo nero.
- **Soluzione Substrato TMB:** 1 flacone contenente 15 mL di 3,3`,5,5`-Tetrametilbenzidina (TMB), < 0,1 %; pronto all'uso; tappo giallo.
- **Controllo Positivo:** 1 flacone da 2 mL controllo; colore giallo; tappo rosso; pronto all'uso; ≤ 0,02% (v/v) MIT.
- **Controllo Cut-off:** 1 flacone da 3 mL controllo; colore giallo; tappo verde; pronto all'uso; ≤ 0,02% (v/v) MIT.
- **Controllo Negativo:** 1 flacone da 2 mL controllo; colore giallo; tappo blu; pronto all'uso; ≤ 0,0015% (v/v) CMIT/ MIT (3:1).

Le indicazioni di pericolo e consigli di prudenza vedi capitolo 12.1.

Per le sostanze potenziali pericolose si prega di leggere la scheda di dati di sicurezza.

4.2. Accessori forniti

- 1 pellicola adesiva
- 1 istruzione per l'uso
- 1 schema della piastra

4.3. Materiali e attrezzature necessari

- Fotometro per Piastre di Microtitolazione con filtri da 450/620 nm
- Incubatrice 37°C
- Lavatore, manuale o automatico, di Piastre di Microtitolazione
- Micropipette per l'uso tra 10-1000 µL
- Vortex-Mixer
- Acqua distillata
- Provette monouso

5. MODALITÀ DI CONSERVAZIONE

Conservare il kit a 2...8 °C. I reagenti aperti sono stabili fino alla data di scadenza indicata sull'etichetta quando sono conservati a 2...8 °C.

6. PREPARAZIONE DEI REAGENTI

È molto importante, portare tutti i reagenti e campioni a temperatura ambiente (20…25 °C) e mescolare prima di iniziare il test.

6.1. Piastre di Microtitolazione

Le strisce divisibili sono rivestite con gli antigeni della Chlamydia pneumoniae. Immediatamente dopo la rimozione degli strisce necessarie, le strisce rimanenti devono essere sigillare nuovamente in un foglio di alluminio insieme con il sacchetto di gel di silice conservati a 2...8 °C.

6.2. Tampone di Lavaggio (20x conc.)

Diluire il Tampone di Lavaggio 1+19; per esempio. 10 mL del Tampone di Lavaggio + 190 mL di acqua distillata. Il Tampone di Lavaggio diluito è stabile per 5 giorni a temperatura ambiente (20…25 °C). Se cristalli appaiono nel concentrato, riscaldare la soluzione a 37 °C per esempio in un bagnomaria. Mescolare bene prima della diluizione.

6.3. Soluzione Substrato TMB

La soluzione sta pronta all'uso e deve essere conservata a 2...8 °C, al riparo dalla luce. La soluzione deve essere incolore o potrebbe avere un leggero colore blu chiaro. Se il substrato diventa blu, potrebbe essere stato contaminato e non può essere utilizzato nel test.

7. PRELIEVO E PREPARAZIONE DEI CAMPIONI

Per questo test si prega di usare campioni di siero o plasma (citrato, eparina) umano. Se il test è fatto entro 5 giorni dal prelievo i campioni possono essere conservati tra 2...8 °C. Altrimenti devono essere aliquotati e congelati tra (-70…-20 °C). Se i campioni sono conservati congelati, mescolare bene i campioni scongelati prima del test. Evitare cicli ripetuti di congelamento/scongelamento.
L'inattivazione dei campioni per mezzo del calore non è raccomandata.

7.1. Diluizione dei campioni

Prima del test, diluire i campioni 1+100 con Tampone di Diluizione del Campione IgM. Per esempio, pipettare nelle provette 10 µL di campione + 1 mL di Tampone di Diluizione del Campione IgM e mescolare bene (Vortex).

8. PROCEDIMENTO

Leggere bene le istruzioni per l'uso **prima** di iniziare il teste. L'affidabilità dei risultati dipende dalla stretta aderenza le istruzioni per l'uso di prova come descritto. La seguente procedura è stata validata per l'esecuzione manuale. Per un'esecuzione su strumentazione automatica si consiglia di incrementare il numero di lavaggi de 3 a 5 volte e il volume del Tampone di Lavaggio da 300 a 350 µL per evitare effetti di lavaggio. Prestare attenzione al capitolo 12. Stabilire innanzitutto il piano di distribuzione e identificazione dei campioni e standards/controlli (è raccomandato determinare in duplicato) sullo schema della piastra fornito con il kit. Inserire i pozzetti necessari nel supporto.

Eseguire il test nell'ordine stabilito dalle istruzioni, senza ritardi.

Sul pipettaggio utilizzare puntali nuovi e puliti per ogni campione e standard/controllo.

Regolare l'incubatore a 37 ± 1 °C.

1. Pipettare 100 µL di standard/controllo e di campione diluito nei relativi pozzetti. Usare il pozzetto A1 per il Bianco-substrato.
2. Coprire i pozzetti con la pellicola adesiva, fornita nel kit.
3. **Incubare 1 ora ± 5 min a 37 ± 1°C.**
4. Al termine dell'incubazione, togliere la pellicola e aspirare il liquido dai pozzetti. Successivamente lavare i pozzetti tre volte con 300 µL di Tampone di Lavaggio. Evitare che la soluzione trabocchi dai pozzetti. L'intervallo tra il lavaggio e l'aspirazione deve essere > 5 sec. Dopo il lavaggio picchiettare delicatamente i pozzetti su una carta assorbente per togliere completamente il liquido, prima del passo successivo.

   Attenzione: Il lavaggio è una fase molto importante. Da lavaggio insufficiente risulta una bassa precisione e risultati falsi!
5. Pipettare 100 µL di Coniugato in tutti i pozzetti, escludendo quello con il Bianco-substrato (Blank) A1.
6. **Incubare per 30 min a temperatura ambiente (20...25 °C).** Non esporre a fonti di luce diretta.
7. Ripetere il lavaggio secondo punto 4.
8. Pipettare 100 µL di Soluzione Substrato TMB in tutti i pozzetti.
9. **Incubare precisamente per 15 min a temperatura ambiente (20...25 °C) al buio.** Un colore blu verifica a causa della reazione enzimatica.
10. Pipettare 100 µL di Soluzione Bloccante in tutti i pozzetti, nello stesso ordine della Soluzione Substrato TMB, in tal modo un cambiamento di colore dal blu al giallo si verifica.
11. Misurare l'assorbanza a 450/620 nm entro 30 min dopo l'aggiunta della Soluzione Bloccante.

8.1. Misurazione

Regolare il fotometro per le Piastre di Microtitolazione ELISA **a zero** usando il substrato-Bianco (Blank).

Se, per motivi tecnici, non è possibile regolare il fotometro per le Piastre di Microtitolazione a zero usando il Bianco-substrato, il valore de assorbanza de questo deve essere sottratto dai valori dell'assorbanza da tutti i valori delle altre assorbanze per ottenere risultati affidabili!

Misurare l'assorbanza di tutti i pozzetti a **450 nm** e inserire tutti i valori misurati nello schema della piastra.

È raccomandato fare le misurazioni delle onde **bichrome** (due colori). Utilizzando la lunghezza d'onda de 620 nm come misura di riferimento.

Dove sono state misurate in doppio, calcolare **la media delle assorbanze**.

9. RISULTATI

9.1. Validazione del test

Affinché un test possa essere considerato valido, le presenti Istruzioni per l'uso devono essere rigorosamente seguite e devono essere soddisfatti i seguenti criteri:

- **Substrato Bianco (Blank)**: Valore di assorbanza **< 0,100**
- **Controllo Negativo**: Valore di assorbanza **< 0,200 e < Cut-off**
- **Controllo Cut-off**: Valore di assorbanza **0,150 – 1,300**
- **Controllo Positivo**: Valore di assorbanza **> Cut-off**

Se non sono soddisfatti questi criteri, il test non è valido e deve essere ripetuto.

9.2. Calcolo dei risultati

Il Cut-off è la media dei valori di assorbanza dei Controlli Cut-off.

Esempio: Valore di assorbanza del Controllo Cut-off 0,44 + valore di assorbanza del Controllo Cut-off 0,42 = 0,86/2 = 0,43
Cut-off = 0,43

9.2.1. Risultati in unità [NTU]

$$\frac{\text{Assorbanza media del campione} \times 10}{\text{Cut-off}} = \text{[unità NovaTec = NTU]}$$

Esempio: $\frac{1{,}591 \times 10}{0{,}43} = 37$ NTU

9.3. Interpretazione dei risultati

Cut-off	10 NTU	-
Positivo	> 11 NTU	Anticorpi contro il patogeno sono presenti. C'è stato un contatto con l'antigene (patogeno resp. vaccino).
Zonna grigia	9 – 11 NTU	Anticorpi contro il patogeno non è stato possibile rilevare chiaramente. Si consiglia di ripetere il test con un nuovo campione in 2-4 settimane. Se il risultato è nuovamente nella zona grigia il campione viene giudicato come **negativo**.
Negativo	< 9 NTU	Il campione non contiene anticorpi contro il patogeno. Un precedente contatto con l'antigene (patogeno resp. vaccino) è improbabile.
La diagnosi di una malattia infettiva non deve essere fatta soltanto sulla risultanza di un unico test. È importante considerare anche l'anamnesi ed i sintomi del paziente. I risultati del test da pazienti immunosoppressi e neonati hanno un valore limitato.		

9.3.1. Isotipi degli anticorpi e Stato dell'infezione

Sierologia	Significato
IgM	Caratteristica della risposta primaria dell'anticorpo Alto titolo IgM con basso titolo IgG: →suggerisce una infezione molto recente o acuta Raro: → IgM persistente
IgG	Caratteristica della risposta secondaria dell'anticorpo Può persistere per diversi anni Alto titolo IgG con basso titolo IgM: → può indicare un'infezione passata
IgA	Sono prodotte a livello delle mucose in tutto il corpo (⇒ barriera protettiva) Solitamente sono prodotte all'inizio dell'infezione

10. CARATTERISTICHE DEL TEST

I risultati si riferiscono al gruppo di campioni investigato; questi non sono specifiche garantite.

Per ulteriori informazioni su caratteristiche del test, si prega, di contattare NovaTec Immundiagnostica GmbH.

10.1. Precisione

Intradosaggio	**n**	**Media (E)**	**CV (%)**
#1	24	0,470	4,00
#2	24	1,070	5,38
#3	24	1,846	2,96
Interdosaggio	**n**	**Media (NTU)**	**CV (%)**
#1	12	23,38	3,86
#2	12	33,19	7,44
#3	12	4,18	9,88

10.2. Specificità diagnostica

La specificità diagnostica è la probabilità del test di fornire un risultato negativo in assenza di analita specifici. La specificità diagnostica è 99,26% (95% Intervallo di confidenza: 95,97% - 99,98%).

10.3. Sensibilità diagnostica

La sensibilità diagnostica è la probabilità del test di fornire un risultato positivo alla presenza di analita specifici. La sensibilità diagnostica è 90,0% (95% Intervallo di confidenza: 55,5% - 99,75%).

10.4. Possibili interferenze

Campioni emolitici, lipidici et itterici contenenti fino a 10 mg/mL di emoglobina, 5 mg/mL di trigliceridi e 0,5 mg/mL di bilirubina non hanno presentato fenomeni d'interferenza nel presente test.

10.5. Reattività crociata

L'investigazione di un gruppo di campioni con attività di anticorpi contro parametri potenzialmente interferenti non ha rivelato alcuna evidenza rilevante di risultati falsamente positivi dovuto a reattività crociata.
Non è quindi possibile escludere una reazione crociata con Chlamydia trachomatis con sieri contenenti anticorpi per LPS e MOMP.

11. LIMITAZIONI

Una contaminazione da microorganismi o ripetuti cicli di congelamento-scongelamento possono alterare i valori delle assorbanze.

12. PRECAUZIONI E AVVERTENZE

- La procedura analitica, le informazioni, le precauzioni e le avvertenze contenute nelle istruzioni per l'uso devono essere seguite scrupolosamente. L'uso dei kit con analizzatori e attrezzature similari deve essere previamente convalidato. Qualunque cambiamento nello scopo, nel progetto, nella composizione o struttura e nella procedura analitica, così come qualunque uso dei kit in associazione ad altri prodotti non approvati dal produttore non è autorizzato; l'utilizzatore stesso è responsabile di questi eventuali cambiamenti. Il produttore non è responsabile per falsi risultati e incidenti che possano essere causati da queste ragioni. Il produttore non è responsabile per qualunque risultato ottenuto attraverso esame visivo dei campioni dei pazienti.
- Solo per uso diagnostico in-vitro.
- Tutti i materiali di origine umana o animale devono essere considerati potenzialmente contagiosi e infettivi.
- Tutti gli elementi di origine umana sono stati trovati non reattivi con Anti-HIV-Ab, Anti-HCV-Ab e HBsAg.
- Non scambiare reagenti e Piastre di Microtitolazione di lotti diversi.
- Non utilizzare reagenti d'altri produttori insieme con i reagenti di questo kit.
- Non usare dopo la data di scadenza.
- Utilizzare soltanto punte per pipette, distributori, e articoli da laboratorio puliti.
- Non scambiare i tappi dei flaconi, per evitare contaminazione crociata.
- Richiudere i flaconi immediatamente dopo l'uso per evitare la vaporizzazione e contaminazione.
- Una volta aperti e dopo relativo stoccaggio verificare i reagenti per una loro eventuale contaminazione prima dell'uso.
- Per evitare contaminazioni crociate e risultati erroneamente alti pipettare i campioni e reagenti con molta precisione nei pozzetti senza spruzzi.
- L'ELISA è progettato solo per il personale qualificato che segue le norme di buona pratica di laboratorio (Good Laboratory Practice, GLP).
- Per un ulteriore controllo di qualità interno ogni laboratorio dovrebbe inoltre utilizzare campioni noti.

12.1. Nota di sicurezza per i reagenti contenenti sostanze pericolose

I reagenti possono contenere CMIT/MIT (3:1) o MIT (vedi capitolo 4.1)
Pertanto, si applicano le seguenti indicazioni di pericolo e le consigli di prudenza.

Attenzione	H317	Può provocare una reazione allergica cutanea.
	P261	Evitare di respirare gli aerosol.
	P280	Indossare guanti/ indumenti protettivi.
	P302+P352	IN CASO DI CONTATTO CON LA PELLE: lavare abbondantemente con sapone acqua.
	P333+P313	In caso di irritazione o eruzione della pelle: consultare un medico.
	P362+P364	Togliere tutti gli indumenti contaminati e lavarli prima di indossarli nuovamente.

Ulteriori informazioni sono disponibili nella scheda di dati di sicurezza.

12.2. Smaltimento

In genere tutte le sostanze chimiche sono considerati rifiuti pericolosi. Lo smaltimento è regolato da leggi nazionali. Per ulteriori informazioni contattare l'autorità locale.

13. INFORMAZIONI PER GLI ORDINI

Numero del prodotto:	CHLM0510	Chlamydia pneumoniae IgM ELISA (96 determinazioni)

ESPAÑOL

1. INTRODUCCIÓN

Las Chlamydias son bacterias inmóviles, gram-negativas, de hábitat intracelular. En comparación con otras bacterias son muy pequeñas (la unidad mas pequeña es de 0,2 µm), tienen un ciclo de reproducción especial y se manifiestan en dos formas morfológicas diferentes, las partículas elementales e las partículas iniciales. Las ínfimas partículas elementales garantizan la supervivencia fuera de una célula y representan la forma contagiosa. La reproducción es posible solamente cuando las partículas elementales están fagocitadas de la célula huésped donde comienzan a dividirse como partículas iniciales dentro del fagosoma. La célula huésped muere dos o tres días después de la infección y libera partículas elementales que de nuevo infectan a otras células. Se distinguen tres especies patógenos para el hombre: Chlamydia trachomatis, Chlamydia pneumoniae y Chlamydia psittaci.

La C. trachomatis es la causa más frecuente de enfermedades de transmisión sexual en el mundo (400 a 500 millones casos). Pueden resultar neumonías o conjuntivitis en neonatos por la transmisión de la bacteria de la madre al niño durante el nacimiento. Los casos que no reciben tratamiento pueden conducir a una salpingitis crónica con embarazos ectópicos o infertilidad. En el hombre la C. trachomatis es la causa más frecuente de la infección uretral no gonorreica. A menudo el curso asintomático resulta un problema serio ya que puede derivar en una enfermedad crónica. En muchos casos sólo la secuela de la infección primaria es detectada.

Especies	Enfermedad	Síntomas (p.ej.)	Vía de transmisión
Chlamydia trachomatis	Trachoma	Conjuntivitis bilateral con sensación de cuerpo extraño, lagrimeo de los ojos, y la descarga de secreciones serosas del ojo.	Transmisión sexual. Puede transmitirse de una madre infectada a su bebé durante el parto.
	Lymphogranuloma venereum	Lesión genital; inflamación de los ganglios linfáticos inguinales y femorales; formación de bubones supurantes.	
	Infecciones del tracto urogenital	**En los hombres**: inflamación de la uretra (uretritis), inflamación de la próstata (prostatitis) y el epidídimo (epididimitis). Esterilidad (infertilidad). **En mujeres:** inflamación de la uretra (uretritis), las glándulas de Bartolino, inflamación del cuello uterino (cervicitis), membrana mucosa uterina (endometritis) y las trompas de Falopio (salpingitis) y flujo vaginal. Esterilidad (Infertilidad)	
C. pneumoniae	Enfermedades respiratorias (por ejemplo, neumonía, bronquitis) discutido: endocarditis, enfermedades coronarias del corazón	Fiebre, tos (en regel productiva)	De hombre a hombre Aerogena, infección por gotitas
C. psittaci	Psitacosis u ornitosis	Hepatoesplenomegalia; tos no productiva, fiebre y dolor de cabeza severo	La inhalación de las heces de las aves infectadas; en contacto con las vísceras de las aves infectadas.

La infección o la presencia de un patógeno puede identificarse mediante:

- Microscopia
- PCR
- Serología: Detección de antigénos a través de ELISA
  Detección de anticuerpos a través de ELISA

2. USO PREVISTO

El enzimoinmunoensayo Chlamydia pneumoniae IgM ELISA se utiliza para la determinación cualitativa de anticuerpos IgM específicos contra Chlamydia pneumoniae en suero o plasma (citrato, heparina) humano.

3. PRINCIPIO DEL ENSAYO

La determinación inmunoenzimática cualitativa de anticuerpos específicos se basa en la técnica ELISA (Enzyme-linked Immunosorbent Assay).
Las Placas de Microtitulación están recubiertas con antígenos específicos unen a los anticuerpos de la muestra. Después de lavar los pocillos para eliminar todo el material de muestra no unida, el conjugado de peroxidasa de rábano (HRP) se añade. Este conjugado se une a los anticuerpos capturados. En una segunda etapa de lavado se retira el conjugado no unido. El complejo inmune formado por el conjugado unido se visualiza añadiendo substrato tetrametilbencidina (TMB), que da un producto de reacción azul.
La intensidad de este producto es proporcional a la cantidad de anticuerpos específicos en la muestra. se añade ácido sulfúrico para detener la reacción. Esto produce un cambio de color de azul a amarillo. La extinción a 450/620 nm se mide con un fotómetro de Placa de Microtitulación ELISA.

4. MATERIALES

4.1. Reactivos suministrados

- **Placa de Microtitulación:** 12 tiras de 8 pocillos rompibles, recubiertos con antígenos de Chlamydia pneumoniae, en bolsa de aluminio.
- **Tampón de Dilución de Muestras IgM:** 1 botella de 100 mL de solución de tampón de fosfato (10 mM) para diluir la muestra; pH 7,2 ± 0,2; anti-humana IgG (RF Absorbente); color verde; listo para ser utilizado; tapa blanca; ≤ 0,0015% (v/v) CMIT/ MIT (3:1).
- **Solución de Parada:** 1 botella de 15 mL de ácido sulfúrico, 0,2 mol/L, listo para ser utilizado; tapa roja.
- **Tampón de Lavado (20x conc.):** 1 botella de 50 mL de una solución de tampón de fosfato 20x concentrado (0,2 M) para lavar los pocillos; pH 7,2 ± 0,2; tapa blanca.
- **Conjugado:** 1 botella de 20 mL de conjugado de anticuerpos IgM anti-humano con peroxidasa en tampón de fosfato (10 mM); color rojo; tapa negra; listo para ser utilizado.
- **Solución de Sustrato de TMB:** 1 botella de 15 mL 3,3',5,5'-tetrametilbenzindina (TMB), < 0,1 %; listo para ser utilizado; tapa amarilla.
- **Control Positivo:** 1 botella de 2 mL control; color amarillo; tapa roja; listo para ser utilizado; ≤ 0,02% (v/v) MIT.
- **Control Cut-off:** 1 botella de 3 mL control; color amarillo; tapa verde; listo para ser utilizado; ≤ 0,02% (v/v) MIT.
- **Control Negativo:** 1 botella de 2 mL control; color amarillo; tapa azul; listo para ser utilizado; ≤ 0,0015% (v/v) CMIT/ MIT (3:1).

Para indicaciones de peligro y consejos de prudencia consulte el cap. 12.1.
Para sustancias potencialmente peligrosas por favor revise la ficha de datos de seguridad.

4.2. Accesorios suministrados

- 1 lámina autoadhesiva
- 1 instrucciones de uso
- 1 esquema de la placa

4.3. Materiales e instrumentos necesarios

- Fotómetro de Placa de Microtitulación con filtros de 450/620 nm
- Incubadora 37°C
- Dispositivo de lavado manual o automático de Placas de Microtitulación
- Micropipetas para uso de (10-1000 µL)
- Mezcladora Vortex
- Agua destilada
- Tubos de plástico desechables

5. ESTABILIDAD Y ALMACENAJE

Almacene el kit a 2...8 °C. Los reactivos abiertos son estables hasta la fecha de caducidad indicada en la etiqueta cuando se almacena a 2...8 °C.

6. PREPARACIÓN DE LOS REACTIVOS

Es muy importante llevar todos los reactivos y las muestras a temperatura ambiente (20...25 °C) y mezclarlos antes de ser utilizados!

6.1. Placa de Microtitulación

Las tiras rompibles están recubiertas con antígeno de Chlamydia pneumoniae. Inmediatamente después de la eliminación de las tiras, las tiras restantes deben sellarse de nuevo en el papel de aluminio junto con la bolsita di dióxio de silicio y almacenar a 2...8 °C.

6.2. Tampón de Lavado (20x conc.)

Diluir el Tampón de Lavado 1+19; por ejemplo. 10 mL del Tampón de Lavado + 190 mL de agua destilada. El Tampón de Lavado diluido es estable durante 5 días a temperatura ambiente (20…25 °C). Caso aparecen cristales en el concentrado, calentar la solución a 37 °C, por ejemplo, en un baño María. Mezclar bien antes de la dilución.

6.3. Solución de Sustrato de TMB

La solución está lista para su uso y debe almacenarse a 2...8 °C, protegida de la luz. La solución debe ser incolora o podría tener un color ligeramente azul claro. Si el sustrato se convierte en azul, es posible que haya sido contaminado y no puede ser utilizado en el ensayo.

7. TOMA Y PREPARACIÓN DE LAS MUESTRAS

Usar muestras de suero o plasma (citrato, heparina) humano. Si el ensayo se realiza dentro de 5 días después de la toma de sangre, las muestras pueden ser almacenadas a 2...8 °C, en caso contrario deben ser alicuotadas y almacenadas congeladas (-70...-20 °C). Agitar bien las muestras descongeladas antes de diluirlas. Evitar congelaciones y descongelaciones repetidas.
No se recomienda la inactivación por calor de las muestras.

7.1. Dilución de las muestras

Antes del ensayo, las muestras tienen que estar diluidas en relación 1 + 100 con el Tampón de Dilución de Muestras IgM, p. e. 10 µL de la muestra con 1 mL de Tampón de Dilución de Muestras IgM, mezclar bien con la mezcladora Vortex.

8. PROCEDIMIENTO

Por favor, leer cuidadosamente las instrucciones de uso del ensayo **antes** de realizarlo. Para el buen funcionamiento de la técnica es necesario seguir las instrucciones. El siguiente procedimiento es válido solamente para el método manual. Si se realiza el ensayo en los sistemas automáticos de ELISA es aconsejable elevar el número de lavados de tres hasta cinco veces y el volumen de Tampón de Lavado de 300 µL a 350 µL para excluir efectos de lavado. Preste atención al capítulo 12. Antes de comenzar, especificar exactamente la repartición y posición de las muestras y de los estándares/controles (se recomienda determinar en duplicado) en el esquema de la placa suministrada. Usar la cantidad necesaria de tiras o pocillos e insertarlos en el soporte.

Realizar el ensayo en el orden indicado y sin retraso.

Para cada paso de pipeteado en los estándares/controles y en las muestras, usar siempre puntas de pipeta de un solo uso.

Graduar la incubadora a 37 ± 1°C.

1. Pipetear 100 µL de estándares/controles y muestras en los pocillos respectivos. Dejar el pocillo A1 para el blanco.
2. Recubrir las tiras con los autoadhesivos suministrados.
3. **Incubar 1 h ± 5 min a 37 ± 1°C.**
4. Después de la incubación, retirar el autoadhesivo, aspirar el líquido de la tira y lavarla tres veces con 300 µL del Tampón de Lavado. Evitar el rebosamiento de los pocillos. El intervalo entre lavado y aspiración debe ser > 5 segundos. Para sacar el líquido restante de las tiras, es conveniente sacudirlas sobre papel absorbente.

   Nota: El lavado es muy importante! Un mal lavado insuficiente provoca una baja precisión y resultados falsamente elevados!
5. Pipetar 100 µL de conjugado en cada pocillo con excepción del blanco substrato A1.
6. **Incubar 30 min a la temperatura ambiente (20…25 °C).** Evitar la luz solar directa.
7. Repetir el lavado como en el paso numero 4.
8. Pipetar 100 µL de Solución de Sustrato de TMB en todos los pocillos.
9. **Incubar exactamente 15 min en oscuridad a temperatura ambiente (20...25 °C).** Un color azul se produce en las muestras positivas debido a la reacción enzimática.
10. Pipetear en todos los pocillos 100 µL de la Solución de Parada en el mismo orden y mismo intervalo de tiempo como con el Solución de Sustrato de TMB, por lo tanto un cambio de color de azul a amarillo se produce.
11. Medir la extinción con 450/620 nm en un periodo de 30 min después de añadir la Solución de Parada.

8.1. Medición

Ajustar el fotómetro de Placa de Microtitulación ELISA al cero utilizando el Blanco.

Si por razones técnicas el fotómetro de Placa de Microtitulación de ELISA no se puede ajustar a cero utilizando el Blanco, el valor de la absorbancia de este debe ser sustraído de los demás valores de absorbancia medidos con el fin de obtener resultados fiables!

Medir la **extinción** de todos los pocillos con **450 nm** y anotar los resultados de los estándares/controles y de las muestras en el esquema de la placa.

Es aconsejable realizar la medición **bicromática** a una longitud de onda de referencia de 620 nm.

Si se efectuaron análisis en duplicado o múltiples, hay que calcular **el promedio de los valores de extinción** de los pocillos correspondientes.

9. CÁLCULO DE LOS RESULTADOS

9.1. Criterios de validez del ensayo

Para que un ensayo se considere válido, deben seguirse estrictamente las presentes instrucciones de uso y deben cumplirse los siguientes criterios:

- **Blanco:** valor de la extinción **< 0,100**
- **Control Negativo:** valor de la extinción **< 0,200 y < Cut-off**
- **Control Cut-off:** valor de la extinción **0,150 – 1,300**
- **Control Positivo:** valor de la extinción **> Cut-off**

Si estos criterios no se cumplen, la prueba no es váida y deberá repetirse.

9.2. Cálculo del valor de la medición

El Cut-off se obtiene de los valores de la extinción de los dos controles Cut-off.

Ejemplo: 0,42 OD Control Cut-off + 0,44 OD Control Cut-off = 0,86:2 = 0,43

Cut-off = 0,43

9.2.1. Resultados en unidades [NTU]

$$\frac{\text{Promedio valor de la extinción de la muestra x 10}}{\text{Cut-off}} = \text{[NovaTec-unidades = NTU]}$$

Ejemplo: $\frac{1{,}591 \times 10}{0{,}43} = 37$ NTU

9.3. Interpretación de los resultados

Cut-off	10 NTU	-
Positivo	> 11 NTU	Los anticuerpos contra el patógeno están presentes. Ha producido un contacto con el antígeno (patógeno resp. vacuna).
Zona intermedia	9 – 11 NTU	Los anticuerpos contra el patógeno no se pudieron detectar claramente. Se recomienda repetir la prueba con una muestra fresca en 2 a 4 semanas. Si el resultado es de nuevo en la zona intermedia, la muestra se considera como **negativa**.
Negativo	< 9 NTU	La muestra no contiene anticuerpos contra el patógeno. Un contacto previo con el antígeno (patógeno resp. vacuna) es poco probable.
El diagnóstico de una infección no solamente se debe basar en el resultado del ensayo. Es necesario considerar la anamnésis y la sintomatología del paciente junto al resultado serológico. Estos resultados sólo tienen valor restringido en pacientes inmunodeprimidos o en neonatos.		

9.3.1. Isotipos de anticuerpo y Estado de la Infección

Serología	Significado
IgM	Característica de la respuesta primaria del anticuerpo Alto título de IgM con bajo título de IgG → sugieren una infección muy reciente o aguda Raras: → persistente IgM
IgG	Característica de la respuesta secundaria del anticuerpo Pueden persistir por varios años El alto título de IgG con bajo título de IgM: → pueden indicar una infección pasada
IgA	Producida en el revestimiento mucoso en todo el cuerpo (⇒ Barrera Protectora) Usualmente producida tempranamente en el transcurso de la infección

10. CARACTERÍSTICAS DEL ENSAYO

Los resultados están basados en el grupo de pruebas investigado; no se trata de especificaciones garantizadas.

Para obtener más información sobre las características del ensayo, por favor, entre en contacto NovaTec Immundiagnostica GmbH.

10.1. Precisión

Intra ensayo	**n**	**Promedio (E)**	**CV (%)**
#1	24	0,470	4,00
#2	24	1,070	5,38
#3	24	1,846	2,96
Inter ensayo	**n**	**Promedio (NTU)**	**CV (%)**
#1	12	23,38	3,86
#2	12	33,19	7,44
#3	12	4,18	9,88

10.2. Especificad diagnóstica

La especificidad del ensayo se define como la probabilidad que tiene el ensayo de dar un resultado negativo en ausencia del analítico específico. Es 99,26% (95% Intervalo de confianza: 95,97% - 99,98%).

10.3. Sensibilidad de diagnóstico

La sensibilidad del ensayo se define como la probabilidad que tiene el ensayo de dar un resultado positivo en presencia del analítico específico. Es 90,0% (95% Intervalo de confianza: 55,5% - 99,75%).

10.4. Interferencias

Las muestras lipémicas, ictéricas e hemolíticas no mostraron interferencias con este equipo ELISA hasta una concentración de 5 mg/mL para triglicéridos, de 0,5 mg/mL para bilirrubina y de 10 mg/mL hemoglobina.

10.5. Reactividad cruzada

La investigación del panel de muestras con actividad de los anticuerpos en los parámetros con potencial de reacción cruzada no reveló ninguna evidencia significativa de resultados positivos falsos debido a reacciones cruzadas.
Una reacción cruzada con Chlamydia trachomatis no puede excluirse con sueros que contengan anticuerpos contra LPS y MOMP.

11. LIMITACIONES DEL ENSAYO

Una contaminación de las muestras con bacterias, o una congelación y descongelación repetida pueden producir cambios en los valores de la extinción.

12. PRECAUCIONES Y ADVERTENCIAS

- El procedimiento, la información, las precauciones y los avisos de las instrucciones de uso han de ser seguidas estrictamente. La utilización de equipos con analizadores y equipamiento similar tiene que ser validada. No se autorizan cambios en el diseño, composición y procedimiento, así como cualquier utilización en combinación con otros productos no aprobados por el fabricante; el usuario debe hacerse responsable de estos cambios. El fabricante no responderá ante falsos resultados e incidentes debidos a estas razones. El fabricante no responderá ante cualquier resultado por análisis visual de las muestras de los pacientes.
- Solo para diagnostico in vitro.
- Todos los materiales de origen humano o animal deberán ser considerados y tratados como potencialmente infecciosos.
- Todos los componentes de origen humano han sido examinados y resultaron no reactivos a anticuerpos contra el VIH, VHC y HbsAG.
- No intercambiar reactivos y Placa de Microtitulación de cargas diferentes.
- No usar reactivos de otro fabricante para este ensayo.
- No usar después de la fecha de caducidad.
- Sólo usar recambios de pipetas, dispensadores y materiales de laboratorio limpios.
- No intercambiar las tapas de los diferentes reactivos, para evitar la contaminación cruzada.
- Para evitar la evaporación y una contaminación microbiana, cierre inmediatamente las botellas después de usarlas.
- Después de abrirlas y posterior almacenaje, asegurarse de que no existe contaminación microbiana antes de seguir usándolas.
- Para evitar contaminaciones cruzadas y resultados erróneamente aumentados, Pipetear cuidadosamente las muestras y los reactivos en los pocillos sin salpicar.
- El ELISA sólo está diseñado para personal cualificado siguiendo las normas de buenas prácticas de laboratorio (Good Laboratory Practice,GLP).
- Para un mayor control de calidad interno, cada laboratorio deberá utilizar además muestras conocidas.

12.1. Nota de seguridad para los reactivos que contienen sustancias peligrosas

Los reactivos pueden contener CMIT/MIT (3:1) o MIT (consulte el cap. 4.1)
Por lo tanto, se aplican las indicaciones de peligro y consejos de prudencia.

Atención

H317	Puede provocar una reacción alérgica en la piel.
P261	Evitar respirar el aerosol.
P280	Llevar guantes/ prendas de protección.
P302+P352	EN CASO DE CONTACTO CON LA PIEL: Lavar con abundante jabón agua.
P333+P313	En caso de irritación o erupción cutánea: Consultar a un médico.
P362+P364	Quitar las prendas contaminadas y lavarlas antes de volver a usarlas.

Se puede encontrar más información en la ficha de datos de seguridad.

12.2. Indicaciones para la eliminación de residuos

Por regla general, los productos químicos y las preparaciones son residuos peligrosos. Su eliminación esta sometida a las leyes y los decretos nacionales sobre la eliminación de residuos. Las autoridades informan sobre la eliminación de residuos peligroso.

13. INFORMACIONES PARA PEDIDOS

N° del producto:	CHLM0510	Chlamydia pneumoniae IgM ELISA (96 determinaciones)

PORTUGUÊS

1. INTRODUÇÃO

As Chlamydiae são bactérias sem motilidade, Gram negativo e de crescimento intracelular obrigatório que formam inclusões características no citoplasma das células parasitadas. São facilmente visíveis ao microscópio óptico. São conhecidas três espécies diferentes de Chlamydia patogénicas para os humanos: Chlamydia trachomatis, Chlamydia pneumoniae e Chlamydia psittaci, e uma espécie apenas patogénica para os animais (C. pecorum). A Chlamydia trachomatis é o agente mais prevalente das doenças sexualmente transmissíveis em todo o mundo (400-500 milhões de casos) e o número de infecções está a crescer constantemente. As mulheres grávidas infectadas com C. trachomatis podem transmitir estas bactérias durante o parto, causando conjuntivite ou pneumonia nos recém-nascidos. Casos não tratados de infecção clamidial podem levar a salpingite crónica, resultando possivelmente em gravidez ectópica ou infertilidade. Nos indivíduos do sexo masculino, a C. trachomatis é uma das principais causas de uretrite não gonocócica. Um problema grave das infecções por Chlamydia é o curso insidioso frequentemente assintomático que pode resultar no início de doenças crónicas. Em muitos casos, as infecções primárias não são reconhecidas e apenas as sequelas causadas pela ascensão de agentes persistentes são diagnosticados.

Espécies	**Doença**	**Sintomas (p. ex.)**	**Via de transmissão**
C. trachomatis	Tracoma	conjuntivite bilateral com sensação de corpo estranho, olhos lacrimejando e descarga de secreções serosas do olho.	Sexualmente transmissível. Pode ser transmitida da mãe infectada para o bebê durante o parto.
	Linfogranuloma venéreo (LGV)	Lesão genital; inchaço dos linfonodos inguinais e femorais; formação de ínguas supurantes.	
	Infecções do trato urogenital	**Nos homens:** a inflamação da uretra (uretrite), inflamação da próstata (prostatite) e epidídimo (epididimite). Esterilidade (infertilidade). **Nas mulheres:** a inflamação da uretra (uretrite), glândulas de Bartholin, inflamação do colo do útero (cervicite), inflamação membrana mucosa uterina (endometrite), das trompas de Falópio (salpingite) e corrimento vaginal. Esterilidade (infertilidade)	
C. pneumoniae	As doenças respiratórias (por exemplo, pneumonia, bronquite) discutiveis: endocardite, doenças coronárias	Febre, tosse (em produtivo regel)	Homem para Homem Aerogena, gotícula infectadas
C. psittaci	Ornitose (Psitacose)	Hepatoesplenomegalia; tosse improdutiva, febre e dor de cabeça severa	A inalação de fezes de aves infectadas; entre em contato com as vísceras das aves infectadas

Infecção ou presença de patógeno pode ser identificada por:

- Microscopia
- PCR
- Serologia: Detecção de antigénios por ELISA
  Detecção de anticorpos por ELISA

2. UTILIZAÇÃO PRETENDIDA

O kit Chlamydia pneumoniae IgM ELISA destina-se à determinação qualitativa de anticorpos da classe IgM contra Chlamydia pneumoniae no soro ou plasma (citrato, heparina) humanos.

3. PRINCÍPIO DO ENSAIO

A determinação imunoenzimática qualitativa de anticorpos específicos é baseado na técnica de ELISA (do inglês Enzyme-linked Immunosorbent Assay).
As Placas de Microtitulação são revestidas com antigénios específicos que se ligam os anticorpos correspondentes da amostra. Após lavagem dos poços, para remover todo o material de amostra não ligada, um conjugado de peroxidase de rábano (HRP) é adicionado. Este conjugado se liga aos anticorpos capturados. Num segundo passo de lavagem o conjugado não ligado é removido. O complexo imune formado pelo conjugado ligado é visualizado por adição de substrato de tetrametilbenzidina (TMB), o que dá um produto de reacção azul.
A intensidade deste produto é proporcional à quantidade de anticorpos específicos da amostra. O ácido sulfúrico é adicionado para parar a reacção. Isso produz uma mudança de cor de azul para amarelo.
Absorvância a 450/620 nm é lida utilizando um fotômetro de Placa de Microtitulação ELISA.

4. MATERIAIS

4.1. Reagentes fornecidos

- **Placa de Microtitulação:** 12 tiras de 8 poços, destacáveis e quebráveis, revestidas com antigénio de Chlamydia pneumoniae, em bolsas de folha de alumínio com fecho.
- **Tampão de Diluição de Amostra IgM:** 1 frasco contendo 100 mL de tampão fosfato (10 mM) para diluição da amostra, pH 7,2 ± 0,2; anti-humana IgG (RF Absorbent); de cor verde; pronto a usar; tampa branca; ≤ 0,0015% (v/v) CMIT/ MIT (3:1).
- **Solução de Bloqueio:** 1 frasco contendo 15 mL ácido sulfúrico; 0,2 mol/L; pronto a usar; tampa vermelha.
- **Tampão de Lavagem (conc. 20x):** 1 frasco contendo 50 mL de um tampão fosfato (0,2 M); concentrado 20 vezes (pH 7,2 ± 0,2) para a lavagem dos poços; tampa branca.
- **Conjugado:** 1 frasco contendo 20 mL de anticorpo para IgM anti-humana marcados com peroxidase no tampão fosfato (10 mM); de cor vermelho, pronto a usar; tampa preta.
- **Solução SubstratoTMB:** 1 frasco contendo 15 mL de 3,3',5,5'-tetrametilbenzidina (TMB), < 0,1 %; pronto a usar; tampa amarela.
- **Controle Positivo:** 1 frasco contendo 2 mL controle; de cor amarela; pronto a usar; tampa vermelha; ≤ 0,02% (v/v) MIT.
- **Controle Cut-off:** 1 frasco contendo 3 mL controle; de cor amarela; pronto a usar; tampa verde; ≤ 0,02% (v/v) MIT.
- **Controle Negativo:** 1 frasco contendo 2 mL controle; de cor amarela; pronto a usar; tampa azul; ≤ 0,0015% (v/v) CMIT/ MIT (3:1).

Para advertências de perigo e recomendaçaões de prudência ver capitulo 12.1.
Para substâncias potencialmente perigosas verifique a ficha de dados de segurança.

4.2. Materiais fornecidos

- 1 Película de cobertura
- 1 Instruções de uso
- 1 Layout da placa

4.3. Materiais e Equipamento necessários

- Fotômetro de Placa de Microtitulação ELISA, equipado para a medição da absorvância a 450/620 nm
- Incubadora 37 °C
- Equipamento manual ou automático para a lavagem de Placas de Microtitulação
- Pipetas para dispensar volumes entre 10 e 1000 µL
- Agitador de tubos tipo Vortex
- Água destilada
- Tubos descartáveis

5. ESTABILIDADE E ARMAZENAMENTO

Armazene o kit a 2...8 °C. Os reagentes abertos são estáveis até o prazo de validade impresso no rótulo quando armazenado a 2...8 °C.

6. PREPARAÇÃO DOS REAGENTES

É muito importante deixar todos os reagentes e amostras estabilizar à temperatura ambiente (20...25 °C) misturá-los antes de iniciar o teste!

6.1. Placa de Microtitulação

As tiras quebraveis são revestidas com antigénio de Chlamydia pneumoniae. Imediatamente após a remoção das tiras necessarias, as tiras restantes devem ser lacradas de novo na folha de alumínio juntamente com o saquinho de silicio fornecido e armazenadar a 2...8 °C .

6.2. Tampão de Lavagem (conc. 20x)

Diluir o Tampão de Lavagem 1+19; por exemplo. 10 mL do Tampão de Lavagem + 190 mL de água destilada. O Tampão de Lavagem diluído é estavel durante 5 dias à temperatura ambiente (20…25 °C). Caso apareça cristais no concentrado, aquecer a solução a 37 °C por exemplo, em banho Maria. Misture bem antes da diluição.

6.3. Solução SubstratoTMB

A solução está pronta para uso e tem de ser armazenada à 2...8 °C, protegida da luz. A solução deve ser incolor ou poderia ter uma ligeira coloração azul claro. Se o substrato se transforma em azul, pode ter sido contaminado e não pode ser usado no teste.

7. COLHEITA E PREPARAÇÃO DAS AMOSTRAS

Usar com este ensaio amostras de soro ou plasma (citrato, heparina) humanos. Se o ensaio for realizado dentro de 5 dias após colheita da amostra, o espécime deve ser mantido a 2…8 °C; caso contrário devem ser alicotadas e armazenadas congeladas (-70...-20 °C). Se as amostras forem armazenadas congeladas, misturar bem as amostras descongeladas antes de testar. Evitar congelar e descongelar repetidamente.
Não é recomendada a inactivação por calor das amostras.

7.1. Diluição das amostras

Antes de testar todas as amostras devem ser diluídas 1 + 100 com Tampão de Diluição de Amostra IgM. Dispensar 10 µL de amostra e 1 mL de Tampão de Diluição de Amostra IgM em tubos para obter uma diluição 1 + 100 e misturar meticulosamente com um vortex.

8. PROCEDIMENTO DO ENSAIO

Por favor, ler atentamente as instruções de uso **antes** de realizar o teste. A fiabilidade dos resultados depende da adesão estrita ao as instruções de uso, conforme descritas. O procedimento de ensaio a seguir está validado apenas para o procedimento manual. Se o teste for realizado em sistemas automáticos para teste ELISA é recomendável aumentar os passos de lavagem de três até cinco e o volume da Tampão de Lavagem de 300 µL para 350 µL para evitar efeitos de lavagem. Preste atenção ao capítulo 12. Antes de iniciar o teste, o plano de distribuição e identificação de todas as amostras e calibradores/controles (é recomendado determinar em duplicidade) deve ser cuidadosamente estabelecido no Layout da placa fornecida no kit. Seleccionar o número necessário de tiras ou poços e inserir os mesmos no suporte.

Realizar todas as etapas do teste na ordem indicada e sem atrasos significativos.

Na pipetagem deve ser utilizada uma ponta limpa e descartável para dispensar cada controle e amostra.

Ajustar a incubadora para 37 ± 1 °C.

1. Dispensar 100 µL dos calibradores/controles e das amostras diluídas nos poços respectivos. Deixar o poço A1 vazio para o branco substrato.
2. Cobrir os poços com a película fornecida no kit.
3. **Incubar durante 1 hora ± 5 min a 37 ± 1 °C.**
4. Quando terminar a incubação, remover a película, aspirar o conteúdo dos poços e lavar cada poço três vezes com 300 µL de Tampão de Lavagem. Evitar que os poços de reacção transbordem. O intervalo entre a lavagem e a aspiração deve ser > 5 seg. No final, retirar cuidadosamente o fluido restante batendo delicadamente as tiras sobre papel absorvente, antes da próxima etapa!

   Nota: A lavagem é muito importante! Lavagem insuficiente resulta em baixa precisão e falsos resultados.
5. Dispensar 100 µL de Conjugado em todos os poços, excepto no poço do Branco substrato A1.
6. **Incubar durante 30 min à temperatura ambiente (20...25°C).** Não expor diretamente à luz solar.
7. Repetir a etapa 4.
8. Dispensar 100 µL de Solução SubstratoTMB em todos os poços.
9. **Incubar durante exactamente 15 min à temperatura ambiente (20...25°C) e no escuro.** A cor azul devido a uma reacção enzimática.
10. Dispensar 100 µL de Solução de Bloqueio em todos os poços, pela mesma ordem e com a mesma velocidade a que foi dispensada a Solução SubstratoTMB,desse modo uma mudança de cor de azul para amarelo ocorre.
11. Medir a absorvância a 450/620 nm dentro de 30 min após a adição da Solução de Bloqueio.

8.1. Medição

Ajustar o fotômetro para Placa de Microtitulação ELISA **a zero** usando o **Branco substrato .**

Se - devido à razões técnicas – o fotômetro para Placa de Microtitulação ELISA não puder ser ajustado a zero usando o Branco substrato, valor da absorvância deste deve ser subtraido de todos os outros valores de absorvância medidos de forma a obter resultados fiáveis!

Medir a absorvância de todos os poços a **450 nm** e registar os valores da absorvância para cada calibrador/controle e amostra no Layout da placa.

É recomendado fazer a medição **dicromática** usando como referência um comprimento de onda de 620 nm.

Se determinações duplas foram realizadas, calcular **os valores médios de absorvância**.

9. RESULTADOS

9.1. Critérios de validação do ensaio

Para que um ensaio seja considerado válido, estas Instruções de Uso devem ser rigorosamente seguidas, e os seguintes critérios devem ser cumpridos:

- **Branco substrato**: Valor de Absorvância **< 0,100**
- **Controle Negativo**: Valor de Absorvância **< 0,200 e < Cut-off**
- **Controle Cut-off**: Valor de Absorvância **0,150 – 1,300**
- **Controle Positivo**: Valor de Absorvância **> Cut-off**

Se estes critérios não forem cumpridos, o teste não é válido e deve ser repetido.

9.2. Cálculo dos Resultados

O Cut-off é o valor médio da absorvância das determinações do Controle Cut-off.

Exemplo: Valor da absorvância do Controle Cut-off 0,42 + valor da absorvância do Controle Cut-off 0,44 = 0,86 : 2 = 0,43
Cut-off = 0,43

9.2.1. Resultados em Unidades [NTU]

$$\frac{\text{Valor da absorvância (média) da amostra x 10}}{\text{Cut-off}} = \text{[Unidades NovaTec = NTU]}$$

Exemplo: $\frac{1{,}591 \times 10}{0{,}43} = 37$ NTU

9.3. Interpretação dos Resultados

Cut-off	10 NTU	-
Positivo	> 11 NTU	Os anticorpos contra o agente patogênico estão presente. Houve um contacto com o antigénio (patógeno resp vacina).
Zona cinzenta	9 – 11 NTU	Os anticorpos contra o agente patogênico não puderam ser claramente detectados. Recomenda-se a repetir o teste com uma amostra fresca em 2 a 4 semanas. Se o resultado estiver novamente dentro da zona cinzenta, a amostra é julgada como **negativa**.
Negativo	< 9 NTU	A amostra não contém os anticorpos contra o agente patogênico. Um contato prévio com o antígeno (patógeno resp. vacina) é improvável.
O diagnóstico de uma doença infecciosa não deve ser estabelecido com base num único resultado do teste. Um diagnóstico preciso deve ter em consideração a história clínica, a sintomatologia bem como dados serológicos. Em pacientes imunossuprimidos e recém-nascidos os dados serológicos têm apenas valor restrito.		

9.3.1. Isotipos de anticorpos e Estado da Infecção

Sorologia	Significado
IgM	Característica da resposta primária do anticorpo Alto título de IgM com baixo título de IgG: → sugere uma infecção muito recente ou aguda Raros: → persistente IgM
IgG	Característica da resposta secundária do anticorpo Podem persistir por vários anos Alto título de IgG com baixo título de IgM: → pode indicar uma infecção passada
IgA	Eles são produzidos a nível das mucosas em todo o corpo (⇒ barreira protectora) Geralmente são producidas no inicio infecção

10. CARACTERÍSTICAS DE DESEMPENHO ESPECÍFICAS

Os resultados referem-se aos grupos de amostras investigados; estas não são especificações garantidas.

Para mais informações sobre as características de desempenho específicas, por favor, entre em contato NovaTec Immundiagnostica GmbH.

10.1. Precisão

Intra ensaio	**n**	**Média (E)**	**CV (%)**
#1	24	0,470	4,00
#2	24	1,070	5,38
#3	24	1,846	2,96
Inter ensaio	**n**	**Média (NTU)**	**CV (%)**
#1	12	23,38	3,86
#2	12	33,19	7,44
#3	12	4,18	9,88

10.2. Especificidade Diagnóstica

A especificidade diagnóstica é definida como a probabilidade do ensaio ser negativo na ausência do analito específico.
É de 99,26% (95% Intervalo de confiança: 95,97% - 99,98%).

10.3. Sensibilidade Diagnóstica

A sensibilidade diagnóstica é definida como a probabilidade do ensaio ser positivo na presença do analito específico.
É de 90,0% (95% Intervalo de confiança: 55,5% - 99,75%).

10.4. Interferências

Não são observadas interferências com amostras hemolisadas, lipémicas ou ictéricas até uma concentração de hemoglobina de 10 mg/mL, de triglicerídeos de 5 mg/mL e de bilirrubina de 0,5 mg/mL.

10.5. Reacção cruzada

A investigação do painel de amostras com atividades de anticorpos em parâmetros com potencial de reação cruzada não revelou nenhuma significante evidencia de resultados falso-positivos devido a reações cruzadas.
Não pode ser excluída uma reacção cruzada com Chlamydia trachomatis com soros contendo anticorpos para LPS e MOMP.

11. LIMITAÇÕES DO PROCEDIMENTO

Contaminação bacteriana ou a repetição de ciclos de congelação-descongelação do espécime podem afectar os valores da absorvância.

12. PRECAUÇÕES E AVISOS

- O procedimento do teste, as informações, as precauções e avisos nas instruções para utilização têm de ser rigorosamente seguidas. O uso de kits de teste com analisadores e equipamento similar tem de ser validado. Qualquer alteração no desenho, composição e procedimento do teste bem como qualquer utilização em combinação com outros produtos não aprovados pelo fabricante não estão autorizados; o próprio utilizador é responsável por tais alterações. O fabricante não é legalmente responsável por resultados falsos e incidentes originados por estes motivos. O fabricante não é legalmente responsável por quaisquer resultados obtidos por análise visual das amostras dos pacientes.
- Apenas para uso no diagnóstico in-vitro.
- Todos os materiais de origem humana ou animal devem ser considerados e tratados como potencialmente infectantes.
- Todos os componentes de origem humana usados para a produção destes reagentes foram testados para anticorpos anti-HIV, anticorpos anti-HCV e HBsAg e foram considerados não-reactivos.
- Não trocar e/ou juntar reagentes ou Placa de Microtitulação de lotes de produção diferentes.
- Nenhuns reagentes de outros fabricantes devem ser usados juntamente com reagentes deste kit de teste.
- Não usar reagentes após a data de validade indicada no rótulo.
- Usar apenas pontas de pipeta, dispensadores e material de laboratório limpos.
- Não trocar as tampas dos frascos dos reagentes para evitar contaminação cruzada.
- Fechar firmemente os frascos dos reagentes imediatamente após a utilização para evitar evaporação e contaminação microbiana.
- Após a primeira abertura e armazenamento subsequente verificar se existe contaminação microbiana dos frascos do conjugado e dos calibradores/controles antes de utiliza-los novamente.
- Para evitar contaminação-cruzada e resultados falsamente elevados, pipetar as amostras dos pacientes e dispensar o reagentes precisamente nos poços sem salpicar.
- O ELISA é projetado apenas para pessoal qualificado seguindo os padrões de boas práticas de laboratório (Good Laboratory Practice, GLP).
- Para um controle de qualidade interno adicional cada laboratório deve utilizar amostras conhecidas.

12.1. Nota de segurança para reagentes que contenham substâncias perigosas

Os reagentes podem conter CMIT/MIT (3:1) ou MIT (ver capitulo 4.1)
Portanto, as seguintes advertências de perigo e recomendações de prudência aplicam-se.

Atenção

H317	Pode provocar uma reacção alérgica cutânea.
P261	Evitar respirar os aerossóis.
P280	Usar luvas de protecção/ vestuário de protecção.
P302+P352	SE ENTRAR EM CONTACTO COM A PELE: lavar abundantemente com sabão água.
P333+P313	Em caso de irritação ou erupção cutânea: consulte um médico.
P362+P364	Retirar a roupa contaminada e lavá-la antes de a voltar a usar.

Mais informações podem ser encontradas na ficha de dados de segurança.

12.2. Considerações de Eliminação

Resíduos de químicos e preparações são geralmente considerados como resíduos perigosos. A eliminação deste tipo de resíduos está regulada por leis e normativas nacionais e regionais. Contactar as autoridades locais ou empresas de gestão de resíduos as quais podem aconselhar sobre como eliminar resíduos perigosos.

13. INFORMAÇÃO DE PEDIDO

Prod. No.:	CHLM0510	Chlamydia pneumoniae IgM ELISA (96 Determinações)

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ABBREVIATIONS / ABKÜRZUNGEN / ABRÉVIATIONS / ABBREVIAZIONI / ABREVIACIÓNES / ABREVIATURAS

CMIT	5-chloro-2-methyl-4-isothiazolin-3-one
MIT	2-methyl-2H-isothiazol-3-one

SYMBOLS KEY / SYMBOLSCHLÜSSEL / EXPLICATION DES SYMBOLES / LEGENDA / SIMBOLOS / TABELA DE SIMBOLOS

	Manufactured by / Hergestellt von / Fabriqué par / Prodotto da / Fabricado por / Fabricado por
IVD	In Vitro Diagnostic Medical Device / In Vitro Diagnosticum / Dispositif médical de diagnostic in vitro / Diagnostico in vitro / Producto para diagnóstico In vitro / Dispositivo Médico para Diagnóstico In Vitro
LOT	Lot Number / Chargenbezeichnung / Numéro de lot / Lotto / Número de lote / Número de lote
	Expiration Date / Verfallsdatum / Date de péremption / Scadenza / Fecha de caducidad / Data de Validade
	Storage Temperature / Lagertemperatur / Température de conservation / Temperatura di conservazione / Temperatura de almacenamiento / Temperatura de Armazenamento
CE	CE Mark / CE-Zeichen / Marquage CE / Marchio CE / Marca CE / Marca CE
REF	Catalogue Number / Katalog Nummer / Référence du catalogue / Numero di codice / Número de Catálogo / Número de Catálogo
	Consult Instructions for Use / Arbeitsanleitung beachten / Consulter la notice d'utilisation / Consultare le istruzioni per l'uso / Consulte las Instrucciones de Uso / Consultar as Instruções de Utilização
MTP	Microtiterplate / Mikrotiterplatte / Plaque de Microtitrage / Piastre di Microtitolazione / Placa de Microtitulación / Placa de Microtitulação
CONJ	Conjugate / Konjugat / Conjugué / Coniugato / Conjugado / Conjugado
CONTROL | -	Negative Control / Negativkontrolle / Contrôle Négatif / Controllo Negativo / Control Negativo / Controle Negativo
CONTROL | +	Positive Control / Positivkontrolle / Contrôle Positif / Controllo Positivo / Control Positivo / Controle Positivo
CUT OFF	Cut-off Control / Cut-off Kontrolle / Contrôle Cut-off / Controllo Cut-off / Control Cut-off / Controle Cut-off
DIL | M	IgM Sample Dilution Buffer / IgM-Probenverdünnungspuffer / Tampon de Dilution d'Échantillon IgM / Tampone di Diluizione del Campione IgM / Tampón de Dilución de Muestras IgM / Tampão de Diluição de AmostraIgM
SOLN | STOP	Stop Solution / Stopplösung / Solution d'Arrêt / Soluzione Bloccante / Solución de Parada/ Solução de Bloqueio
SUB | TMB	TMB Substrate Solution / TMB-Substratlösung / Solution de Substrat TMB / Soluzione Substrato TMB / Solución de Sustrato de TMB / Solução SubstratoTMB
WASH | BUF | 20x	Washing Buffer 20x concentrated / Waschpuffer 20x konzentriert / Tampon de Lavage concentré 20 x / Tampone di Lavaggio concentrazione x20 / Tampón de Lavado concentrado x20 / Tampão de Lavagem concentrada 20x
	Contains sufficient for "n" tests / Ausreichend für "n" Tests / Contenu suffisant pour "n" tests / Contenuto sufficiente per "n" saggi / Contenido suficiente para "n" tests / Conteúdo suficiente para "n" testes

SUMMARY OF TEST PROCEDURE / KURZANLEITUNG TESTDURCHFÜHRUNG / RÉSUMÉ DE LA PROCEDURE DE TEST / SCHEMA DELLA PROCEDURA / RESUMEN DE LA TÉCNICA / RESUMO DO PROCEDIMENTO DE TESTE

SCHEME OF THE ASSAY

Chlamydia pneumoniae IgM ELISA

Test Preparation

Prepare reagents and samples as described. Establish the distribution and identification plan for all samples and standards/controls on the plate layout supplied in the kit. Select the required number of microtiter strips or wells and insert them into the holder.

Assay Procedure

	Substrate Blank (A1)	Negative Control	Cut-off Control	Positive Control	Sample (diluted 1+100)
Negative Control	-	100 µL	-	-	-
Cut-off Control	-	-	100 µL	-	-
Positive Control	-	-	-	100 µL	-
Sample (diluted 1+100)	-	-	-	-	100 µL
Cover wells with foil supplied in the kit **Incubate for 1 h at 37 ± 1 °C** Wash each well three times with 300 µL of Washing Buffer					
Conjugate	-	100 µL	100 µL	100 µL	100 µL
Incubate for 30 min at room temperature (20...25 °C) Do not expose to direct sunlight Wash each well three times with 300 µL of Washing Buffer					
TMB Substrate Solution	100 µL	100 µL	100 µL	100 µL	100 µL
Incubate for exactly 15 min at room temperature (20...25 °C) in the dark					
Stop Solution	100 µL	100 µL	100 µL	100 µL	100 µL
Photometric measurement at 450 nm (reference wavelength: 620 nm)					

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