CHROMATIC™ ESBL AGAR BASE

Chromogenic medium for detection of ESBLs and AmpC in Enterobacteriaceae directly from clinical specimen.

TYPICAL FORMULA	(g/l)
Peptone Mix	43.2
Chromogenic Mix	1.0
Agar	15.0
Final pH 7.2 ± 0.2 at 25°C	

DESCRIPTION

CHROMATIC™ ESBL AGAR BASE is a chromogenic medium used with supplements for detection of Extended-spectrum β-lactamase (ESBL) and AmpC producers.

ESBLs are enzymes that hydrolyze most penicillins and cephalosporins, inhibited by β-lactamase inhibitors such as clavulanic acid, sulbactam and tazobactam. AmpC-type enzymes hydrolyze penicillins, cephalosporins and monobactam and are poorly inhibited by the classical ESBL inhibitors, especially clavulanic acid.

PRINCIPLE

Peptones supply amino acids, nitrogen, carbon, minerals, vitamins and other nutrients which support the growth of microorganisms. Chromogenic mix allows the identification of microorganisms on the basis of the colony color and morphology. Agar is the solidifying agent.

The medium must be supplied with one of the following supplements:

- Chromatic™ ESBL Supplement (ref. 81089) contains a selective mix that inhibits the ESBL-non-producing organisms, including AmpC producers
- Chromatic™ ESBL+AmpC Supplement (ref. 81090) contains a selective mix that inhibits the ESBL-non-producing organisms, but allows the growth of AmpC producers.

PREPARATION

Suspend 59.2 g of powder in one liter of deionized or distilled water. Bring to boil and shake until completely dissolved. Sterilize at 121°C for 15 minutes. Cool up to 45-50°C. Aseptically, add rehydrated content of 2 vials (10 ml) of either Chromatic™ ESBL Supplement (ref. 81089) or Chromatic™ ESBL+AmpC Supplement (ref. 81090), as desired*. Pour in Petri dishes.

*Notice that Chromatic™ ESBL Supplement gives more selectivity compared to Chromatic™ ESBL+AmpC Supplement.

TECHNIQUE

Inoculate the plates by streaking directly the specimen onto the agar surface. Incubate aerobically at 37°C for 18-24 hours.

INTERPRETATION OF RESULTS

E. coli produces pink-reddish-mauve colonies.
Klebsiella spp, *Enterobacter* spp, *Serratia* spp produce green-blue colonies.
Proteus spp produces brown colonies.

STORAGE AND TRANSPORT CONDITIONS

The powder is very hygroscopic, store the powder at 10-30°C, in a dry environment, in its original container tightly closed and use it before the expiry date on the label or until sings of deterioration or contamination are evident. Store prepared plates at 2-8°C away from light.

WARNING AND PRECAUTIONS

The product does not contain hazardous substances in concentrations exceeding the limits set by current legislation and therefore is not classified as dangerous. it is nevertheless recommended to consult the safety data sheet for its correct use. The product is designed for *in vitro* diagnostic use only and must be used by properly trained operators.

DISPOSAL OF WASTE

Disposal of waste must be carried out according to the national and local regulations in force.

REFERENCES

- EUCAST guidelines for detection of resistance mechanisms and specific resistances of clinical and/or epidemiological importance. Version 1.0, 2013.
- Podschun R, Ullman U. Klebsiella spp as Nosocomial Pathogens: Epidemiology, Taxonomy, Typing Methods, and Pathogenicity Factors. Clinical Microbiology Reviews. 1998; 11 (4): 589–603.
- Geiss H.K. Comparison of two test kits for rapid identification of Escherichia coli by a beta-glucuronidase assay. European Journal of Clinical Microbiology & Infections Diseases. 1990; 9 (2):151-152.

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PRODUCT SPECIFICATIONS

NAME
CHROMATIC™ ESBL AGAR BASE

PRESENTATION
Dehydrated medium

STORAGE
10-30°C

PACKAGING

Ref.	Content	Packaging
610629	500 g	500 g of powder in plastic bottle
620629	100 g	100 g of powder in plastic bottle

pH OF THE MEDIUM
7.2 ± 0.2

USE
CHROMATIC™ ESBL AGAR BASE is a chromogenic medium used with supplements for detection of Extended-spectrum β-lactamase (ESBL) and AmpC producers

TECHNIQUE
Refer to technical sheet of the product

APPEARANCE OF THE MEDIUM
Powder medium
Appearance: fine, dry, homogeneous, free of extraneous material
Colour: beige
Ready-to-use medium
Appearance: slightly opalescent
Colour: amber

SHELFLIFE
2 years

QUALITY CONTROL
1. Control of general characteristics, label and print
2. Microbiological control
   Supplement: Chromatic™ ESBL Supplement
   Inoculum for productivity: 10-100 CFU/ml
   Inoculum for selectivity: 10^4-10^5 CFU/ml
   Incubation Conditions: 18-24 h at 35 ± 2°C, in aerobiosis

Microorganism		Growth	Colony colour
Escherichia coli (ESBL+, AmpC−)	DSM 22311	Good	Reddish
Klebsiella pneumoniae (ESBL+, AmpC−)	ATCC® 700603	Good	Green-blue
Klebsiella pneumoniae (ESBL−, AmpC+)	ATCC® BAA-1144	Inhibited	---
Escherichia coli (ESBL−, AmpC−)	ATCC® 25922	Inhibited	---

TABLE OF SYMBOLS

LOT Batch code	IVD *In vitro* Diagnostic Medical Device	Manufacturer	Use by	Fragile, handle with care
REF Catalogue number	Temperature limitation	Contains sufficient for <n> tests	Caution, consult instructions for use	Do not reuse

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CE IVD

CHROMATIC™ ESBL AGAR BASE

Terreno cromogenico per la ricerca di ESBL ed AmpC in Enterobacteriaceae direttamente da campioni clinici.

FORMULA TIPICA (g/l)

Miscela di Peptoni	43.2
Miscela Cromogenica	1.0
Agar	15.0
pH Finale 7.2 ± 0.2 a 25°C	

DESCRIZIONE

CHROMATIC™ ESBL AGAR BASE è un terreno cromogenico utilizzato con supplementi per la ricerca di Enterobacteriaceae produttrici di β-lattamasi a spettro esteso ed AmpC.

ESBL sono enzimi che idrolizzano la maggior parte delle penicilline e cefalosporine, inibiti da inibitori delle β-lattamasi come acido clavulanico, sulbactam e tazobactam. AmpC sono enzimi che idrolizzano penicilline, cefalosporine e monobattami e sono debolmente inibiti dai classici inibitori degli ESBL, l'acido clavulanico in particolare.

PRINCIPIO

I peptoni forniscono amino acidi, azoto, carbonio, minerali, vitamine ed altri nutrienti che supportano la crescita dei microrganismi. La miscela cromogenica permette l'identificazione dei microrganismi sulla base del colore e della morfologia delle colonie. L'agar è l'agente solidificante.

Al terreno deve essere aggiunto uno dei seguenti supplementi:

- Chromatic™ ESBL Supplement (ref. 81089) contiene una miscela selettiva che inibisce gli organismi che non producono ESBL, inclusi i produttori di AmpC
- Chromatic™ ESBL+AmpC Supplement (ref. 81090) contiene una miscela selettiva che inibisce gli organismi che non producono ESBL, ma permette la crescita dei produttori di AmpC.

PREPARAZIONE

Sospendere 59.2 g di polvere in un litro di acqua distillata o deionizzata. Portare ad ebollizione ed agitare fino a completo scioglimento. Sterilizzare a 121°C per 15 minuti. Lasciar raffreddare fino a 45-50°C. Asetticamente, ricostituire 2 fiale del supplemento desiderato*, Chromatic™ ESBL Supplement (ref. 81089) o Chromatic™ ESBL+AmpC Supplement (ref. 81090) ed aggiungere il contenuto (10 ml) al terreno in preparazione. Distribuire in piastre Petri.

*Notare che Chromatic™ ESBL Supplement conferisce maggiore selettività rispetto a Chromatic™ ESBL+AmpC Supplement.

TECNICA

Inoculare le piastre strisciando direttamente il campione clinico sulla superficie dell'agar. Incubare a 37°C per 18-24 ore.

INTERPRETAZIONE DEI RISULTATI

E. coli produce colonie rosa-rossastre-malva.
Klebsiella spp, *Enterobacter* spp, *Serratia* spp producono colonie verdi-blue.
Proteus spp produce colonie marroni.

CONDIZIONI DI CONSERVAZIONE E TRASPORTO

Il prodotto è molto igroscopico, conservare la polvere a 10-30°C, in un ambiente asciutto, nel suo contenitore originale chiuso ermeticamente, fino alla data di scadenza indicata in etichetta. Eliminare se vi sono segni evidenti di deterioramento o contaminazione. Conservare le piastre pronte a 2-8°C al riparo dalla luce.

AVVERTENZE E PRECAUZIONI

Il prodotto non contiene sostanze nocive in concentrazioni superiori ai limiti fissati dalla normativa vigente, perciò non è classificato come pericoloso; per il suo impiego si consiglia comunque di consultare la scheda di sicurezza. Il prodotto è destinato esclusivamente per Uso Diagnostico *in vitro* e deve essere utilizzato da parte di personale qualificato.

SMALTIMENTO DEI RIFIUTI

Lo smaltimento del prodotto deve essere effettuato secondo le vigenti regolamentazioni nazionali e locali.

RIFERIMENTI BIBLIOGRAFICI

1. EUCAST guidelines for detection of resistance mechanisms and specific resistances of clinical and/or epidemiological importance. Version 1.0, 2013.
2. Podschun R, Ullman U. Klebsiella spp as Nosocomial Pathogens: Epidemiology, Taxonomy, Typing Methods, and Pathogenicity Factors. Clinical Microbiology Reviews. 1998; 11 (4): 589–603.
3. Geiss H.K. Comparison of two test kits for rapid identification of Escherichia coli by a beta-glucuronidase assay. European Journal of Clinical Microbiology & Infections Diseases. 1990; 9 (2):151-152.

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SPECIFICHE DI PRODOTTO

DENOMINAZIONE
CHROMATIC™ ESBL AGAR BASE

PRESENTAZIONE
Terreno in polvere

CONSERVAZIONE
10-30°C

CONFEZIONAMENTO

Ref.	Contenuto	Confezionamento
610629	500 g	500 g di povere in contenitore di plastica
620629	100 g	100 g di povere in contenitore di plastica

pH DEL TERRENO
7.2 ± 0.2

IMPIEGO
CHROMATIC™ ESBL AGAR BASE è un terreno cromogenico utilizzato con supplementi per la ricerca di Enterobacteriaceae produttrici di β-lattamasi a spettro esteso ed AmpC

TECNICA
Fare riferimento alla scheda tecnica del prodotto

ASPETTO DEL TERRENO
Terreno in polvere
Aspetto: fine, asciutto, omogeneo, privo di materiale estraneo
Colore: beige
Terreno pronto
Aspetto: leggermente opalescente
Colore: ambra

VALIDITÀ DALLA DATA DI PRODUZIONE
2 anni

CONTROLLO DI QUALITÀ
1. Controllo caratteristiche generali, etichettatura e stampa
2. Controllo microbiologico
   Supplemento: Chromatic™ ESBL Supplement
   Dimensione dell'inoculo per produttività: 10-100 UFC/ml
   Dimensione dell'inoculo per selettività : 10^4-10^5 UFC/ml
   Condizioni di incubazione: 18-24 h a 35 ± 2°C in aerobiosi

Microrganismo		Crescita	Colore colonie
Escherichia coli (ESBL+, AmpC−)	DSM 22311	Buona	Rossastro
Klebsiella pneumoniae (ESBL+, AmpC−)	ATCC® 700603	Buona	Verde-blu
Klebsiella pneumoniae (ESBL−, AmpC+)	ATCC® BAA-1144	Inibita	---
Escherichia coli (ESBL−, AmpC−)	ATCC® 25922	Inibita	---

TABELLA DEI SIMBOLI

LOT Numero di lotto	IVD Per uso diagnostico *in vitro*	Fabbricante	Data di scadenza	Fragile, maneggiare con cura
REF Numero di catalogo	Limiti di temperatura	Contenuto sufficiente per <n> test	Attenzione, consultare le istruzioni per l'uso	Non riutilizzare

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508_4

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REF 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
	Sufficient for ... determinations
LOT	Batch code
	Use by
	Temperature limitation
	Keep away from sunlight
	Do not reuse
	Date of manufacture
CE	CE marked according to 98/79/EC
	Consult electronic Instructions For Use
508_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
50 x	50 x concentrate

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	▽ 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.

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.

REFERENCES

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Change Control

Former version: *ORG 508_IFU_EN_QM113135_2018-01-02_3* Reason for revision: *Definition of symbols used and symbols updated*

1. **100 µl** Standards, Kontrollen und verdünnte Patientenproben pipettieren
   → **30 Minuten** bei Raumtemperatur inkubieren
   → Inhalt der Platte verwerfen und 3 mal mit **300 µl** Waschpuffer waschen
2. **100 µl** Enzymkonjugatlösung pipettieren
   → **15 Minuten** bei Raumtemperatur inkubieren
   → Inhalt der Platte verwerfen und 3 mal mit **300 µl** Waschpuffer waschen
3. **100 µl** Substratlösung pipettieren
   → **15 Minuten** bei Raumtemperatur inkubieren
4. **100 µl** Stopplösung zugeben
   → Platte **5 Minuten** stehenlassen
   → Bei **450 nm** messen

ORGENTEC Diagnostika GmbH
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509_4

www.orgentec.com

REF 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
E	Sufficient for ... determinations
LOT	Batch code
	Use by
	Temperature limitation
	Keep away from sunlight
	Do not reuse
	Date of manufacture
CE	CE marked according to 98/79/EC
	Consult electronic Instructions For Use
509_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
50 x	50 x concentrate

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	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.

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 µl** 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 µl** 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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   → **15 Minuten** bei Raumtemperatur inkubieren
4. **100 µl** Stopplösung zugeben
   → Platte **5 Minuten** stehenlassen
   → Bei **450 nm** messen

HBcAb

Competitive Enzyme Immunoassay for the determination of antibodies to Hepatitis B core Antigen in human serum and plasma

- for “in vitro” diagnostic use only -

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e-mail: info@diapro.it

REF. BCAB.CE
96 Tests

HBcAb

A. INTENDED USE

Competitive Enzyme ImmunoAssay (ELISA) for the determination of antibodies to Hepatitis B core Antigen in human plasma and sera.
The kit is intended for the screening of blood units and the follow-up of HBV-infected patients.
For "in vitro" diagnostic use only.

B. INTRODUCTION

The World Health Organization (WHO) defines Hepatitis B as follows:

"Hepatitis B is one of the major diseases of mankind and is a serious global public health problem. Hepatitis means inflammation of the liver, and the most common cause is infection with one of 5 viruses, called hepatitis A,B,C,D, and E. All of these viruses can cause an acute disease with symptoms lasting several weeks including yellowing of the skin and eyes (jaundice); dark urine; extreme fatigue; nausea; vomiting and abdominal pain. It can take several months to a year to feel fit again. Hepatitis B virus can cause chronic infection in which the patient never gets rid of the virus and many years later develops cirrhosis of the liver or liver cancer.

HBV is the most serious type of viral hepatitis and the only type causing chronic hepatitis for which a vaccine is available. Hepatitis B virus is transmitted by contact with blood or body fluids of an infected person in the same way as human immunodeficiency virus (HIV), the virus that causes AIDS. However, HBV is 50 to 100 times more infectious than HIV. The main ways of getting infected with HBV are: (a) perinatal (from mother to baby at the birth); (b) child- to-child transmission; (c) unsafe injections and transfusions; (d) sexual contact.

Worldwide, most infections occur from infected mother to child, from child to child contact in household settings, and from reuse of un-sterilized needles and syringes. In many developing countries, almost all children become infected with the virus. In many industrialized countries (e.g. Western Europe and North America), the pattern of transmission is different. In these countries, mother-to-infant and child-to-child transmission accounted for up to one third of chronic infections before childhood hepatitis B vaccination programmes were implemented. However, the majority of infections in these countries are acquired during young adulthood by sexual activity, and injecting drug use. In addition, hepatitis B virus is the major infectious occupational hazard of health workers, and most health care workers have received hepatitis B vaccine.

Hepatitis B virus is not spread by contaminated food or water, and cannot be spread casually in the workplace. High rates of chronic HBV infection are also found in the southern parts of Eastern and Central Europe. In the Middle East and Indian sub-continent, about 5% are chronically infected. Infection is less common in Western Europe and North America, where less than 1% are chronically infected.

Young children who become infected with HBV are the most likely to develop chronic infection. About 90% of infants infected during the first year of life and 30% to 50% of children infected between 1 to 4 years of age develop chronic infection. The risk of death from HBV-related liver cancer or cirrhosis is approximately 25% for persons who become chronically infected during childhood.

Chronic hepatitis B in some patients is treated with drugs called *interferon or lamivudine*, which can help some patients. Patients with cirrhosis are sometimes given liver transplants, with varying success. It is preferable to prevent this disease with vaccine than to try and cure it.

Hepatitis B vaccine has an outstanding record of safety and effectiveness. Since 1982, over one billion doses of hepatitis B vaccine have been used worldwide. The vaccine is given as a series of three intramuscular doses. Studies have shown that the vaccine is 95% effective in preventing children and adults from developing chronic infection if they have not yet been infected. In many countries where 8% to 15% of children used to become chronically infected with HBV, the rate of chronic infection has been reduced to less than 1% in immunized groups of children. Since 1991, WHO has called for all countries to add hepatitis B vaccine into their national immunization programmes."

Hepatitis B core Antigen (or HBcAg) is the major component of the core particles of HBV.
HBcAg is composed of a single polypeptide of about 17 kD that is released upon disaggregating the core particles; the antigen contains at least one immunological determinant.
Upon primary infection, anti HBcAg antibodies are one of the first markers of HBV hepatitis appearing in the serum of the patient, slightly later than HBsAg, the viral surface antigen.
Anti HBcAg antibodies are produced usually at high titers and their presence is detectable even years after infection. Isolated HBcAb, in absence of other HBV markers, have been observed in infected blood units, suggesting the use of this test for screening HBV, in addition of HBsAg.
The determination of HBcAb has become important for the classification of the viral agent, together with the detection of the other markers of HBV infection, in sera and plasma.

C. PRINCIPLE OF THE TEST

The assay is based on the principle of competition where the antibodies in the sample compete with a monoclonal antibody for a fixed amount of antigen on the solid phase.
A purified recombinant HBcAg is coated to the microwells.
The patient's serum/plasma is added to the microwell together with an additive able to block interferences present in the sample.
In the second incubation after washing, a monoclonal antibody, conjugated with Horseradish Peroxidase (HRP) and specific for HBcAg is added and binds to the free rec-HBcAg coated on the plastic.
After incubation, microwells are washed to remove any unbound conjugate and then the chromogen/substrate is added. In the presence of peroxidase enzyme the colorless substrate is hydrolyzed to a colored end-product.
The color intensity is inversely proportional to the amount of antibodies to HBcAg present in the sample.

D. COMPONENTS

Each kit contains sufficient reagents to perform 96 tests.

1. Microplate MICROPLATE
8x12 microwell strips coated with recombinant HBcAg 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 2..8°C.

2. Negative Control CONTROL -
1x1.0ml/vial. Ready to use. Contains 5% bovine serum albumin, 10 mM phosphate buffer pH 7.4 +/-0.1, 0.09% sodium azide and 0.045% ProClin 300 as preservatives. The negative control is pale yellow color coded.

3. Positive Control CONTROL +
1x1.0ml/vial. Ready to use. Contains 5% bovine serum albumin, anti HBcAg antibodies at a concentration of about 10 PEI U/ml, (calibrated on PEI HBc Reference Material 82), 10 mM phosphate buffer pH 7.4 +/-0.1, 0.09% sodium azide and 0.045% ProClin 300 as preservatives. The positive control is green color coded.

4. Calibrator CAL ….
n° 1 vial. Lyophilised. To be dissolved with EIA grade water as reported in the label. Contains fetal bovine serum, human antibodies to HBcAg at a concentration of 2 PEI U/ml +/-10% (calibrated on PEI HBc Reference Material 82) and 0.045% ProClin 300 as preservative.
Note: The volume necessary to dissolve the content of the vial may vary from lot to lot. Please use the right volume reported on the label .

5. 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, 0.05% Tween 20 and 0.045% ProClin 300.

6. Enzyme Conjugate CONJ
1x16ml/vial. Ready-to-use solution. Contains 5% bovine serum albumine, 10 mM tris buffer pH 6.8 +/-0.1, Horseradish peroxidase conjugated mouse monoclonal antibody to HBcAg in presence of 0.3 mg/ml gentamicine sulphate and 0.045% ProClin 300. as preservatives. The component is red colour coded.

7. Chromogen/Substrate SUBS TMB
1x16ml/vial. Contains a 50 mM citrate-phosphate buffered solution at pH 3.6 +/-0.1, 0.03% tetra-methyl-benzidine (TMB), 0.02% hydrogen peroxide (H_2O_2) and 4% dimethylsulphoxide
Note: To be stored protected from light as sensitive to strong illumination.

8. Specimen Diluent DILSPE
4x3ml/vial. 10 mM tris buffered solution pH 8.0 +/-0.1 containing 0.045% ProClin 300 for the pre-treatment of samples and controls in the plate, blocking interference. The component is blue colour coded.

Note: Use all the content of one vial before opening a second one. The reagent is sensitive to oxidation.

9. Sulphuric Acid H_2SO_4 O.3 M
1x15ml/vial. Contains 0.3 M H_2SO_4 solution.
Attention: Irritant (H315; H319; P280; P302+P352; P332+P313; P305+P351+P338; P337+P313; P362+P363)

10. Plate sealing foil n° 2

11. Instruction manual n° 1

E. MATERIALS REQUIRED BUT NOT PROVIDED
1. Calibrated Micropipettes (100ul and 50ul) and 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.

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. When the kit is used for the screening of blood units and blood components, it has to be used in a laboratory certified and qualified by the national authority in that field (Ministry of Health or similar entity) to carry out this type of analysis.
3. 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.
4. All the personnel involved in sample handling should be vaccinated for HBV and HAV, for which vaccines are available, safe and effective.
5. 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 (TMB) from strong light and avoid vibration of the bench surface where the test is undertaken.
6. Upon receipt, store the kit at 2-8°C into a temperature controlled refrigerator or cold room.
7. 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.
8. Check that the reagents are clear and do not contain visible heavy particles or aggregates. If not, advise the laboratory supervisor to initiate the necessary procedures.
9. Avoid cross-contamination between serum/plasma samples by using disposable tips and changing them after each sample. Do not reuse disposable tips.
10. Avoid cross-contamination between kit reagents by using disposable tips and changing them between the use of each one. Do not reuse disposable tips.
11. Do not use the kit after the expiration date stated on external (primary container) and internal (vials) labels.
12. Treat all specimens as potentially infective. All human serum specimens should be handled at Biosafety Level 2, as recommended by the Center for Disease Control, Atlanta, U.S. in compliance with what reported in the Institutes of Health's publication: "Biosafety in Microbiological and Biomedical Laboratories", ed. 1984.
13. The use of disposable plastic-ware is recommended in the preparation of the washing solution or in transferring components into other containers of automated workstations, in order to avoid contamination.
14. Waste produced during the use of the kit has 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. 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..
15. Accidental spills 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.

16. The Sulphuric Acid is an irritant. In case of spills, wash the surface with plenty of water.
17. Other waste materials generated from the use of the kit (example: tips used for samples and controls, used microplates) should be handled as potentially infective and disposed according to national directives and laws concerning laboratory wastes.

G. SPECIMEN: PREPARATION AND RECOMMANDATIONS

1. Blood is drawn aseptically by venepuncture and plasma or serum is prepared using standard techniques of preparation of samples for clinical laboratory analysis. No influence has been observed in the preparation of the sample with citrate, EDTA and heparin.
2. Avoid any addition of preservatives to samples; especially sodium azide as this chemical would affect the enzymatic activity of the conjugate.
3. Samples have to be clearly identified with codes or names in order to avoid misinterpretation of results. When the kit is used for the screening of blood units, bar code labeling and electronic reading is strongly recommended.
4. Haemolysed (red) and visibly hyperlipemic ("milky") samples have to be discarded as they could generate false results. Samples containing residues of fibrin or heavy particles or microbial filaments and bodies should be discarded as they could give rise to false results.
5. Sera and plasma can be stored at +2°…+8°C in primary collection tubes for up to five days after collection.
   Do not freeze primary tubes of collection. For longer storage periods, sera and plasma samples, carefully removed from the primary collection tube, can be stored frozen at –20°C for at least 12 months. Any frozen samples should not be frozen/thawed more than once as this may generate particles that could affect the test result.
6. If particles are present, centrifuge at 2.000 rpm for 20 min or filter using 0.2-0.8u filters to clean up the sample for testing.

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 6 months.

1. 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 storage.
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. After first opening, remaining strips are stable until the humidity indicator inside the desiccant bag turns from yellow to green.

2. Negative Control:
Ready to use. Mix well on vortex before use.

3. Positive Control:
Ready to use. Mix well on vortex before use.

4. Calibrator:
Add the volume of ELISA grade water, reported on the label, to the lyophilised powder; let fully dissolve and then gently mix on vortex.
Note: *The dissolved calibrator is not stable. Store it frozen in aliquots at –20°C.*

5. Wash buffer concentrate:
The whole content of the concentrated solution has to be diluted 20x with bidistilled 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.
Note: *Once diluted, the wash solution is stable for 1 week at +2..8° C.*

6. Enzyme conjugate:
Ready to use. Mix well on vortex before use.
Avoid contamination of the liquid with oxidizing chemicals, dust or microbes. If this component has to be transferred, use only plastic, and if possible, sterile disposable containers.

7. 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.

8. Specimen Diluent
Ready to use solution. Mix gently on vortex before use. Use all the content of one vial before opening a second one. The reagent is sensitive to oxidation.

9. 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.

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 "Assay Procedure". 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 work station, 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 "Validation of Test" and "Assay Performances". 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 for blood screening and when the number of samples to be tested exceed 20-30 units per run.
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.

L. PRE ASSAY CONTROLS AND OPERATIONS

1. Check the expiration date of the kit printed on the external label (primary container). Do not use if expired.
2. Check that the liquid components are not contaminated by visible particles or aggregates. Check that the Chromogen (TMB) is colourless or pale blue by aspirating a small volume of it with a sterile plastic pipette. Check that no breakage occurred in transportation and no spillage of liquid is present inside the box (primary container). Check that the aluminium pouch, containing the microplate, is not punctured or damaged.
3. Dilute all the content of the 20x concentrated Wash Solution as described above.
4. Dissolve the Calibrator as described above and gently mix.
5. Allow all the other components to reach room temperature (about 1 hr) and then mix gently on vortex all liquid reagents.
6. 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 as reported in the specific section.
7. Check that the ELISA reader is turned on or ensure it will be turned on at least 20 minutes before reading.
8. If using an automated work station, turn on, check settings and be sure to use the right assay protocol.
9. Check that the micropipettes are set to the required volume.
10. Check that all the other equipment is available and ready to use.
11. In case of problems, do not proceed further with the test and advise the supervisor.

M. ASSAY PROCEDURE

The assay has to be performed according to the procedure given below, taking care to maintain the same incubation time for all the samples being tested.

1. Place the required number of strips in the plastic holder and carefully identify the wells for controls, calibrator and samples.
2. Leave the A1 well empty for blanking purposes.
3. Dispense 50 ul Specimen Diluent into all the control and sample wells.
4. Pipette 50 µl of the Negative Control in triplicate, 50 ul of the Calibrator in duplicate and then 50 ul of the Positive Control in single. Then dispense 50 ul of each of the samples.
5. Incubate the microplate for **60 min at +37°C.**

Important note: *Strips have to be sealed with the adhesive sealing foil, only when the test is performed manually. Do not cover strips when using ELISA automatic instruments.*

6. When the first incubation is finished, wash the microwells as previously described (section I.3)
7. Pipette 100 µl Enzyme Conjugate in all the wells, except A1; incubate the microplate for **60 min at +37°C.**

Important note: *Be careful not to touch the plastic inner surface of the well with the tip filled with the Enzyme Conjugate. Contamination might occur.*

8. When the second incubation is finished, wash the microwells as previously described (section I.3)
9. Pipette 100 µl Chromogen/Substrate into all the wells, A1 included.

Important note: *Do not expose to strong direct light. as a high background might be generated.*

10. Incubate the microplate protected from light at **room temperature (18-24°C) for 20 minutes**. Wells dispensed with negative control and negative samples will turn from clear to blue (competitive method).
11. Pipette 100 µl Sulphuric Acid into all the wells using the same pipetting sequence as in step 9 to stop the enzymatic reaction. Addition of the stop solution will turn the negative control and negative samples from blue to yellow.
12. Measure the colour 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.

Important notes:

1. *Ensure that no finger prints are present on the bottom of the microwell before reading. Finger prints could generate false positive results on reading.*
2. *Reading has should ideally be performed immediately after the addition of the Stop Solution but definitely no longer than 20 minutes afterwards. Some self oxidation of the chromogen can occur leading to a higher background.*
3. *The Calibrator (CAL) does not affect the cut-off calculation and therefore the test results calculation. The Calibrator*

may be used only when a laboratory internal quality control is required by the management.

N. ASSAY SCHEME

Specimen Diluent	50 ul
Controls&calibrator and samples	50 ul
1st incubation	**60 min**
Temperature	+37°C
Wash	n° 5 cycles with 20'' of soaking OR n° 6 cycles without soaking
Enzyme Conjugate	100 ul
2nd incubation	**60 min**
Temperature	+37°C
Wash	n° 5 cycles with 20'' of soaking OR n° 6 cycles without soaking
TMB/H2O2 mix	100 ul
3rd incubation	**20 min**
Temperature	r.t.
Sulphuric Acid	100 ul
Reading OD	450nm / 620-630nm

An example of dispensation scheme is reported below:

Microplate

	1	2	3	4	5	6	7	8	9	10	11	12
A	BLK	S2										
B	NC	S3										
C	NC	S4										
D	NC	S5										
E	CAL	S6										
F	CAL	S7										
G	PC	S8										
H	S1	S9										

Legenda: BLK = Blank NC = Negative Control
CAL = Calibrator PC = Positive Control S = Sample

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 Co/S values have been matched in the analysis.
Ensure that the following parameters are met:

Parameter	Requirements
Blank well	< 0.050 OD450nm value
Negative Control (NC)	> 1.000 OD450nm after blanking coefficient of variation < 20%
Calibrator (about 2 PEI U/ml)	Co/S > 1
Positive Control	< 0.200 OD450nm

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 > 0.050 OD450nm	that the Chromogen/Substrate solution has not become contaminated during the assay
Negative Control (NC) < 1.000 OD450nm after blanking coefficient of variation > 20%	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 control instead of negative control; 4. that no contamination of the negative control or of the wells where the control was dispensed has occurred due to positive samples, to spills or to 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.
Calibrator Co/S < 1	1. that the procedure has been correctly performed; 2. that no mistake has occurred during its distribution (ex.: dispensation of negative control 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.
Positive Control > 0.200 OD450nm	1. that the procedure has been correctly performed; 2. that no mistake has occurred during the distribution of the control (dispensation of negative control instead of positive control). 3. that the washing procedure and the washer settings are as validated in the pre qualification study; 4. that no external contamination of the positive control 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, point 12.

P. RESULTS

The results are calculated by means of a cut-off value determined with the following formula:

Cut-Off = (NC + PC) / 5

***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

Results are interpreted as ratio between the cut-off value and the sample OD450nm/620-630nm or Co/S.

Results are interpreted according to the following table:

Co/S	Interpretation
< 0.9	Negative
0.9 - 1.1	Equivocal
> 1.1	Positive

A negative result indicates that the patient has not been infected by HBV.
Any patient showing an equivocal result should be re-tested on a second sample taken 1-2 weeks after the initial sample.
The blood unit should not be transfused.
A positive result is indicative of HBV infection and therefore the patient should be treated accordingly or the blood unit should be discarded.

Important notes:

1. *Interpretation of results should be done under the supervision of the laboratory supervisor to reduce the risk of judgement errors and misinterpretations.*
2. *When test results are transmitted from the laboratory to another facility, attention must be paid to avoid erroneous data transfer.*
3. *Diagnosis of viral hepatitis infection has to be taken by and released to the patient by a suitably qualified medical doctor.*

An example of calculation is reported below (data obtained proceeding as the the reading step described in the section M, point 12):

The following data must not be used instead or real figures obtained by the user.

Negative Control: 2.000 – 2.200 – 2.000 OD450nm
Mean Value: 2.100 OD450nm
Higher than 1.000 – Accepted

Positive Control: 0.100 OD450nm
Lower than 0.200 – Accepted

Cut-Off = (2.100 + 0.100) / 5 = 0.440

Calibrator: 0.400-0.360 OD450nm
Mean value: 0.380 OD450nm
Co/S>1 – Accepted

Sample 1: 0.028 OD450nm
Sample 2: 1.890 OD450nm
Sample 1 Co/S > 1.1 positive
Sample 2 Co/S < 0.9 negative

R. PERFORMANCES

Evaluation of Performances has been conducted in accordance to what reported in the Common Technical Specifications or CTS (art. 5, Chapter 3 of IVD Directive 98/79/EC).

1. LIMIT OF DETECTION:

The sensitivity of the assay has been calculated by means of the reference preparation for HBcAb supplied by Paul Erlich Institute (PEI HBc Reference Material 82). The assay shows a sensitivity of about 1.25 PEI U/ml.
The table below reports the Co/S values shown by the PEI standard diluted as suggested by the manufacturer to prepare a limiting dilution curve in Fetal Calf Serum (FCS).

PEI U/ml	Lot 1001	Lot 0702	Lot 0702/2	Lot 1202
5	**22.6**	**18.0**	**19.0**	**17.7**
2.5	**8.0**	**5.5**	**5.4**	**5.0**
1.25	**1.1**	**1.3**	**1.0**	**1.0**
0.625	0.4	0.4	0.4	0.4

In addition Accurun 1 – series 3000 – supplied by Boston Biomedica Inc., USA, was tested to determine its Co/S value. Results are reported in the table below:

Accurun 1 – series 3000

Value	Lot 1001	Lot 0702	Lot 1202
Co/S	2.9	2.3	2.2

2. DIAGNOSTIC SPECIFICITY AND SENSITIVITY

The Performance Evaluation of the device was carried out in a trial conducted on more than total 6000 samples.

2.1 Diagnostic Specificity

It is defined as the probability of the assay of scoring negative in the absence of specific analyte. In addition to the first study, where a total of 5179 unselected donors, including 1st time donors, 206 samples from hospitalized patients and 164 potentially interfering specimen were examined, the diagnostic specificity was recently assessed by testing a total of 1498 negative samples on seven different lots. A value of specificity of 100% was observed. In addition to the above population, 189 potentially interfering samples (other liver diseases,pregnant women, hemolized, lipemic, RF positives) have been tested and found negative, confirming a 100% of specificity of the device.
Finally, both human plasma, derived with different standard techniques of preparation (citrate, EDTA and heparin), and human sera have been used to determine the specificity.
No false reactivity due to the method of specimen preparation has been observed.

2.2 Diagnostic Sensitivity

It defined as the probability of the assay of scoring positive in the presence of specific analyte.
In addition to the first Performance Evaluation Study, in order to further evaluate the diagnostic sensitivity of the device, a total of 262 positive samples were recently evaluated. The respective results, collected from seven different lots of the device show a diagnostic sensitivity of 100%.

3. PRECISION

The mean values obtained from a study conducted on three lots and on two samples of different anti-HBcAg reactivity, examined in 16 replicates in three separate runs is reported below:

BCAB.CE: lot # 1202

Negative Control (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	1.943	1.939	1.924	1.935
Std.Deviation	0.081	0.078	0.103	0.087
CV %	4.2	4.0	5.3	4.5

Calibrator (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.143	0.147	0.148	0.146
Std.Deviation	0.014	0.017	0.018	0.016
CV %	9.8	11.4	12.1	11.1
Co/S	2.8	2.7	2.6	2.7

BCAB.CE: lot # 0702

Negative Control (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	2.163	2.110	2.106	2.126
Std.Deviation	0.105	0.088	0.139	0.111
CV %	4.9	4.2	6.6	5.2

Calibrator (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.182	0.193	0.195	0.190
Std.Deviation	0.018	0.023	0.019	0.020
CV %	10.0	12.0	9.9	10.6
Co/S	2.5	2.2	2.3	2.3

BCAB.CE: lot # 0702/2

Negative Control (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	2.278	2.098	2.130	2.169
Std.Deviation	0.135	0.126	0.159	0.140
CV %	5.9	6.0	7.5	6.5

Calibrator (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.193	0.190	0.199	0.134
Std.Deviation	0.023	0.023	0.027	0.025
CV %	12.1	12.3	13.5	12.6
Co/S	2.4	2.2	2.2	2.3

The variability shown in the tables did not result in sample misclassification.

Important note:
The performance data have been obtained proceeding as the reading step described in the section M, point 12.

S. LIMITATIONS OF THE PROCEDURE

Bacterial contamination or heat inactivation of the specimen may affect the absorbance values of the samples with consequent alteration of the level of the analyte. This test is suitable only for testing single samples and not pooled ones.
Diagnosis of an infectious disease should not be established on the basis of a single test result. The patient's clinical history, symptomatology, as well as other diagnostic data should be considered.

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All the IVD Products manufactured by the company are under the control of a certified Quality Management System approved by an EC Notified Body. 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:
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CE
0318

HBsAb

Enzyme Immunoassay for qualitative/quantitative determination of antibodies to Hepatitis B surface Antigen in human serum and plasma

- for “in vitro” diagnostic use only -

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REF SAB.CE
96 Tests

HBs Ab

A. INTENDED USE

Enzyme ImmunoAssay (ELISA) for both the quantitative and qualitative determination of antibodies to the Surface Antigen of Hepatitis B Virus in human plasma and sera.
For "in vitro" diagnostic use only.

B. INTRODUCTION

The World Health Organization (WHO) defines Hepatitis B Virus infection as follows:

"Hepatitis B is one of the major diseases of mankind and is a serious global public health problem. Hepatitis means inflammation of the liver, and the most common cause is infection with one of 5 viruses, called hepatitis A,B,C,D, and E. All of these viruses can cause an acute disease with symptoms lasting several weeks including yellowing of the skin and eyes (jaundice); dark urine; extreme fatigue; nausea; vomiting and abdominal pain. It can take several months to a year to feel fit again. Hepatitis B virus can cause chronic infection in which the patient never gets rid of the virus and many years later develops cirrhosis of the liver or liver cancer.

HBV is the most serious type of viral hepatitis and the only type causing chronic hepatitis for which a vaccine is available. Hepatitis B virus is transmitted by contact with blood or body fluids of an infected person in the same way as human immunodeficiency virus (HIV), the virus that causes AIDS. However, HBV is 50 to 100 times more infectious than HIV. The main ways of getting infected with HBV are: (a) perinatal (from mother to baby at the birth); (b) child- to-child transmission; (c) unsafe injections and transfusions; (d) sexual contact.

Worldwide, most infections occur from infected mother to child, from child to child contact in household settings, and from reuse of un-sterilized needles and syringes. In many developing countries, almost all children become infected with the virus. In many industrialized countries (e.g. Western Europe and North America), the pattern of transmission is different. In these countries, mother-to-infant and child-to-child transmission accounted for up to one third of chronic infections before childhood hepatitis B vaccination programmes were implemented. However, the majority of infections in these countries are acquired during young adulthood by sexual activity, and injecting drug use. In addition, hepatitis B virus is the major infectious occupational hazard of health workers, and most health care workers have received hepatitis B vaccine.

Hepatitis B virus is not spread by contaminated food or water, and cannot be spread casually in the workplace. High rates of chronic HBV infection are also found in the southern parts of Eastern and Central Europe. In the Middle East and Indian sub-continent, about 5% are chronically infected. Infection is less common in Western Europe and North America, where less than 1% are chronically infected.

Young children who become infected with HBV are the most likely to develop chronic infection. About 90% of infants infected during the first year of life and 30% to 50% of children infected between 1 to 4 years of age develop chronic infection. The risk of death from HBV-related liver cancer or cirrhosis is approximately 25% for persons who become chronically infected during childhood.

Chronic hepatitis B in some patients is treated with drugs called *interferon or lamivudine*, which can help some patients. Patients with cirrhosis are sometimes given liver transplants, with varying success. It is preferable to prevent this disease with vaccine than to try and cure it.

Hepatitis B vaccine has an outstanding record of safety and effectiveness. Since 1982, over one billion doses of hepatitis B vaccine have been used worldwide. The vaccine is given as a series of three intramuscular doses. Studies have shown that the vaccine is 95% effective in preventing children and adults from developing chronic infection if they have not yet been infected. In many countries where 8% to 15% of children used to become chronically infected with HBV, the rate of chronic infection has been reduced to less than 1% in immunized groups of children. Since 1991, WHO has called for all countries to add hepatitis B vaccine into their national immunization programmes."

Hepatitis B surface Antigen (HBsAg) is the major structural polypeptide of the envelope of the Hepatitis B Virus (HBV).
This antigen is composed mainly of the type common determinant "a" and the type specific determinants "d" and "y", present only on the specific serotypes.
Upon infection, a strong immunological response develops firstly against the type specific determinants and in a second time against the "a" determinant.
Anti "a" antibodies are however recognised to be most effective in the neutralisation of the virus, protecting the patient from other infections and leading it to convalescence.
The detection of HBsAb has become important for the follow up of patients infected by HBV and the monitoring of recipients upon vaccination with synthetic and natural HBsAg.

C. PRINCIPLE OF THE TEST

Microplates are coated with a preparation of highly purified HBsAg that in the first incubation with sample specifically captures anti HBsAg antibodies to the solid phase.
After washing, captured antibodies are detected by an HBsAg, labelled with peroxidase (HRP), that specifically binds the second available binding site of these antibodies.
The enzyme specifically bound to wells, by acting on the substrate/chromogen mixture, generates an optical signal that is proportional to the amount of HBsAb in the sample and can be detected by an ELISA reader.
The amount of antibodies may be quantitated by means of a standard curve calibrated against the W.H.O reference preparation.
Samples are pre treated in the well with an specimen diluent able to block interference present in vaccinated individuals.

D. COMPONENTS

Each kit contains sufficient reagents to perform 96 tests.

1. Microplate: MICROPLATE
8x12 microwell strips coated with purified heat-inactivated HBsAg of both subtypes (ad and ay) from human origin 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 Curve: CAL N° …
5x2.0 ml/vial. Ready to use and colour coded standard curve, derived from HBsAb positive plasma titrated on WHO standard for anti HBsAg (1st reference preparation 1977, lot 17-2-77), ranging: CAL1 = 0 mIU/ml // CAL2 = 10 mIU/ml // CAL3 = 50 mIU/ml // CAL4 = 100 mIU/ml // CAL 5 = 250 mIU/ml.
Contains human serum proteins, 5% BSA, 10 mM phosphate buffer pH 7.4+/-0.1, 0.09% sodium azide and 0.045% ProClin 300 as preservatives. Standards are blue coloured.

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, 0.05% Tween 20 and 0.045% ProClin 300.

4. Enzyme conjugate : CONJ
1x16.0 ml/vial. Ready-to-use solution and red color coded.
It contains inactivated purified HBsAg of both subtypes ad and ay, labelled with HRP, 5% BSA, 10 mM Tris buffer pH 6.8+/-0.1, 0.3 mg/ml gentamicine sulphate and 0.045% ProClin 300 as preservatives.

5. Chromogen/Substrate: SUBS TMB
1x16ml/vial. Contains a 50 mM citrate-phosphate buffered solution at pH 3.5-3.8, 4% dimethylsulphoxide, 0.03% tetra-methyl-benzidine (TMB) and 0.02% hydrogen peroxide (H2O2).
Note: To be stored protected from light as sensitive to strong illumination.

6. Sulphuric Acid: H2SO4 0.3 M
1x15ml/vial. Contains 0.3 M H_2SO_4 solution.
Attention: Irritant (H315, H319; P280, P302+P352, P332+P313, P305+P351+P338, P337+P313, P362+P363).

7. Specimen Diluent: DILSPE
1x8ml. 10 mM Tris Buffered solution ph 7.4 +/-0.1,suggested to be used in the follow up of vaccination. It contains 0.09% sodium azide as preservatives.

8. Control Serum: CONTROL …ml
1 vial. Lyophilized.
Contains fetal bovine serum proteins, human anti HBsAg antibodies calibrated at 50 ± 10% WHO mIU/ml. 0.3 mg/ml gentamicine sulphate and 0.045% ProClin 300 as preservatives.

9. Plate sealing foil n° 2

10. Package insert n° 1

E. MATERIALS REQUIRED BUT NOT PROVIDED

1. Calibrated Micropipettes (100ul and 50ul) and 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 (+/-1°C tolerance)..
6. Calibrated ELISA microwell reader with 450nm (reading) and with 620-630nm (blanking, strongly recommended) filters.
7. Calibrated ELISA microplate washer.
8. Vortex or similar mixing tools.

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. All the personnel involved in sample handling should be vaccinated for HBV and HAV, for which vaccines are available, safe and effective.
4. 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 (TMB) from strong light and avoid vibration of the bench surface where the test is undertaken.
5. Upon receipt, store the kit at 2..8°C into a temperature controlled refrigerator or cold room.
6. 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.
7. Check that the reagents 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.
8. Avoid cross-contamination between serum/plasma samples by using disposable tips and changing them after each sample. Do not reuse disposable tips.
9. Avoid cross-contamination between kit reagents by using disposable tips and changing them between the use of each one. Do not reuse disposable tips.
10. Do not use the kit after the expiration date stated on the external container and internal (vials) labels. A study conducted on an opened kit did not pointed out any relevant loss of activity up to six 6 uses of the device and up to 6 months.
11. Treat all specimens as potentially infective. All human serum specimens should be handled at Biosafety Level 2, as recommended by the Center for Disease Control, Atlanta, U.S. in compliance with what reported in the Institutes of Health's publication: "Biosafety in Microbiological and Biomedical Laboratories", ed. 1984.
12. 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.
13. Waste produced during the use of the kit has 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..
14. 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.
15. The Sulphuric Acid is an irritant. In case of spills, wash the surface with plenty of water
16. Other waste materials generated from the use of the kit (example: tips used for samples and controls, used microplates) should be handled as potentially infective and disposed according to national directives and laws concerning laboratory wastes.

G. SPECIMEN: PREPARATION AND WARNINGS

1. Blood is drawn aseptically by venipuncture and plasma or serum is prepared using standard techniques of preparation of samples for clinical laboratory analysis. No influence has been observed in the preparation of the sample with citrate, EDTA and heparin.

2. Samples have to be clearly identified with codes or names in order to avoid misinterpretation of results. Bar code labeling and electronic reading is strongly recommended.
3. Haemolysed ("red") and visibly hyperlipemic ("milky") samples have to be discarded as they could generate false results. Samples containing residues of fibrin or heavy particles or microbial filaments and bodies should be discarded as they could give rise to false results.
4. Sera and plasma can be stored at +2°…+8°C in primary collection tubes for up to five days after collection. Do not freeze primary tubes of collection. For longer storage periods, sera and plasma samples, carefully removed from the primary collection tube, can be stored frozen at –20°C for at least 12 months. Any frozen samples should not be frozen/thawed more than once as this may generate particles that could affect the test result.
5. If particles are present, centrifuge at 2.000 rpm for 20 min or filter using 0.2-0.8u filters to clean up the sample for testing.
6. Samples whose anti-HBsAg antibody concentration is expected to be higher than 250 mIU/ml should be diluted before use either 1:10 or 1:100 in the Calibrator 0 mIU/ml. Dilutions have to be done in clean disposable tubes by diluting 50 ul of each specimen with 450 ul of Cal 0 (1:10). Then 50 ul of the 1:10 dilution are diluted with 450 ul of the Cal 0 (1:100). Mix tubes thoroughly on vortex when preparing the diluted samples.

H. PREPARATION OF COMPONENTS AND WARNINGS

1. Microplate:
Allow the microplate to reach room temperature (about 1 hr) before opening the container. Check that the desiccant has not turned green, indicating a defect in conservation.
In this case, call Dia.Pro's customer service.
Unused strips have to be placed back into the aluminum pouch, with the desiccant supplied, firmly zipped and stored at +2°-8°C. After first opening, remaining strips are stable until the humidity indicator inside the desiccant bag turns from yellow to green.

2. Calibration Curve
Ready to use. Mix well on vortex before use.

3. Control Serum
Add the volume of ELISA grade water, reported on the label, to the lyophilised powder; let fully dissolve and then gently mix on vortex.
Note: *The control after dissolution is not stable. Store frozen in aliquots at –20°C.*

4. Wash buffer concentrate:
The whole content of the concentrated solution has to be diluted 20x with bidistilled 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.
Note: Once diluted, the wash solution is stable for 1 week at +2..8° C.

5. Enzyme conjugate:
Ready to use. Mix well on vortex before use.
Avoid contamination of the liquid with oxidising chemicals, dust or microbes. If this component has to be transferred, use only plastic, and if possible, sterile disposable containers.

6. Specimen Diluent:
Ready to use. Mix well on vortex before use.

7. Chromogen/Substrate:
Ready to use. Mix well on vortex before use.
Avoid contamination of the liquid with oxidising chemicals, air-driven dust or microbes. Do not expose to strong light, oxidising agents and metallic surfaces.
If this component has to be transferred use only plastic, and if possible, sterile disposable container

8. 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$1°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.
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 "Assay Procedure". 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 “Validation of Test” and “Assay Performances”. 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 for blood screening and when the number of samples to be tested exceed 20-30 units per run.
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.

L. PRE ASSAY CONTROLS AND OPERATIONS

1. Check the expiration date of the kit printed on the external label of the kit box. Do not use if expired.
2. 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.
3. Dilute all the content of the 20x concentrated Wash Solution as described above.
4. Dissolve the Control Serum as described above.
5. Allow all the other components to reach room temperature (about 1 hr) and then mix as described.
6. 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 as reported in the specific section.
7. Check that the ELISA reader has been turned on at least 20 minutes before reading.
8. If using an automated workstation, turn it on, check settings and be sure to use the right assay protocol.
9. Check that the micropipettes are set to the required volume.
10. Check that all the other equipments are available and ready to use.

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 can be carried out with the device according to the request of the clinician.

M.1 Quantitative analysis

1. Place the required number of strips in the microplate holder. Leave A1 and B1 wells empty for the operation of blanking.
Store the other strips into the bag in presence of the desiccant at 2..8°C, sealed.
Then Dispense in all the wells to be used for the test, except for A1 and B1, 50µl of the Specimen Diluent.

Important note: *This additive is added before distributing samples and controls into specific wells and is particularly intended for blocking some substances present in people undergoing vaccination and capable to mask antibodies.*

2. Pipette 100µl of all the Calibrators, 100µl of Control Serum in duplicate and then 100ul of samples. The Control Serum is used to verify that the whole analytical system works as expected. Check that Calibrators, Control Serum and samples have been correctly added. Then incubate the microplate at **+37°C for 60 min**.

Important note: *Strips have to be sealed with the adhesive sealing foil only when the test is performed manually. Do not cover strips when using ELISA automatic instruments.*

3. Wash the microplate as reported in section I.3.

4. In all the wells except A1 and B1, pipette 100 µl Enzyme Conjugate. Check that the reagent has been correctly added. Incubate the microplate at **+37°C for 60 minutes.**

Important notes:

1) *Be careful not to touch the inner surface of the well with the pipette tip when dispensing the Enzyme Conjugate. Contamination might occur.*
2) *Mix thoroughly the Enzyme Conjugate on vortex before use.*

5. Wash the microplate as described.
6. Pipette 100µl TMB/H_2O_2 mixture in each well, the blank wells included. Check that the reagent has been correctly added. Then incubate the microplate at **room temperature for 20 minutes**.

Important note: *Do not expose to strong direct light as a high background might be generated.*

7. Stop the enzymatic reaction by pipette 100µl Sulphuric Acid into each well and using the same pipetting sequence as in step 6. Then measure the colour intensity with a microplate reader at 450nm (reading) and at 620-630nm (blanking, mandatory), blanking the instrument on A1 and B1 wells.

M.2 Qualitative analysis

1. Place the required number of strips in the microplate holder. Leave A1 well empty for the operation of blanking.
Store the other strips into the bag in presence of the desiccant at 2..8°C, sealed.
2. Dispense 50 ul Specimen Diluent in all the wells, except for the blank A1. Then pipette 100µl of the Calibrator 0 mIU/ml in duplicate, 100µl of the Calibrator 10 mIU/ml in duplicate, 100µl of the Calibrator 250 mIU/ml in single, and then 100ul of samples. Check that Calibrators and samples have been correctly added. Then incubate the microplate at **+37°C for 60 min**.
3. Wash the microplate as reported in section I.3.
4. In all the wells except A1, pipette 100 µl Enzyme Conjugate. Check that the reagent has been correctly added. Incubate the microplate at **+37°C for 60 minutes.**

Important notes:

1) *Be careful not to touch the inner surface of the well with the pipette tip when dispensing the Enzyme Conjugate. Contamination might occur.*
2) *Mix thoroughly the Enzyme Conjugate on vortex before use.*

5. Wash the microplate as described.
6. Pipette 100µl TMB/H_2O_2 mixture in each well, the blank wells included. Check that the reagent has been correctly added. Then incubate the microplate at **room temperature for 20 minutes**.

Important note: *Do not expose to strong direct light as a high background might be generated.*

7. Stop the enzymatic reaction by pipette 100µl Sulphuric Acid into each well and using the same pipetting sequence as in step 6. Then measure the colour intensity with a microplate reader at 450nm (reading) and at 620-630nm (blanking, mandatory), blanking the instrument on A1 and B1 wells.

Important general notes:

1. *Ensure that no finger prints are present on the bottom of the microwell before reading. Finger prints could generate false positive results on reading.*
2. *Reading has should ideally be performed immediately after the addition of the Stop Solution but definitely no longer than 20 minutes afterwards. Some self oxidation of the chromogen can occur leading to a higher background.*
3. *The Control Serum (CS) does not affect the cut-off calculation and therefore the test results calculation. The Control Serum may be used only when a laboratory internal quality control is required by the management.*

N. ASSAY SCHEME (standard procedure)

Specimen Diluent Calibrators Control Serum Samples	50 ul 100 ul 100 ul 100 ul
1st incubation	**60 min**
Temperature	+37°C
Wash step	n° 5 cycles with 20'' of soaking OR n° 6 cycles without soaking
Enzyme Conjugate	100 ul
2nd incubation	**60 min**
Temperature	+37°C
Wash step	n° 5 cycles with 20'' of soaking OR n° 6 cycles without soaking
TMB/H2O2 mix	100 ul
3rd incubation	**20 min**
Temperature	r.t.
Sulphuric Acid	100 ul
Reading OD	450nm / 620-630nm

An example of dispensation scheme in quantitative assays is reported below:

Microplate

	1	2	3	4	5	6	7	8	9	10	11	12
A	BLK	CAL4	S3									
B	BLK	CAL4	S4									
C	CAL1	CAL5	S5									
D	CAL1	CAL5	S6									
E	CAL2	CS	S7									
F	CAL2	CS	S8									
G	CAL3	S1	S9									
H	CAL3	S2	S10									

Legenda: BLK = Blank // CAL = Calibrators // CS = Control Serum // S = Sample

An example of dispensation scheme in qualitative assays is reported below:

Microplate

	1	2	3	4	5	6	7	8	9	10	11	12
A	BLK	S 3	S 11									
B	CAL1	S 4	S 12									
C	CAL1	S 5	S 13									
D	CAL2	S 6	S 14									
E	CAL2	S 7	S 15									
F	CAL5	S 8	S 16									
G	S1	S 9	S 17									
H	S2	S 10	S 18									

Legenda: BLK = Blank // CAL = Calibrators // S = Sample

O. INTERNAL QUALITY CONTROL

A validation check is carried out on the controls any time the kit is used in order to verify whether the performances of the assay are as qualified.
Control that the following data are matched:

Parameters	Requirements
Blank well	< 0.100 OD450nm
Calibrator 0 WHO mIU/ml	< 0.200 OD450nm after blanking
Calibrator 10 WHO mIU/ml	OD450nm higher than the OD450nm of the Calibrator 0 mIU/ml + 0.100
Calibrator 250 WHO mIU/ml	> 1.500 OD450nm
Control Serum	OD450nm = OD450nm CAL 50 mIU/ml ± 10%
Coefficient of variation	< 30% for the Calibrator 0 mIU/ml

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
Calibrator 0 mIU/ml > 0.200 coefficient of variation > 30%	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 when the dispensation of standards is carried out; 4. that no contamination of the Cal 0 mIU/ml or of the wells where it was dispensed has occurred due to positive samples, to spills or to 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.

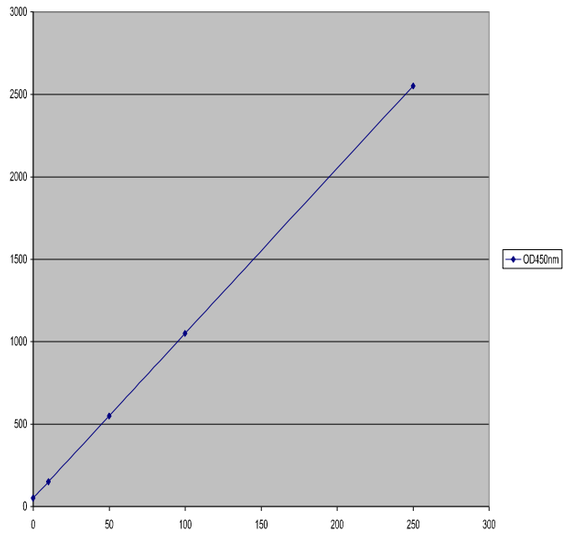
Calibrator 10 mIU/ml OD450nm < Cal 0 + 0.100	1. that the procedure has been correctly performed; 2. that no mistake has occurred during its distribution (e.g.: dispensation of a wrong calibrator); 3. that the washing procedure and the washer settings are as validated in the pre qualification study; 4. that no external contamination of the standard has occurred.
Calibrator 250 mIU/ml < 1.500 OD450nm	1. that the procedure has been correctly performed; 2. that no mistake has occurred during its distribution; 3. that the washing procedure and the washer settings are as validated in the pre qualification study; 4. that no external contamination of the standard has occurred.
Control Serum Different from expected value	First verify that: 1. the procedure has been correctly performed; 2. no mistake has occurred during its distribution (e.g.: dispensation of a wrong sample); 3. the washing procedure and the washer settings are correct; 4. no external contamination of the standard has occurred. 5. the Control Serum has been dissolved with the right volume reported on the label. If a mistake has been pointed out, the assay has to be repeated after eliminating the reason of this error. If no mistake has been found, proceed as follows: **a**) a value up to +/-20% is obtained: the overall Precision of the laboratory might not enable the test to match the expected value +/-10%. Report the problem to the Supervisor for acceptance or refusal of this result. **b**) a value higher than +/-20% is obtained: in this case the test is invalid and the DiaPro's customer service has to be called.

Important note:
The analysis must be done proceeding as the reading step described in the section M, point 7.

P. RESULTS

P.1 Quantitative method
If the test turns out to be valid, use for the quantitative method an approved curve fitting program to draw the calibration curve from the values obtained by reading at 450nm (4-parameters interpolation is suggested).
Then on the calibration curve calculate the concentration of anti HBsAg antibody in samples.
An example of Calibration curve is reported in the next page.

Example of Calibration Curve :



Important Note:
Do not use the calibration curve above to make calculations.

P.2 Qualitative method
In the qualitative method, calculate the mean OD450nm/620-630nm values for the Calibrators 0 and 10 mIU/ml and then check that the assay is valid.

Example of calculation (data obtained proceeding as the reading step described in the section M, point 7).

The following data must not be used instead of real figures obtained by the user.

Calibrator 0 mIU/ml: 0.020 – 0.024 OD450nm
Mean Value: 0.022 OD450nm
Lower than 0.200 – Accepted

Calibrator 10 mIU/ml: 0.250 – 0.270 OD450nm
Mean Value: 0.260 OD450nm
Higher than Cal 0 + 0.100 – Accepted

Calibrator 250 mIU/ml: 2.845 OD450nm
Higher than 1.500 – Accepted

Q. INTERPRETATION OF RESULTS
Samples with a concentration lower than 10 WHO mIU/ml are considered negative for anti HBsAg antibody by most of the international medical literature.
Samples with a concentration higher than 10 WHO mIU/ml are considered positive for anti HBsAg antibody.
In the follow up of vaccination recipients, however, the value of 20 WHO mIU/ml is usually accepted by the medical literature as the minimum concentration at which the patient is considered clinically protected against HBV infection.

Important notes:
1. *Interpretation of results should be done under the supervision of the laboratory supervisor to reduce the risk of judgement errors and misinterpretations.*
2. *When test results are transmitted from the laboratory to another facility, attention must be paid to avoid erroneous data transfer.*

3. *Diagnosis has to be done and released to the patient by a suitably qualified medical doctor.*

R. PERFORMANCES

Evaluation of Performances has been conducted in accordance to what reported in the Common Technical Specifications or CTS (art. 5, Chapter 3 of IVD Directive 98/79/EC).

1. LIMIT OF DETECTION:

The limit of detection of the assay has been calculated by means of the HBsAb international preparation supplied by CLB on behalf of WHO (1st reference preparation 1977, lot 17-2-77), on which Calibration Curve has been calibrated. HBV negative serum was used as diluent, as recommended by the supplier.
Results of Quality Control are given in the following table:

WHO mIU/ml	SAB.CE Lot # 1002	SAB.CE Lot # 1001	SAB.CE Lot # 1002/2
50	0.933	0.812	0.846
10	0.219	0.192	0.194
5	0.110	0.096	0.104
2.5	0.057	0.058	0.067
Std 0	0.021	0.015	0.023

2. DIAGNOSTIC SPECIFICITY AND SENSITIVITY

A Performance Evaluation has been conducted on a total number of more than 700 samples.

2.1 Diagnostic Specificity

It is defined as the probability of the assay of scoring negative in the absence of specific analyte.
More than 500 negative specimens were tested, internally and externally, against a European company.
A diagnostic specificity of 98.8% was assessed. .
Moreover, diagnostic specificity was assessed by testing 113 potentially interfering specimens (other infectious diseases, patients affected by non viral hepatic diseases, dialysis patients, pregnant women, hemolized, lipemic, etc.) against the European company. A value of specificity of 100% was assessed.
Finally, both human plasma, derived with different standard techniques of preparation (citrate, EDTA and heparin), and human sera have been used to determine the specificity.
No false reactivity due to the method of specimen preparation has been observed.

2.2 Diagnostic Sensitivity

It defined as the probability of the assay of scoring positive in the presence of specific analyte.
106 vaccinated patients were evaluated providing a diagnostic sensitivity of 100%.
More than 100 HBV naturally infected patients were tested, internally and externally, against the European company; a diagnostic sensitivity of 100% was found.

3. PRECISION:

The mean values obtained from a study conducted on three samples of different anti-HBsAg reactivity, examined in 16 replicates in three separate runs is reported below:

SAB.CE: lot # 1202

Calibrator 0 mIU/ml (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.038	0.038	0.039	0.039
Std.Deviation	0.003	0.004	0.005	0.004
CV %	8.8	9.5	11.8	10.0

Calibrator 10 mIU/ml (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.250	0.243	0.244	0.246
Std.Deviation	0.020	0.023	0.017	0.020
CV %	8.0	9.3	7.0	8.1

Calibrator 250 mIU/ml (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	2.998	3.000	3.259	3.085
Std.Deviation	0.152	0.151	0.158	0.153
CV %	5.1	5.0	4.8	5.0

SAB.CE: lot # 1002

Calibrator 0 mIU/ml (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.048	0.048	0.050	0.049
Std.Deviation	0.005	0.004	0.006	0.005
CV %	9.4	8.4	11.5	9.8

Calibrator 10 mIU/ml (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.249	0.252	0.242	0.248
Std.Deviation	0.021	0.020	0.023	0.021
CV %	8.3	7.9	9.6	8.6

Calibrator 250 mIU/ml (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	3.544	3.653	3.612	3.603
Std.Deviation	0.153	0.176	0.138	0.156
CV %	4.3	4.8	3.8	4.3

SAB.CE: lot # 1002/2

Calibrator 0 mIU/ml (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.050	0.051	0.050	0.050
Std.Deviation	0.005	0.006	0.006	0.005
CV %	10.0	10.9	11.9	10.9

Calibrator 10 mIU/ml (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.226	0.238	0.239	0.234
Std.Deviation	0.015	0.017	0.018	0.016
CV %	6.5	7.0	7.5	7.0

Calibrator 250 mIU/ml (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	3.526	3.457	3.499	3.494
Std.Deviation	0.137	0.143	0.162	0.147
CV %	3.9	4.1	4.6	4.2

The variability shown in the tables did not result in sample misclassification.

4. ACCURACY

The assay accuracy has been checked by the dilution and recovery tests. Any “hook effect”, underestimation likely to happen at high doses of analyte, was ruled out up to 10.000 mIU/ml.

Important note:

The performance data have been obtained proceeding as the reading step described in the section M, point 7.

S. LIMITATIONS OF THE PROCEDURE

Bacterial contamination or heat inactivation of the specimen may affect the absorbance values of the samples with consequent alteration of the level of the analyte.
This test is suitable only for testing single samples and not pooled ones.
Diagnosis of an infectious disease should not be established on the basis of a single test result. The patient's clinical history, symptomatology, as well as other diagnostic data should be considered.

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All the IVD Products manufactured by the company are under the control of a certified Quality Management System approved by an EC Notified Body. Each lot is submitted to a quality control and released into the market only if conforming with the EC technical specifications and acceptance criteria.

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REF ORG 538 ANAscreen

INTENDED PURPOSE

ANAscreen is an ELISA-based test system for the qualitative measurement of IgG class autoantibodies against SS-A 60, SS-A 52, SS-B, RNP-70, Sm, RNP/Sm, Scl-70, centromere B, Jo-1 in human serum or plasma samples. This product is intended for professional in vitro diagnostic use only.

The test is used for screening of patients with suspected autoimmune connective tissue diseases, e.g. systemic lupus erythematosus, mixed connective tissue disease, Sjoegren's syndrome, scleroderma, and polymyositis/dermatomyositis. 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	Sufficient for ... determinations
LOT	Batch code
	Use by
	Temperature limitation
	Keep away from sunlight
	Do not reuse
	Date of manufacture
CE	CE marked according to 98/79/EC
	Consult electronic Instructions For Use
538_4	Electronic Instruction For Use: version

MICROPLATE	Microplate
CALIBRATOR	Calibrator
CONTROL –	Control negative
DILUENT	Sample Buffer P
CONJUGATE	Enzyme Conjugate
TMB	TMB Substrate
STOP	Stop solution
WASH	Wash Buffer
RTU	Ready to use
50 x	50 x concentrate

PRINCIPLE OF THE TEST

A mixture of purified antigens SS-A 60, SS-A 52, SS-B, RNP-70, Sm, RNP/Sm, Scl-70, Centromere B and Jo-1 is coated on 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 538	▽E 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: ***Asc***
CALIBRATOR	1x 1.5 ml	Calibrator, containing ANA antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use.
CONTROL –	1x 1.5 ml	Control negative, containing ANA antibodies serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use.
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.

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 µl** 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 µl** 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	CAL											
B	C-											
C	P1											
D	P2											
E	P3											
F												
G												
H												

P1, ... patient sample CAL calibrator C- Control negative

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

First optical density (OD) of cut-off is calculated by multiplying optical density of the calibrator by the test specific factor 0.5:
OD cut-off = OD Calibrator * 0.5
Then the optical density of a sample is compared to the optical density of the cut-off:
Negative: OD sample < OD cut-off
Positive: OD sample ≥ OD cut-off
For detailed results the optical density of a sample is expressed as Index value:
Index = OD sample / OD cut-off

PERFORMANCE CHARACTERISTICS

Calibration

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

Measuring range

not applicable

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 Index 1.0

Interpretation of results

Negative: Index < 1.0
Borderline: Index 1.0 - 1.2
Positive: Index > 1.2

Linearity

Patient samples containing high levels of specific antibody were serially diluted in sample buffer. Activity for each dilution step was calculated as Index-Value.

Sample	Dilution	Observed Index	Expected Index	O/E [%]
1	1:100	5.8	5.8	100
.	1:200	2.7	2.9	93
.	1:400	1.6	1.5	110
.	1:800	0.8	0.7	110
.	1:1600	0.4	0.4	106
2	1:100	4.9	4.9	100
.	1:200	2.7	2.5	110
.	1:400	1.3	1.2	106
.	1:800	0.6	0.6	98
.	1:1600	0.3	0.3	90

Limit of detection

not applicable

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 Index	CV %
1	1.1	3.5
2	1.9	2.4
3	3.2	2.2

Inter-Assay		
Sample	Mean Index	CV %
1	1.2	6.5
2	1.9	4.0
3	3.3	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	%
SLE	63	60	95.2
Sjogren's syndrome	10	10	100.0
MCTD	10	10	100.0
Poly-/dermatomyositis	8	7	87.5
Scleroderma	10	10	100.0
CREST syndrome	9	9	100.0
Normal human sera	148	3	2.0

		Clinical Diagnosis		
		POS	NEG	
ORG 538	POS	106	3	
	NEG	4	145	
		110	148	258

Sensitivity:	96.4	%
Specificity:	98.0	%
Overall agreement:	97.3	%

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 538_IFU_EN_QM113172_2018-01-02_3* Reason for revision: *Definition of symbols used and symbols updated*

1. **100 µl** Standards, Kontrollen und verdünnte Patientenproben pipettieren
   → **30 Minuten** bei Raumtemperatur inkubieren
   → Inhalt der Platte verwerfen und
   3 mal mit **300 µl** Waschpuffer waschen
2. **100 µl** Enzymkonjugatlösung pipettieren
   → **15 Minuten** bei Raumtemperatur inkubieren
   → Inhalt der Platte verwerfen und
   3 mal mit **300 µl** Waschpuffer waschen
3. **100 µl** Substratlösung pipettieren
   → **15 Minuten** bei Raumtemperatur inkubieren
4. **100 µl** Stopplösung zugeben
   → Platte **5 Minuten** stehenlassen
   → Bei **450 nm** messen

HBsAg one

Version ULTRA

Fourth generation Enzyme Immunoassay (ELISA) for the determination of Hepatitis B surface Antigen or HBsAg in human serum and plasma

- for “in vitro” diagnostic use only -

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REF SAG1ULTRA.CE
96/192/480/960 Tests

HBsAg One version ULTRA

A. INTENDED USE

Fourth generation Enzyme Immunoassay (ELISA) for the one-step determination of Hepatitis B surface Antigen or HBsAg in human plasma and sera.
The kit is intended for the screening of blood units, is able to detect HBsAg mutants and finds application in the follow-up of HBV-infected patients.
For "in vitro" diagnostic use only.

B. INTRODUCTION

The World Health Organization (WHO) defines Hepatitis B Virus infection as follows:

"Hepatitis B is one of the major diseases of mankind and is a serious global public health problem. Hepatitis means inflammation of the liver, and the most common cause is infection with one of 5 viruses, called hepatitis A,B,C,D, and E. All of these viruses can cause an acute disease with symptoms lasting several weeks including yellowing of the skin and eyes (jaundice); dark urine; extreme fatigue; nausea; vomiting and abdominal pain. It can take several months to a year to feel fit again. Hepatitis B virus can cause chronic infection in which the patient never gets rid of the virus and many years later develops cirrhosis of the liver or liver cancer.

HBV is the most serious type of viral hepatitis and the only type causing chronic hepatitis for which a vaccine is available. Hepatitis B virus is transmitted by contact with blood or body fluids of an infected person in the same way as human immunodeficiency virus (HIV), the virus that causes AIDS. However, HBV is 50 to 100 times more infectious than HIV. The main ways of getting infected with HBV are: (a) perinatal (from mother to baby at the birth); (b) child- to-child transmission; (c) unsafe injections and transfusions; (d) sexual contact.

Worldwide, most infections occur from infected mother to child, from child to child contact in household settings, and from reuse of un-sterilized needles and syringes. In many developing countries, almost all children become infected with the virus. In many industrialized countries (e.g. Western Europe and North America), the pattern of transmission is different. In these countries, mother-to-infant and child-to-child transmission accounted for up to one third of chronic infections before childhood hepatitis B vaccination programmes were implemented. However, the majority of infections in these countries are acquired during young adulthood by sexual activity, and injecting drug use. In addition, hepatitis B virus is the major infectious occupational hazard of health workers, and most health care workers have received hepatitis B vaccine.

Hepatitis B virus is not spread by contaminated food or water, and cannot be spread casually in the workplace. High rates of chronic HBV infection are also found in the southern parts of Eastern and Central Europe. In the Middle East and Indian sub-continent, about 5% are chronically infected. Infection is less common in Western Europe and North America, where less than 1% are chronically infected.

Young children who become infected with HBV are the most likely to develop chronic infection. About 90% of infants infected during the first year of life and 30% to 50% of children infected between 1 to 4 years of age develop chronic infection. The risk of death from HBV-related liver cancer or cirrhosis is approximately 25% for persons who become chronically infected during childhood. Chronic hepatitis B in some patients is treated with drugs called *interferon or lamivudine*, which can help some patients. Patients with cirrhosis are sometimes given liver transplants, with varying success. It is preferable to prevent this disease with vaccine than to try and cure it.

Hepatitis B vaccine has an outstanding record of safety and effectiveness. Since 1982, over one billion doses of hepatitis B vaccine have been used worldwide. The vaccine is given as a series of three intramuscular doses. Studies have shown that the vaccine is 95% effective in preventing children and adults from developing chronic infection if they have not yet been infected. In many countries where 8% to 15% of children used to become chronically infected with HBV, the rate of chronic infection has been reduced to less than 1% in immunized groups of children. Since 1991, WHO has called for all countries to add hepatitis B vaccine into their national immunization programs."

Hepatitis B surface Antigen or HBsAg is the most important protein of the envelope of Hepatitis B Virus, responsible for acute and chronic viral hepatitis.
The surface antigen contains the determinant "a", common to all the known viral subtypes, immunologically distinguished by two distinct subgroups (ay and ad).
The ability to detect HBsAg with high sensitive immunoassays in the last years has led to an understanding of its distribution and epidemiology worldwide and to radically decrease the risk of infection in transfusion.

C. PRINCIPLE OF THE TEST

A mix of mouse monoclonal antibodies specific to the determinants "a", "d" and "y" of HBsAg is fixed to the surface of microwells. Patient's serum/plasma is added to the microwell together with a second mix of mouse monoclonal antibodies, conjugated with Horseradish Peroxidase (HRP) and directed against a different epitope of the determinant "a" and against "preS".
The specific immunocomplex, formed in the presence of HBsAg in the sample, is captured by the solid phase.
At the end of the one-step incubation, microwells are washed to remove unbound serum proteins and HRP conjugate.
The chromogen/substrate is then added and, in the presence of captured HBsAg immunocomplex, the colorless substrate is hydrolyzed by the bound HRP conjugate to a colored end-product. After blocking the enzymatic reaction, its optical density is measured by an ELISA reader.
The color intensity is proportional to the amount of HBsAg present in the sample.
The version ULTRA is particularly suitable for automated screenings and is able to detect "s" mutants.

D. COMPONENTS

The standard configuration contains reagents to perform 192 tests and is made of the following components:

1. Microplate MICROPLATE
n° 2. 12 strips of 8 breakable wells coated with anti HBsAg, affinity purified mouse monoclonal antibodies, specific to "a", "y" and "d" determinants, and sealed into a bag with desiccant.

2. Negative Control CONTROL -
1x4.0ml/vial. Ready to use control. It contains goat serum, 10 mM phosphate buffer pH 7.4+/-0.1, 0.09% Na-azide and 0.045% ProClin 300 as preservatives. The negative control is pale yellow color coded.

3. Positive Control CONTROL +
1x4.0ml/vial. Ready to use control. It contains goat serum, non infectious recombinant HBsAg, 10 mM phosphate buffer pH 7.4+/-0.1, 0.02% gentamicine sulphate and 0.045% ProClin 300 as preservatives. The positive control is color coded green.

4. Calibrator CAL ….
n° 2 vials. Lyophilized calibrator. To be dissolved with EIA grade water as reported in the label. Contains fetal bovine serum, non infectious recombinant HBsAg at 0.5 IU/ml (2nd WHO international standard for HBsAg, NIBSC code 00/588), 10 mM phosphate buffer pH 7.4+/-0.1, 0.02% gentamicine sulphate and 0.045% ProClin 300 as preservatives.
Note: The volume necessary to dissolve the content of the vial may vary from lot to lot. Please use the right volume reported on the label .

5. Wash buffer concentrate WASHBUF 20X
2x60ml/bottle. 20X concentrated solution. Once diluted, the wash solution contains 10 mM phosphate buffer pH 7.0+/-0.2, 0.05% Tween 20 and 0.045% ProClin 300.

6. Enzyme Conjugate Diluent CONJ DIL
2x16ml/vial. Ready to use and pink/red color coded regent. It contains 10 mM Tris buffer pH 6.8+/-0.1, 1% normal mouse serum, 5% BSA, 0.045% ProClin 300 and 0.02% gentamicine sulphate as preservatives. The solution is normally opalescent.

7. Enzyme Conjugate CONJ 20X
2x1ml/vial. 20X concentrated reagent. It contains Horseradish Peroxidase (HRP) labeled mouse monoclonal antibodies to HBsAg, determinant "a" and "preS", 10 mM Tris buffer pH 6.8+/-0.1, 5% BSA, 0.045% ProClin 300 and 0.02% gentamicine sulphate as preservatives.

8. Chromogen/Substrate SUBS TMB
2x25ml/bottle. It contains a 50 mM citrate-phosphate buffered solution at pH 3.5-3.8, 4% dimethylsulphoxide, 0.03% tetra-methyl-benzidine (TMB) and 0.02% hydrogen peroxide (H2O2).
Note: To be stored protected from light as sensitive to strong illumination.

9. Sulphuric Acid H_2SO_4 O.3 M
1x25ml/bottle. It contains 0.3 M H2SO4 solution.
Note: Attention: Irritant (H315; H319; P280; P302+P352; P332+P313; P305+P351+P338; P337+P313; P362+P363)

10. Plate sealing foils n° 4

11. Package insert

Important note:
Only upon specific request , Dia.Pro can supply reagents for 96, 480, 960 tests , as reported below**:**

Microplates	N°1	N°5	N°10
Negative Control	1x2ml/vial	1x10ml/vial	1x20ml/vial
Positive Control	1x2ml/vial	1x10ml/vial	1x20ml/vial
Calibrator	N° 1 vial	N° 5 vials	N° 10 vials
Wash buffer concentrate	1x60ml/vial	5x60ml/vial	4x150ml/vial
Enzyme conjugate	1x0.8ml/vial	1x4ml/vial	2x4ml/vial
Conjugate Diluent	1x16ml/vial	2x40ml/vial	2x80ml/vial
Chromogen/Substrate	1x25ml/vial	3x42ml/vial	2x125ml/vial
Sulphuric Acid	1x15ml/vial	2x40ml/vial	2x80ml/vial
Plate sealing foils	N° 2	N° 10	N° 20
Package insert	N° 1	N° 1	N° 1
Number of tests	96	480	960
Code SAG1ULTRA.CE	96	480	960

E. MATERIALS REQUIRED BUT NOT PROVIDED
1. Calibrated Micropipettes (150ul, 100ul and 50ul) and 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), capable to provide shaking at 1300 rpm+/-150, 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.

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. When the kit is used for the screening of blood units and blood components, it has to be used in a laboratory certified and qualified by the national authority in that field (Ministry of Health or similar entity) to carry out this type of analysis.
3. 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.
4. All the personnel involved in sample handling should be vaccinated for HBV and HAV, for which vaccines are available, safe and effective.
5. 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 (TMB) from strong light and avoid vibration of the bench surface where the test is undertaken.
6. Upon receipt, store the kit at 2..8°C into a temperature controlled refrigerator or cold room.
7. 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.
8. Check that the reagents 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.
9. Avoid cross-contamination between serum/plasma samples by using disposable tips and changing them after each sample. Do not reuse disposable tips.
10. Avoid cross-contamination between kit reagents by using disposable tips and changing them between the use of each one. Do not reuse disposable tips.
11. Do not use the kit after the expiration date stated on the external container and internal (vials) labels. A study conducted on an opened kit has not pointed out any relevant loss of activity up to 6 re-use of the device and up to 6 months.
12. Treat all specimens as potentially infective. All human serum specimens should be handled at Biosafety Level 2, as recommended by the Center for Disease Control, Atlanta, U.S. in compliance with what reported in the Institutes of Health's publication: "Biosafety in Microbiological and Biomedical Laboratories", ed. 1984.
13. 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.
14. Waste produced during the use of the kit has 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..
15. 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.
16. The Stop Solution is an irritant. In case of spills, wash the surface with plenty of water
17. Other waste materials generated from the use of the kit (example: tips used for samples and controls, used microplates) should be handled as potentially infective and disposed according to national directives and laws concerning laboratory wastes.

G. SPECIMEN: PREPARATION AND WARNINGS

1. Blood is drawn aseptically by venepuncture and plasma or serum is prepared using standard techniques of preparation of samples for clinical laboratory analysis. No influence has been observed in the preparation of the sample with citrate, EDTA and heparin.
2. 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.
3. Samples have to be clearly identified with codes or names in order to avoid misinterpretation of results. When the kit is used for the screening of blood units, bar code labeling and electronic reading is strongly recommended.
4. Haemolysed (red) and lipemic ("milky") samples have to be discarded as they could generate false results. Samples containing residues of fibrin or heavy particles or microbial filaments and bodies should be discarded as well as they could give rise to false positive results. Specimens with an altered pathway of coagulation, presenting particles after blood collection and preparation of serum/plasma as those coming from hemodialized patients, could give origin to false positive results.
5. Sera and plasma can be stored at +2°…+8°C in primary collection tubes for up to five days after collection. Do not freeze primary tubes of collection. For longer storage periods, sera and plasma samples, carefully removed from the primary collection tube, can be stored frozen at –20°C for at least 12 months. Any frozen sample should not be frozen/thawed more than once as this may generate particles that could affect the test result.
6. If some turbidity is present or presence of microparticles is suspected after thawing, filter the sample on a disposable 0.2-0.8u filter to clean it up for testing or use the two-steps alternative method.

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 6 months.

1. Microplates:
Allow the microplate to reach room temperature (about 1 hr) before opening the container. Check that the desiccant has not turned green, indicating a defect in conservation.
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. After first opening, remaining strips are stable until the humidity indicator inside the desiccant bag turns from yellow to green.

2. Negative Control:
Ready to use. Mix well on vortex before use.

3. Positive Control:
Ready to use. Mix well on vortex before use. The positive control does not contain any infective HBV as it is composed of recombinant synthetic HBsAg.

4. Calibrator:
Add the volume of ELISA grade water, reported on the label, to the lyophilized powder; let fully dissolve and then gently mix on vortex. The solution is not stable. Store the Calibrator frozen in aliquots at –20°C.

5. Wash buffer concentrate:
The 20x concentrated solution has to be diluted with EIA grade water up to 1200 ml and mixed gently end-over-end before use. As some salt crystals may be present into the vial, take care to dissolve all the content when preparing the solution.
In the preparation avoid foaming as the presence of bubbles could give origin to a bad washing efficiency.

Note: *Once diluted, the wash solution is stable for 1 week at +2..8° C.*

6. Enzyme conjugate:
The working solution is prepared by diluting the 20X concentrated reagent into the Conjugate
Mix well on vortex before use.
Avoid any contamination of the liquid with oxidizing chemicals, dust or microbes. If this component has to be transferred, use only plastic sterile disposable containers.
Important note*: The working solution is not stable. Prepare only the volume necessary for the work of the day. As an example when the kit is used in combination with other instruments or manually, dilute 0.1 ml 20X Conjugate with 1.9 ml Conjugate Diluent into a disposable plastic vial and mix carefully before use.*

7. Chromogen/Substrate:
Ready to use. Mix well by end-over-end mixing.
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.

8. Sulphuric Acid:
Ready to use. Mix well by end-over-end mixing.
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$1°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. In case of **shaking** during incubations, the instrument has to ensure 350 rpm $\pm$150. Amplitude of shaking is very important as a wrong one could give origin to splashes and therefore to some false positive result.
4. 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.

5. **Incubation times** have a tolerance of ±5%.
6. The **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 ≤ 10 nm; (b) absorbance range from 0 to ≥ 2.0; (c) linearity to ≥ 2.0; (d) repeatability ≥ 1%. Blanking is carried out on the well identified in the section "Assay Procedure". 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.
7. When using **ELISA automated workstations**, all critical steps (dispensation, incubation, washing, reading, shaking, data handling, etc.) 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 by checking full matching the declared performances of the kit. In addition, the liquid handling part of the station (dispensation and washing) has to be validated and correctly set paying particular attention to avoid carry over by the needles used for dispensing samples and for washing. The carry over effect 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 for blood screening and when the number of samples to be tested exceed 20-30 units per run.
8. When using automatic devices, in case the vial holder of the instrument does not fit with the vials supplied in the kit, transfer the solution into appropriate containers and label them with the same label peeled out from the original vial. This operation is important in order to avoid mismatching contents of vials, when transferring them. When the test is over, return the secondary labeled containers to 2..8°C, firmly capped.
9. **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 essential requirements of the assay. Support is also provided for the installation of new instruments to be used in combination with the kit.

L. PRE ASSAY CONTROLS AND OPERATIONS

1. Check the expiration date of the kit printed on the external label of the kit box. Do not use if expired.
2. 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. 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.
3. Dilute all the content of the 20x concentrated Wash Solution as described above.
4. Dilute the 20X concentrated Enzyme Conjugate with its Diluent as reported.
5. Dissolve the Calibrator 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 as 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.

Automated assay:

In case the test is carried out automatically with an ELISA system, we suggest to make the instrument dispense first 150 ul controls & calibrator, then all the samples and finally 100 ul diluted Enzyme Conjugate.
For the pre-washing step (point 1 of the assay procedure) and all the next operations follow the operative instructions reported below for the Manual Assay.
It is strongly recommended to check that the time lap between the dispensation of the first and the last sample will be calculated by the instrument and taken into consideration by delaying the first washing operation accordingly.

Manual Assay:

1. Place the required number of strips in the plastic holder and wash them once to hydrate wells. Carefully identify the wells for controls, calibrator and samples.

Important note: *Pre washing (1 cycle: dispensation of 350ul/well of washing solution+ aspiration) is fundamental to obtain reliable and specific results both in the manual and in the automatic procedures. Do not omit it !*

2. Leave the A1 well empty for blanking purposes.
3. Pipette 150µl of the Negative Control in triplicate, 150ul of the Calibrator in duplicate and then 150ul of the Positive Control in single followed by 150ul of each of the samples.
4. 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).
5. Dispense 100ul diluted 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.*

6. Following addition of the conjugate, check that the color of the samples have changed from yellowish to pink/red and then incubate the microplate for **120 min at +37°C**.

Important notes:

a. Strips have to be sealed with the adhesive sealing foil, only when the test is performed manually. Do not cover strips when using ELISA automatic instruments.
b. If the procedure is carried out on shaking, be sure to deliver the rpm reported for in Section I.3 as otherwise intra-well contamination could occur.

7. When the first incubation is over, wash the microwells as previously described (section I.4)
8. Pipette 200 µl Chromogen/Substrate into all the wells, A1 included.

Important note: *Do not expose to strong direct light as a high background might be generated.*

9. Incubate the microplate protected from light at **18-24°C for 30 min.** Wells dispensed with the positive control, the calibrator and positive samples will turn from clear to blue.
10. Pipette 100 µl Sulphuric Acid into all the wells to stop the enzymatic reaction, using the same pipetting sequence as in step 8. Addition of the acid solution will turn the positive control, the calibrator and positive samples from blue to yellow/brown.
11. Measure the color intensity of the solution in each well, as described in section I.6 using a 450nm filter (reading) and a 620-630nm filter (background subtraction, mandatory), blanking the instrument on A1.

Important general 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.*
3. *When samples to be tested are not surely clean or have been stored frozen, the assay procedure reported below is recommended as long as it is far less sensitive to interferences due to hemolysis, hyperlipaemia, bacterial contamination and fibrin microparticles. The assay is carried out in two-steps at +37°C on shaking at* 350 rpm ±150 *as follows:*
   - *dispense 100 ul of controls, calibrator and samples*
   - *incubate 60 min at +37°C on shaking*
   - *wash according to instructions (section I.4)*
   - *dispense 100 ul diluted enzyme tracer*
   - *incubate 30 min at +37°C on shaking*
   - *wash*
   - *dispense 100 ul TMB&H2O2 mix*
   - *incubate 30 min at r.t. on shaking*
   - *stop and read*

   In this procedure the pre-wash can be omitted.
   This method shows performances similar to the standard one and therefore can be used in alternative.
4. *The Calibrator (CAL) does not affect the cut-off calculation and therefore the test results calculation. The Calibrator may be used only when a laboratory internal quality control is required by the management.*

N. ASSAY SCHEME

Operations	Procedure
Pre-Washing step	n° 1 cycle
Controls&Calibrator&samples Diluted Enzyme Conjugate	150 ul 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	**30 min**
Temperature	room
Sulphuric Acid	100 ul
Reading OD	450nm / 620-630nm

An example of dispensation scheme is reported in the following section:

Microplate

	1	2	3	4	5	6	7	8	9	10	11	12
A	BLK	S2										
B	NC	S3										
C	NC	S4										
D	NC	S5										
E	CAL	S6										
F	CAL	S7										
G	PC	S8										
H	S1	S9										

Legenda: BLK = Blank NC = Negative Control
CAL = Calibrator PC = Positive Control S = Sample

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 or S/Co values have been matched in the analysis.

Ensure that the following results are met:

Parameter	Requirements
Blank well	< 0.100 OD450nm value
Negative Control (NC)	< 0.050 mean OD450nm value after blanking
Calibrator 0.5 IU/ml	S/Co ≥ 2
Positive Control	> 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
Negative Control (NC) > 0.050 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 control instead of the negative one); 4. that no contamination of the negative control or of the wells where the control 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.

Calibrator S/Co < 2	1. that the procedure has been correctly performed; 2. that no mistake has occurred during its distribution (ex.: dispensation of negative control instead of calibrator) 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.
Positive Control < 1.000 OD450nm	1. that the procedure has been correctly performed; 2. that no mistake has occurred during the distribution of the control (dispensation of negative control instead of positive control. In this case, the negative control will have an OD450nm value > 0.050). 3. that the washing procedure and the washer settings are as validated in the pre qualification study; 4. that no external contamination of the positive control 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, point 11.

P. CALCULATION OF THE CUT-OFF
The test results are calculated by means of a cut-off value determined on the mean OD450nm/620-630nm value of the negative control (NC) with the following formula:

NC + 0.050 = Cut-Off (Co)

The value found for the test is used for the interpretation of results as described in the next paragraph.

***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
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
< 0.9	Negative
0.9 – 1.1	Equivocal
> 1.1	Positive

A negative result indicates that the patient is not infected by HBV and that the blood unit may be transfused.
Any patient showing an equivocal result should be retested on a second sample taken 1-2 weeks after the initial sample; the blood unit should not be transfused.
A positive result is indicative of HBV infection and therefore the patient should be treated accordingly or the blood unit should be discarded.

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 must be confirmed first by repeating the test on the sample, after having filtered it on 0.2-0.8 u filter to remove any microparticles interference. Then, if still positive, the sample has to be submitted to a confirmation test before a diagnosis of viral hepatitis is released.*
3. *When test results are transmitted from the laboratory to another department, attention must be paid to avoid erroneous data transfer.*
4. *Diagnosis of viral hepatitis infection has to be taken and released to the patient by a suitably qualified medical doctor.*

An example of calculation is reported below (data obtained proceeding as the the reading step described in the section M, point 11):

The following data must not be used instead or real figures obtained by the user.

Negative Control: 0.012 – 0.008 – 0.010 OD450nm
Mean Value: 0.010 OD450nm
Lower than 0.050 – Accepted
Positive Control: 2.489 OD450nm
Higher than 1.000 – Accepted
Cut-Off = 0.010+0.050 = 0.060
Calibrator: 0.350 - 0.370 OD450nm
Mean value: 0.360 OD450nm S/Co = 6.0
S/Co higher than 2.0 – Accepted
Sample 1: 0.028 OD450nm
Sample 2: 1.690 OD450nm
Sample 1 S/Co < 0.9 = negative
Sample 2 S/Co > 1.1 = positive

R. PERFORMANCE CHARACTERISTICS
Evaluation of Performances has been conducted in accordance to what reported in the Common Technical Specifications or CTS (art. 5, Chapter 3 of IVD Directive 98/79/EC). Version ULTRA proved to be at least equivalent to the original design in a study conducted for the validation of the new version.

1. Analytical Sensitivity
The limit of detection of the assay has been calculated on the 2nd WHO international standard, NIBSC code 00/588.
In the following table, results are given for three lots (P1, P2 and P3) of the version ULTRA in comparison with the reference device (Ref.):

WHO IU/ml	Lot # P1 S/Co	Lot # P2 S/Co	Lot # P3 S/Co	Ref. S/Co
0.4	**4.6**	**4.8**	**4.6**	**4.6**
0.2	**2.3**	**2.4**	**2.4**	**2.4**
0.1	**1.4**	**1.4**	**1.5**	**1.2**
0.05	0.8	0.8	***1.0***	0.7
0.025	0.6	0.6	0.6	0.4
FCS (NC)	0.3	0.2	0.3	0.1

The assay shows an Analytical Sensitivity better than 0.1 WHO IU/ml of HBsAg.

In addition two panels of sensitivity supplied by EFS, France, and by SFTS, France, were tested and gave in the best conditions the following results:

Panel EFS Ag HBs HB1-HB6 lot n° 04

Sample ID	Characteristics	ng/ml	S/Co
HB1	diluent	/	0,2
HB2	adw2+ayw3	0.05	0,6
HB3	adw2+ayw3	0.1	**1,0**
HB4	adw2+ayw3	0.2	**1,8**
HB5	adw2+ayw3	0.3	**2,4**
HB6	adw2+ayw3	0.5	**4,2**

Sensitivity panel SFTS, France, Ag HBs 2005

Sample ID	Characteristics	ng/ml	S/Co
171	Adw2 + ayw3	2.21 ± 0.15	**15,4**
172	Adw2 + ayw3	1.18 ± 0.10	**8,7**
173	Adw2 + ayw3	1.02 ± 0.05	**6,1**
174	Adw2 + ayw3	0.64 ± 0.04	**4,0**
175	Adw2 + ayw3	0.49 ± 0.03	**3,4**
176	Adw2 + ayw3	0.39 ± 0.02	**2,6**
177	Adw2 + ayw3	0.25 ± 0.02	**2,0**
178	Adw2 + ayw3	0.11 ± 0.02	**1,3**
179	Adw2 + ayw3	0.06 ± 0.01	***0,9***
180	Adw2 + ayw3	0.03 ± 0.01	0,8
181	Adw2	0.5 – 1.0	**4,7**
182	Adw4	0.5 – 1.0	**3,6**
183	Adr	0.5 – 1.0	**4,5**
184	Ayw1	0.5 – 1.0	**5,1**
185	Ayw2	0.5 – 1.0	**6,4**
186	Ayw3	0.5 – 1.0	**7,3**
187	Ayw3	0.5 – 1.0	**5,8**
188	Ayw4	0.5 – 1.0	**6,9**
189	Ayr	0.5 – 1.0	**6,1**
190	diluent	/	0,6

The panel # 808, supplied by Boston Biomedical Inc., USA, was also tested to define the limit of sensitivity.
Results in the best conditions are as follows :

BBI panel PHA 808

Sample ID	Characteristics	ng/ml	S/Co
01	ad	2,49	**10,2**
02	ad	1,17	**4,8**
03	ad	1,02	**4,3**
04	ad	0,96	**3,8**
05	ad	0,69	**2,9**
06	ad	0,50	**2,2**
07	ad	0,41	**1,5**
08	ad	0,37	**1,3**
09	ad	0,30	**1,2**
10	ad	0,23	***1,0***
11	ay	2,51	**11,2**
12	ay	1,26	**5,9**
13	ay	0,97	**4,1**
14	ay	0,77	**3,7**
15	ay	0,63	**2,0**
16	ay	0,48	**2,4**
17	ay	0,42	**2,0**
18	ay	0,33	**1,8**
19	ay	0,23	**1,6**
20	ay	0,13	**1,1**
21	negative	/	0,6

2. Diagnostic Sensitivity:
The diagnostic sensitivity was tested according to what required by Common Technical Specifications (CTS) of the directive 98/79/EC on IVD for HBsAg testing.
Positive samples, including HBsAg subtypes and a panel of "s" mutants from most frequent mutations, were collected from different HBV pathologies (acute, a-symptomatic and chronic hepatitis B) or produced synthetically, and were detected positive in the assay.
All the HBsAg known subtypes, "ay" and "ad", and isoforms "w" and "r", supplied by CNTS, France, were tested in the assay and determined positive by the kit as expected.
An overall value of 100% has been found in a study conducted on a total number of more than 400 samples positive with the original reference IVD code SAG1.CE, CE marked.
A total of 30 sero-conversions were studied, most of them produced by Boston Biomedica Inc., USA.
Results obtained by examining eight panels supplied by Boston Biomedica Inc., USA, are reported below for the version ULTRA in comparison with the reference device code SAG1.CE.

Panel ID	1st sample positive	HBsAg subtype	HBsAg ng/ml	Version ULTRA S/Co	Ref. device S/Co
PHM 906	02	ad	0.5	**3.7**	**1.4**
PHM 907 (M)	06	ay	1.0	**4.4**	**2.9**
PHM 909	04	ad	0.3	**1.2**	0.8
PHM 914	04	ad	0.5	**1.1**	**1.1**
PHM 918	02	ad	0.1	**1.8**	0.5
PHM 923	03	ay	< 0.2	**2.2**	**1.2**
PHM 925	03	Ind.	n.d.	**1.4**	*0.9*
PHM 934	01	ad	n.d.	**1.0**	0.8

3. Diagnostic Specificity:
It is defined as the probability of the assay of scoring negative in the absence of specific analyte. In addition to the first study, where more than 5000 negative samples from blood donors (two blood centers), classified negative with a CE marked device in use at the laboratory of collection were examined, the diagnostic specificity was recently assessed by testing a total of 2288 negative blood donors on seven different lots. A value of specificity of 100% was found.
Both plasma, derived with different standard techniques of preparation (citrate, EDTA and heparin), and sera have been used to determine the specificity.
No false reactivity due to the method of specimen preparation has been observed.
Frozen specimens have also been tested to check whether samples freezing interferes with the performance of the test. No interference was observed on clean and particle free samples.
Samples derived from patients with different viral (HCV, HAV) and non viral pathologies of the liver that may interfere with the test were examined. No cross reaction were observed.

4. Precision:
It has been calculated for the version ULTRA on two samples examined in 16 replicates in 3 different runs for three lots.
Results are reported in the following tables:

Average values Total n = 144	Negative Sample	Calibrator 0.5 IU/ml
OD450nm	0.026	0.332
Std.Deviation	0.004	0.027
CV %	16%	8%

The variability shown in the tables did not result in sample misclassification.

S. LIMITATIONS

Repeatable false positive results were assessed on freshly collected specimens in less than 0.1% of the normal population, mostly due to high titers Heterophilic Anti Mouse Antibodies (HAMA).

Interferences in fresh samples were also observed when they were not particles-free or were badly collected (see chapter G).

Old or frozen samples, presenting fibrin clots, crioglobulins, lipid-containing micelles or microparticles after storage or thawing, can generate false positive results.

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All the IVD Products manufactured by the company are under the control of a certified Quality Management System approved by an EC Notified Body. 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 S.r.l.
Via G. Carducci n° 27 – Sesto San Giovanni (MI) – Italy

CE
0318

HDV Ab

Competitive Enzyme Immunoassay for the qualitative determination of antibodies to Hepatitis Delta Virus in human serum and plasma

- for “in vitro” diagnostic use only -

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REF DAB.CE
96 Tests

HDV Ab

A. INTENDED USE

Competitive Enzyme ImmunoAssay (ELISA) for the qualitative determination of antibodies to Hepatitis Delta Virus or HDV in human plasma and sera with a "two-steps" methodology.
The kit is used for the follow-up of patients infected by HDV.
For "in vitro" diagnostic use only.

B. INTRODUCTION

The Hepatitis Delta Virus or HDV is a RNA defective virus composed of a core presenting the delta-specific antigen, encapsulated by HBsAg, that requires the helper function of HBV to support its replication.
Infection by HDV occurs in the presence of acute or chronic HBV infection. When acute delta and acute HBV simultaneously occur, the illness becomes severe and clinical and biochemical features may be indistinguishable from those of HBV infection alone. In contrast, a patient with chronic HBV infection can support HDV replication indefinitely, usually with a less severe illness appearing as a clinical exacerbation.
The determination of HDV specific serological markers (HDV Ag, HDV Ab, HDV IgM and HDV IgG) represents in these cases an important tool to the clinician for the classification of the etiological agent, for the follow up of infected patients and their treatment. The detection of HDV total antibodies allows the classification of the illness and the monitoring of the seroconversion event.

C. PRINCIPLE OF THE TEST

Anti-HDV antibodies, if present in the sample, compete with a virus-specific polyclonal IgG, labeled with peroxidase (HRP), for a fixed amount of rec-HDV coated on the microplate. The test is carried out with a two steps incubation competitive system. First the sample is added to the plate and specific anti HDV antibodies bind to the adsorbed antigen. After washing, an enzyme conjugated antibody to HDV is added and binds to the free portion of the antigen coated. After washing a chromogen/substrate mixture is dispensed. The concentration of the bound enzyme on the solid phase becomes inversely proportional to the amount of anti-HDV antibodies in the sample and its activity is detected by the added chromogen/substrate. The concentration of HDV-specific antibodies in the sample is determined by means of a cut-off value that allows for the semi quantitative detection of anti-HDV antibodies.

D. COMPONENTS

Each kit contains sufficient reagents to perform 96 tests.

1. Microplate: MICROPLATE
8x12 microwell strips coated with recombinant HDV-specific antigen 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. Negative Control: CONTROL -
1x2.0ml/vial. Ready to use. Contains goat serum proteins, 100 mM Tris-HCl buffer pH 7.4 +/-0.1, 0.09% Sodium Azide and 0.045% ProClin 300 as preservatives. The negative control is colour coded pale yellow.

3. Positive Control: CONTROL +
1x2.0ml/vial. Ready to use. Contains goat serum proteins, high titer anti HDV antibodies, 100 mM Tris-HCl buffer pH 7.4 +/-0.1, 0.09% Sodium Azide and 0.045% ProClin 300 as preservatives. The positive control is colour coded green.

4. Calibrator: CAL ….
n° 1 vial. Lyophilised. To be dissolved with EIA grade water as reported in the label. Contains bovine serum proteins, low titer human antibodies to HDV, 0.2 mg/ml gentamicine sulphate and 0.045% ProClin 300 as preservatives.
Note: The volume necessary to dissolve the content of the vial may vary from lot to lot. Please use the right volume reported on the label.

5. 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, 0.05% Tween 20 and 0.045% ProClin 300.

6. Enzyme conjugate: CONJ
1x16ml/vial. Ready-to-use solution. Contains 5% bovine serum albumine, 10 mM tris buffer pH 6.8 +/-0.1, Horseradish peroxidase conjugated antibody to HDV in presence of 0.2 mg/ml gentamicine sulphate and 0.045% ProClin 300 as preservatives. The component is colour coded red.

7. Chromogen/Substrate: SUBS TMB
1x16ml/vial. Contains a 50 mM citrate-phosphate buffered solution at pH 3.5-3.8, 4% DMSO, 0.03% tetra-methyl-benzidine or TMB and 0.02% hydrogen peroxide of H_2O_2.
Note: To be stored protected from light as sensitive to strong illumination.

8. Sulphuric Acid: H_2SO_4 0.3 M
1x15ml/vial. Contains 0.3 M H_2SO_4 solution.
Attention: Irritant (H315, H319; P280, P302+P352, P332+P313, P305+P351+P338, P337+P313, P362+P363).

Plate sealers n° 2

Instructions for Use n° 1

E. MATERIALS REQUIRED BUT NOT PROVIDED

1. Calibrated Micropipettes in the range 10-1000 ul and disposable plastic tips.
2. EIA grade water (double distilled or deionized, 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.

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. All the personnel involved in sample handling should be vaccinated for HBV and HAV, for which vaccines are available, safe and effective.

4. 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 (TMB/H_2O_2) from strong light and avoid vibration of the bench surface where the test is undertaken.
5. Upon receipt, store the kit at +2..8°C into a temperature controlled refrigerator or cold room.
6. 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.
7. Check that the reagents are clear and do not contain visible heavy particles or aggregates. If not, advise the laboratory supervisor to initiate the necessary procedures.
8. Avoid cross-contamination between serum/plasma samples by using disposable tips and changing them after each sample. Do not reuse disposable tips.
9. Avoid cross-contamination between kit reagents by using disposable tips and changing them between the use of each one. Do not reuse disposable tips.
10. Do not use the kit after the expiration date stated on external (primary container) and internal (vials) labels.
11. Treat all specimens as potentially infective. All human serum specimens should be handled at Biosafety Level 2, as recommended by the Center for Disease Control, Atlanta, U.S. in compliance with what reported in the Institutes of Health's publication: "Biosafety in Microbiological and Biomedical Laboratories", ed. 1984.
12. The use of disposable plastic labware is recommended in the preparation of the washing solution or in transferring components into other containers of automated workstations, in order to avoid contamination.
13. Waste produced during the use of the kit has 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. 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..
14. Accidental spills 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.
15. The Sulphuric Acid is an irritant. In case of spills, wash the surface with plenty of water.
16. Other waste materials generated from the use of the kit (example: tips used for samples and controls, used microplates) should be handled as potentially infective and disposed according to national directives and laws concerning laboratory wastes.

G. SPECIMEN: PREPARATION AND RECOMMANDATIONS

1. Blood is drawn aseptically by venepuncture and plasma or serum is prepared using standard techniques of preparation of samples for clinical laboratory analysis. No influence has been observed in the preparation of the sample with citrate, EDTA and heparin.
2. Avoid any addition of preservatives to samples; especially sodium azide as this chemical would affect the enzymatic activity of the conjugate.
3. Samples have to be clearly identified with codes or names in order to avoid misinterpretation of results. When the kit is used for the screening of blood units, bar code labeling and electronic reading is strongly recommended.
4. Haemolysed (red) and visibly hyperlipemic ("milky") samples have to be discarded as they could generate false results. Samples containing residues of fibrin or heavy particles or microbial filaments and bodies should be discarded as they could give rise to false results.
5. Sera and plasma can be stored at +2°…+8°C in primary collection tubes for up to five days after collection.
   Do not freeze primary tubes of collection. For longer storage periods, sera and plasma samples, carefully removed from the primary collection tube, can be stored frozen at –20°C for at least 12 months. Any frozen samples should not be frozen/thawed more than once as this may generate particles that could affect the test result.
6. If particles are present, centrifuge at 2.000 rpm for 20 min or filter using 0.2-0.8u filters to clean up the sample for testing.

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.

1. Antigen coated microwells:
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 manufacturing.
In this case, call Dia.Pro's customer service.
Unused strips have to be placed back into the aluminium pouch, with the desiccant supplied, firmly zipped and stored at +2°-8°C. When opened the first time, unused strips are stable until the humidity indicator inside the desiccant bag turns from yellow to green.

2. Negative Control:
Ready to use. Mix well on vortex before use.

3. Positive Control:
Ready to use. Mix well on vortex before use.

4. Calibrator:
Low positive control. Add precisely the volume of EIA grade water, reported on its label, to the lyophilized powder; let fully dissolve and then gently mix on vortex.
Note: *The dissolved calibrator is not stable. Store it frozen in aliquots at –20°C. When thawed do not freeze again; discard it.*

5. Wash buffer concentrate:
The whole content of the 20x concentrated solution has to be diluted with EIA grade water up to 1200 ml 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.
Note: *Once diluted, the wash solution is stable for 1 week at +2..8° C.*

6. Enzyme conjugate:
Ready to use. Mix well on vortex before use.
Avoid contamination of the liquid with oxidizing chemicals, dust or microbes. If this component has to be transferred, use only plastic, and if possible, sterile disposable containers.

7. 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

8. 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 ±2%.
2. The ELISA incubator has to be set at +37°C (tolerance of ±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.
4. Incubation times have a tolerance of ±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 ≤ 10 nm; (b) absorbance range from 0 to 4; (c) linearity to 4; repeatability ≥ 1%. Blanking is carried out on the well identified in the section "Assay Procedure". 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 work station, 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 "Validation of Test" and "Assay Performances". 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 for blood screening and when the number of samples to be tested exceed 20-30 units per run.
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.

L. PRE ASSAY CONTROLS AND OPERATIONS

1. Check the expiration date of the kit printed on the external label (primary container). Do not use if expired.
2. Check that the liquid components are not contaminated by visible particles or aggregates. Check that the Chromogen/Substrate is colorless or pale blue by aspirating a small volume of it with a sterile plastic pipette. Check that no breakage occurred in transportation and no spillage of liquid is present inside the box (primary container). Check that the aluminum pouch, containing the microplate, is not punctured or damaged.
3. Dilute all the content of the 20x concentrated Wash Solution as described above.
4. Dissolve the Calibrator as described above and gently mix.
5. Allow all the other components to reach room temperature (about 1 hr) and then mix gently on vortex all liquid reagents.
6. 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 as reported in the specific section.
7. Check that the ELISA reader is turned on or ensure it will be turned on at least 20 minutes before reading.
8. If using an automated work station, turn on, check settings and be sure to use the right assay protocol.
9. Check that the micropipettes are set to the required volume.
10. Check that all the other equipment is available and ready to use.
11. 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.

1. Place the required number of strips in the microplate holder. Leave A1 well empty for the operation of blanking.
Store the other strips into the bag in presence of the desiccant at +2..8°C, sealed.

2. Pipette 100 µl of Negative Control in triplicate, 100 µl Positive Control in single and then 100 µl of samples. Check that controls and samples have been correctly added.
Then incubate the microplate at **+37°C for 60 min**.

3. Wash the microplate as reported in section I.3.

4. In all the wells except A1, pipette 100 µl Enzyme Conjugate. Check that the reagent has been correctly added.
Then incubate the microplate at **+37°C for 60 min.**

Important note: *Be careful not to touch the inner surface of the well with the pipette tip when dispensing the Enzyme Conjugate. Contamination might occur.*

5. Wash the microplate as described.

6. Pipette 100 µl TMB/H_2O_2 mixture in each well, the blank wells included. Check that the reagent has been correctly added. Then incubate the microplate at **room temperature 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 using the same pipetting sequence as in step n° 6 to stop the enzymatic reaction. Addition of the stop solution will turn the negative control and negative samples from blue to yellow.

8. Measure the colour 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.

Important notes:

1. *Ensure that no finger prints are present on the bottom of the microwell before reading. Finger prints could generate false positive results on reading.*
2. *Reading has should ideally be performed immediately after the addition of the Stop Solution but definitely no longer than 20 minutes afterwards. Some self oxidation of the chromogen can occur leading to a higher background.*
3. *The use of the Calibrator, a low positive control, is not mandatory for the assay as the CAL does not enter into the cut-off calculation. The CAL may be used as a low titer positive control when a laboratory internal quality verification is required by the management. When used for such purpose, dispense 100 ul of it, possibly in duplicate.*

N. ASSAY SCHEME

Controls/Calibrator Samples	100 ul 100 ul
1st incubation	**60 min**
Temperature	+37°C
Washing step	n° 5 cycles with 20'' of soaking OR n° 6 cycles without soaking
Enzyme Conjugate	100 ul
2nd incubation	**60 min**
Temperature	+37°C
Washing step	n° 5 cycles with 20'' of soaking OR n° 6 cycles without soaking
TMB/H2O2 mix	100 ul
3rd incubation	**20 min**
Temperature	r.t.
Sulphuric Acid	100 ul
Reading OD	450nm / 620-630nm

An example of dispensation scheme (including CAL) is reported in the table below:

Microplate

	1	2	3	4	5	6	7	8	9	10	11	12
A	BLK	S2										
B	NC	S3										
C	NC	S4										
D	NC	S5										
E	CAL	S6										
F	CAL	S7										
G	PC	S8										
H	S1	S9										

Legenda: BLK = Blank NC = Negative Control
CAL = Calibrator PC = Positive Control S = Sample

O. INTERNAL QUALITY CONTROL

A check is performed on the negative and positive controls any time, and on the Calibrator in addition when the kit is used for the first time, in order to verify whether the expected OD450nm / 620-630nm or Co/S values have been matched in the analysis. Ensure that the following parameters are met:

Parameter	Requirements
Blank well	< 0.100 OD450nm value
Negative Control (NC)	> 1.000 OD450nm after blanking If lower carefully control the washing procedure and decrease the number of cycles or the soaking time coefficient of variation < 30%
Positive Control (PC)	OD450 nm < NC/10
Calibrator (CAL)	PC < OD450nm < (NC+PC)/5

If the results of the test match the requirements stated above, proceed to the next section.
If they don't, do not proceed any further and perform the following checks:

Problem	Check
Blank well > 0.100 OD450nm	that the Chromogen/Substrate solution has not become contaminated during the assay
Negative Control (NC) < 1.000 OD450nm after blanking coefficient of variation > 30%	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 control instead of negative control); 4. that no contamination of the negative control or of the wells where the control was dispensed has occurred due to positive samples, to spills or to 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.

Calibrator OD450nm Outside the range	1. that the procedure has been correctly performed; 2. that no mistake has occurred during its distribution (ex.: dispensation of negative control instead of Calibrator); 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.
Positive Control OD450nm > NC/10	1. that the procedure has been correctly performed; 2. that no mistake has occurred during the distribution of the control (dispensation of negative control instead of positive control). 3. that the washing procedure and the washer settings are as validated in the pre qualification study; 4. that no external contamination of the positive control 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, point 8.

P. RESULTS
The results are calculated by means of a cut-off value determined with the following formula:

Cut-Off = (NC + PC) / 5

***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
Results are interpreted as ratio between the cut-off value and the sample OD450nm / 620-630nm or Co/S. Results are interpreted according to the following table:

Co/S	Interpretation
< 0.9	Negative
0.9 – 1.1	Equivocal
> 1.1	Positive

A negative result indicates that the patient has not been infected by HDV.
Any patient showing an equivocal result should be re-tested on a second sample taken 1-2 weeks after the initial sample.
A positive result is indicative of HDV 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 judgement errors and misinterpretations.*
2. *When test results are transmitted from the laboratory to another facility, attention must be paid to avoid erroneous data transfer.*
3. *Diagnosis of viral hepatitis infection has to be taken by and released to the patient by a suitably qualified medical doctor.*

An example of calculation is reported below (data obtained proceeding as the the reading step described in the section M, point 8).

The following data must not be used instead of real figures obtained by the user.

Negative Control: 2.100 – 2.200 – 2.000 OD450nm
Mean Value: 2.100 OD450nm
Higher than 1.000 – Accepted

Positive Control: 0.100 OD450nm
Lower than NC/10 – Accepted

Cut-Off = (2.100 + 0.100) / 5 = 0.440

Calibrator: 0.300-0.260 OD450nm
Mean value: 0.280 OD450nm
Within the range PC ≤ OD450nm < (NC+PC)/5 – *Accepted*

Sample 1: 0.020 OD450nm
Sample 2: 1.900 OD450nm
Sample 1 Co/S > 1.1 positive
Sample 2 Co/S < 0.9 negative

R. PERFORMANCES
Evaluation of Performances has been conducted in accordance to what reported in the Common Technical Specifications or CTS (art. 5, Chapter 3 of IVD Directive 98/79/EC)

1. LIMIT OF DETECTION:
In absence of an international standard, the sensitivity of the assay has been calculated by means of the product named Accurun n° 127 supplied by Boston Biomedica Inc. – USA .
The table below reports the OD450nm shown by this preparation when diluted in Fetal Calf Serum to prepare a limiting dilution curve, in three different lots.

Co/S values

	DAB.CE	Lot # 1102	DAB.CE	Lot # 0103	DAB.CE	Lot # 0403
Accurun # 127	**OD450 nm**	**Co/S value**	**OD450 nm**	**Co/S value**	**OD450 nm**	**Co/S value**
1x	0.171	**3.0**	0.163	**2.9**	0.156	**2.8**
2x	0.187	**2.7**	0.176	**2.6**	0.179	**2.5**
4x	0.230	**2.2**	0.220	**2.1**	0.202	**2.2**
8x	0.298	**1.7**	0.285	**1.6**	0.271	**1.6**
16x	0.417	**1.2**	0.405	**1.1**	0.402	**1.1**
32x	0.514	**1.0**	0.490	***0.9***	0.482	***0.9***
64x	0.717	0.7	0.700	0.7	0.705	0.6
128x	1.063	0.5	1.006	0.5	1.015	0.4
CTRL (-)	2.484	//////////	2.261	//////////	2.114	//////////

2. DIAGNOSTIC SPECIFICITY AND SENSITIVITY
The diagnostic performances were evaluated in a clinical trial conducted by the Department of Gastro-Hepatology, Prof. M.Rizzetto, S.Giovanni Battista hospital, Torino, Italy, on more than 400 samples against a reference kit.
Negative, positive and potentially interfering samples were examined in the trial.
Both plasma, derived with different standard techniques of preparation (citrate, EDTA and heparin), and sera have been used to determine the specificity. No false reactivity due to the method of specimen preparation has been observed.
Results are briefly reported in the tables below:

Sensitivity	> 98 %
Specificity	> 98 %

3. PRECISION

The mean values obtained from a study conducted on two samples of different anti-HDV antibody reactivity, examined in 16 replicates in three separate runs for three lots of product, is reported below:

DAB.CE: lot #1102

Negative Control (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	2.342	2.428	2.433	2.401
Std.Deviation	0.113	0.106	0.122	0.114
CV %	4.8	4.4	5.0	4.7

Calibrator (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.298	0.289	0.286	0.291
Std.Deviation	0.023	0.027	0.026	0.025
CV %	7.7	9.3	9.1	8.7
Co/S	1.6	1.7	1.7	1.7

DAB.CE: lot #0103

Negative Control (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	2.208	2.237	2.246	2.230
Std.Deviation	0.105	0.108	0.108	0.107
CV %	4.7	4.8	4.8	4.8

Calibrator (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.269	0.277	0.266	0.271
Std.Deviation	0.026	0.024	0.025	0.025
CV %	9.8	8.5	9.5	9.3
Co/ S	1.7	1.7	1.7	1.7

DAB.CE: lot # 0403

Negative Control (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	2.246	2.221	2.182	2.216
Std.Deviation	0.097	0.103	0.118	0.106
CV %	4.3	4.6	5.4	4.8

Calibrator (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.286	0.273	0.280	0.280
Std.Deviation	0.027	0.023	0.026	0.025
CV %	9.3	8.5	9.1	9.0
Co/S	1.6	1.7	1.6	1.6

The variability shown in the tables did not result in sample misclassification.

Important note:
The performance data have been obtained proceeding as the reading step described in the section M, point 8.

S. LIMITATIONS

Bacterial contamination or heat inactivation of the specimen may affect the absorbance values of the samples with consequent alteration of the level of the analyte.
This test is suitable only for testing single samples and not pooled ones.
Diagnosis of an infectious disease should not be established on the basis of a single test result. The patient's clinical history, symptomatology, as well as other diagnostic data should be considered.

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All the IVD Products manufactured by the company are under the control of a certified Quality Management System approved by an EC Notified Body. Each lot is submitted to a quality control and released into the market only if conforming with the EC technical specifications and acceptance criteria.

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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.

18. BIBLIOGRAFÍA

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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**

Fabricante legal
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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

HCV Ab

Version 4.0 Enzyme Immunoassay for the determination of anti Hepatitis C Virus antibody in human serum and plasma

- for “in vitro” diagnostic use only -

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REF CVAB.CE
96,192,480,960 Tests

HCV Ab

A. INTENDED USE

Version 4.0 Enzyme ImmunoAssay (ELISA) for the determination of antibodies to Hepatitis C Virus in human plasma and sera. The kit is intended for the screening of blood units and the follow-up of HCV-infected patients.
For "in vitro" diagnostic use only.

B. INTRODUCTION

The World Health Organization (WHO) define Hepatitis C infection as follows:

"Hepatitis C is a viral infection of the liver which had been referred to as parenterally transmitted "non A, non B hepatitis" until identification of the causative agent in 1989. The discovery and characterization of the hepatitis C virus (HCV) led to the understanding of its primary role in post-transfusion hepatitis and its tendency to induce persistent infection.

HCV is a major cause of acute hepatitis and chronic liver disease, including cirrhosis and liver cancer. Globally, an estimated 170 million persons are chronically infected with HCV and 3 to 4 million persons are newly infected each year. HCV is spread primarily by direct contact with human blood. The major causes of HCV infection worldwide are use of unscreened blood transfusions, and re-use of needles and syringes that have not been adequately sterilized. No vaccine is currently available to prevent hepatitis C and treatment for chronic hepatitis C is too costly for most persons in developing countries to afford. Thus, from a global perspective, the greatest impact on hepatitis C disease burden will likely be achieved by focusing efforts on reducing the risk of HCV transmission from nosocomial exposures (e.g. blood transfusions, unsafe injection practices) and high-risk behaviours (e.g. injection drug use).

Hepatitis C virus (HCV) is one of the viruses (A, B, C, D, and E), which together account for the vast majority of cases of viral hepatitis. It is an enveloped RNA virus in the *flaviviridae* family which appears to have a narrow host range. Humans and chimpanzees are the only known species susceptible to infection, with both species developing similar disease. An important feature of the virus is the relative mutability of its genome, which in turn is probably related to the high propensity (80%) of inducing chronic infection. HCV is clustered into several distinct genotypes which may be important in determining the severity of the disease and the response to treatment.

The incubation period of HCV infection before the onset of clinical symptoms ranges from 15 to 150 days. In acute infections, the most common symptoms are fatigue and jaundice; however, the majority of cases (between 60% and 70%), even those that develop chronic infection, are a symptomatic. About 80% of newly infected patients progress to develop chronic infection. Cirrhosis develops in about 10% to 20% of persons with chronic infection, and liver cancer develops in 1% to 5% of persons with chronic infection over a period of 20 to 30 years. Most patients suffering from liver cancer who do not have hepatitis B virus infection have evidence of HCV infection. The mechanisms by which HCV infection leads to liver cancer are still unclear. Hepatitis C also exacerbates the severity of underlying liver disease when it coexists with other hepatic conditions. In particular, liver disease progresses more rapidly among persons with alcoholic liver disease and HCV infection. HCV is spread primarily by direct contact with human blood. Transmission through blood transfusions that are not screened for HCV infection, through the reuse of inadequately sterilized needles, syringes or other medical equipment, or through needle-sharing among drug-users, is well documented. Sexual and perinatal transmission may also occur, although less frequently. Other modes of transmission such as social, cultural, and behavioural practices using percutaneous procedures (e.g. ear and body piercing, circumcision, tattooing) can occur if inadequately sterilized equipment is used. HCV is not spread by sneezing, hugging, coughing, food or water, sharing eating utensils, or casual contact.

In both developed and developing countries, high risk groups include injecting drug users, recipients of unscreened blood, haemophiliacs, dialysis patients and persons with multiple sex partners who engage in unprotected sex. In developed countries, it is estimated that 90% of persons with chronic HCV infection are current and former injecting drug users and those with a history of transfusion of unscreened blood or blood products. In many developing countries, where unscreened blood and blood products are still being used, the major means of transmission are unsterilized injection equipment and unscreened blood transfusions. In addition, people who use traditional scarification and circumcision practices are at risk if they use or re-use unsterilized tools.

WHO estimates that about 170 million people, 3% of the world's population, are infected with HCV and are at risk of developing liver cirrhosis and/or liver cancer. The prevalence of HCV infection in some countries in Africa, the Eastern Mediterranean, South-East Asia and the Western Pacific (when prevalence data are available) is high compared to some countries in North America and Europe.

Diagnostic tests for HCV are used to prevent infection through screening of donor blood and plasma, to establish the clinical diagnosis and to make better decisions regarding medical management of a patient. Diagnostic tests commercially available today are based on Enzyme immunosorbent assays (EIA) for the detection of HCV specific antibodies. EIAs can detect more than 95% of chronically infected patients but can detect only 50% to 70% of acute infections. A recombinant immunoblot assay (RIBA) that identifies antibodies which react with individual HCV antigens is often used as a supplemental test for confirmation of a positive EIA result. Testing for HCV circulating by amplification tests RNA (e.g. polymerase chain reaction or PCR, branched DNA assay) is also being utilized for confirmation of serological results as well as for assessing the effectiveness of antiviral therapy. A positive result indicates the presence of active infection and a potential for spread of the infection and or/the development of chronic liver disease.

Antiviral drugs such as interferon taken alone or in combination with ribavirin, can be used for the treatment of persons with chronic hepatitis C, but the cost of treatment is very high. Treatment with interferon alone is effective in about 10% to 20% of patients. Interferon combined with ribavirin is effective in about 30% to 50% of patients. Ribavirin does not appear to be effective when used alone.

There is no vaccine against HCV. Research is in progress but the high mutability of the HCV genome complicates vaccine development. Lack of knowledge of any protective immune response following HCV infection also impedes vaccine research. It is not known whether the immune system is able to eliminate the virus.

Some studies, however, have shown the presence of virus neutralizing antibodies in patients with HCV infection. In the absence of a vaccine, all precautions to prevent infection must be taken including (a) screening and testing of blood and organ donors; (b) Virus inactivation of plasma derived products; (c) implementation and maintenance of infection control practices in health care settings, including appropriate sterilization of medical and dental equipment; (d) promotion of behaviour change among the general public and health care workers to reduce overuse of injections and to use safe injection practices; and (e) Risk reduction counselling for persons with high-risk drug and sexual practices. "

The genome encodes for structural components, a nucleocapsid protein and two envelope glycoproteins, and functional constituents involved in the virus replication and protein processing.
The nucleocapsid-encoding region seems to be the most conservative among the isolates obtained all over the world.

C. PRINCIPLE OF THE TEST
Microplates are coated with HCV-specific antigens derived from "core" and "ns" regions encoding for conservative and immunodominant antigenic determinants (Core peptide, recombinant NS3, NS4 and NS5 peptides).
The solid phase is first treated with the diluted sample and HCV Ab are captured, if present, by the antigens.
After washing out all the other components of the sample, in the 2nd incubation bound HCV antibodies, IgG and IgM as well, are detected by the addition of polyclonal specific anti hIgG&M antibodies, labelled with peroxidase (HRP).
The enzyme captured on the solid phase, acting on the substrate/chromogen mixture, generates an optical signal that is proportional to the amount of anti HCV antibodies present in the sample. A cut-off value let optical densities be interpreted into HCV antibody negative and positive results.

D. COMPONENTS
Code CVAB.CE contains reagents for 192 tests.

1. Microplate MICROPLATE
n° 2 microplates
12 strips of 8 microwells coated with Core peptide, recombinant NS3, NS4 and NS5 peptides. Plates are sealed into a bag with desiccant.

2. Negative Control CONTROL -
1x4.0ml/vial. Ready to use control. It contains 1% goat serum proteins, 10 mM Na-citrate buffer pH 6.0 +/-0.1, 0.5% Tween 20, 0.09% Na-azide and 0.045% ProClin 300 as preservatives. The negative control is olive green colour coded.

3. Positive Control CONTROL +
1x4.0ml/vial. Ready to use control. It contains 1% goat serum proteins, human antibodies positive to HCV, 10 mM Na-citrate buffer pH 6.0 +/-0.1, 0.5% Tween 20, 0.09% Na-azide and 0.045% ProClin 300 as preservatives. The Positive Control is blue colour coded.

4. Calibrator CAL ….
n° 2 vials. Lyophilized calibrator. To be dissolved with the volume of EIA grade water reported on the label. It contains foetal bovine serum proteins, human antibodies to HCV whose content is calibrated on the NIBSC Working Standard code 99/588-003-WI, 10 mM Na-citrate buffer pH 6.0 +/-0.1, 0.3 mg/ml gentamicine sulphate and 0.045% ProClin 300 as preservatives.

Note: The volume necessary to dissolve the content of the vial may vary from lot to lot. Please use the right volume reported on the label .

5. Wash buffer concentrate WASHBUF 20X
2x60ml/bottle. 20x concentrated solution. Once diluted, the wash solution contains 10 mM phosphate buffer pH 7.0+/-0.2, 0.05% Tween 20 and 0.045% ProClin 300.

6. Enzyme Conjugate CONJ
2x16ml/vial. Ready to use and pink/red colour coded reagent. It contains Horseradish Peroxidase conjugated goat polyclonal antibodies to human IgG and IgM, 5% BSA, 10 mM Tris buffer pH 6.8+/-0.1, 0.045% ProClin 300 and 0.02% gentamicine sulphate as preservatives.

7. Chromogen/Substrate SUBS TMB
2x16ml/vial. Ready-to-use component. It contains 50 mM citrate-phosphate buffer pH 3.5-3.8, 4% dimethylsulphoxide, 0.03% tetra-methyl-benzidine or TMB and 0.02% hydrogen peroxide or H2O2.
Note: To be stored protected from light as sensitive to strong illumination.

8. Assay Diluent DILAS
1x15ml/vial. 10 mM tris buffered solution pH 8.0 +/-0.1 containing 0.045% ProClin 300 for the pre-treatment of samples and controls in the plate, blocking interference.

9. Sulphuric Acid H_2SO_4 O.3 M
1x32ml/bottle. It contains 0.3 M H_2SO_4 solution.
Attention: Irritant (H315; H319; P280; P302+P352; P332+P313; P305+P351+P338; P337+P313; P362+P363)

10. Sample Diluent: DILSPE
2x50ml/bottle. It contains 1% goat serum proteins, 10 mM Na-citrate buffer pH 6.0 +/-0.1, 0.5% Tween 20, 0.09% Na-azide and 0.045% ProClin 300 as preservatives. To be used to dilute the sample.
Note: The diluent changes colour from olive green to dark bluish green in the presence of sample.

11. Plate sealing foils **n° 4**

12. Package insert **n° 1**

Important note: Only upon specific request , Dia.Pro can supply reagents for 96, 480, 960 tests , as reported below**:**

1. Microplate	n°1	n°5	n°10
2.NegativeControl	1x2.0ml/vial	1x10ml/vial	1x20.ml/vial
3.PositiveControl	1x2.0ml/vial	1x10ml/vial	1x20.ml/vial
4.Calibrator	n° 1 vial	n° 5 vials	n° 10 vials
5.Wash buff conc	1x60ml/bottle	5x60ml/bottles	4x150ml/bottles
6.Enz. Conjugate	1x16ml/vial	2x40ml/bottles	4x40ml/bottles
7.Chromog/Subs	1x16ml/vial	2x40ml/bottles	4x40ml/bottles
8.Assay Diluent	1x8ml/vial	1x40ml/bottle	1x80ml/bottle
9.Sulphuric Acid	1x15ml/vial	2x40ml/bottle	2x80ml/bottles
10.SampleDiluent	1x50ml/vial	5x50ml/bottles	4x125ml/bottles
11.Plate seal foils	n° 2	n° 10	n° 20
12. Pack. insert	n° 1	n° 1	n° 1
Number of tests	**96**	**480**	**960**
Code	**CVAB.CE.96**	**CVAB.CE.480**	**CVAB.CE.960**

E. MATERIALS REQUIRED BUT NOT PROVIDED

1. Calibrated Micropipettes (200ul and 10ul) and disposable plastic tips.
2. EIA grade water (bidistilled 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 capable to provide a temperature of +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.

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. When the kit is used for the screening of blood units and blood components, it has to be used in a laboratory certified and qualified by the national authority in that field (Ministry of Health or similar entity) to carry out this type of analysis.
3. 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.
4. All the personnel involved in sample handling should be vaccinated for HBV and HAV, for which vaccines are available, safe and effective.
5. 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.
6. Upon receipt, store the kit at 2..8°C into a temperature controlled refrigerator or cold room.
7. 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.
8. Check that the reagents 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.
9. Avoid cross-contamination between serum/plasma samples by using disposable tips and changing them after each sample. Do not reuse disposable tips.
10. Avoid cross-contamination between kit reagents by using disposable tips and changing them between the use of each one. Do not reuse disposable tips.
11. Do not use the kit after the expiration date stated on the external container and internal (vials) labels.
12. Treat all specimens as potentially infective. All human serum specimens should be handled at Biosafety Level 2, as recommended by the Center for Disease Control, Atlanta, U.S. in compliance with what reported in the Institutes of Health's publication: "Biosafety in Microbiological and Biomedical Laboratories", ed. 1984.
13. 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.
14. Waste produced during the use of the kit has 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..
15. 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.
16. The Sulphuric Acid is an irritant. In case of spills, wash the surface with plenty of water
17. Other waste materials generated from the use of the kit (example: tips used for samples and controls, used microplates) should be handled as potentially infective and disposed according to national directives and laws concerning laboratory wastes.

G. SPECIMEN: PREPARATION AND RECOMMANDATIONS

1. Blood is drawn aseptically by venipuncture and plasma or serum is prepared using standard techniques of preparation of samples for clinical laboratory analysis. No influence has been observed in the preparation of the sample with citrate, EDTA and heparin.
2. 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.
3. Samples have to be clearly identified with codes or names in order to avoid misinterpretation of results. When the kit is used for the screening of blood units, bar code labeling and electronic reading is strongly recommended.
4. Haemolysed (red) and visibly hyperlipemic ("milky") samples have to be discarded as they could generate false results. Samples containing residues of fibrin or heavy particles or microbial filaments and bodies should be discarded as they could give rise to false results.
5. Sera and plasma can be stored at +2°…+8°C in primary collection tubes for up to five days after collection.
Do not freeze primary tubes of collection. For longer storage periods, sera and plasma samples, carefully removed from the primary collection tube, can be stored frozen at –20°C for several months. Any frozen samples should not be frozen/thawed more than once as this may generate particles that could affect the test result.
6. If particles are present, centrifuge at 2.000 rpm for 20 min or filter using 0.2-0.8u filters to clean up the sample for testing.

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-use of the device and up to 6 months.

1. Microplates:
Allow the microplate to reach room temperature (about 1 hr) before opening the container. Check that the desiccant is not turned to dark green, indicating a defect of manufacturing.
In this case call Dia.Pro's customer service.
Unused strips have to be placed back into the aluminium pouch, in presence of desiccant supplied, firmly zipped and stored at +2°..8°C.
When opened the first time, residual strips are stable till the indicator of humidity inside the desiccant bag turns from yellow to green.

2. Negative Control:
Ready to use. Mix well on vortex before use.

3. Positive Control:
Ready to use. Mix well on vortex before use. Handle this component as potentially infective, even if HCV, eventually present in the control, has been chemically inactivated.

4. Calibrator:
Dissolve carefully the content of the lyophilised vial with the volume of EIA grade water reported on its label.
Mix well on vortex before use.
Handle this component as potentially infective, even if HCV, eventually present in the control, has been chemically inactivated.
***Note**: When dissolved the Calibrator is not stable. Store in aliquots at –20°C.*

5. Wash buffer concentrate:
The 20x concentrated solution has to be diluted with EIA grade water up to 1200 ml and mixed gently end-over-end before use. As some salt crystals may be present into the vial, take care to dissolve all the content when preparing the solution.
In the preparation avoid foaming as the presence of bubbles could give origin to a bad washing efficiency.
***Note:** Once diluted, the wash solution is stable for 1 week at +2..8° C.*

6. Enzyme conjugate:
Ready to use. Mix well on vortex before use.
Be careful not to contaminate the liquid with oxidizing chemicals, air-driven dust or microbes.
If this component has to be transferred use only plastic, possibly sterile disposable containers.

7. Chromogen/Substrate:
Ready to use. Mix well on vortex before use.
Be careful not to contaminate the liquid with oxidizing chemicals, air-driven dust or microbes.
Do not expose to strong illumination, oxidizing agents and metallic surfaces.
If this component has to be transferred use only plastic, possible sterile disposable container.

8. Assay Diluent:
Ready to use. Mix well on vortex before use.

9. 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).

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.

10. Sample Diluent:
Ready to use. Mix well on vortex before use.

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 (household alcohol, 10% solution of bleach, hospital grade disinfectants) of those parts that could accidentally come in contact with the sample. They should also be regularly maintained in order to show a precision of 1% and a trueness of +/-2%. Decontamination of spills or residues of kit components should also be carried out regularly.
2. The ELISA incubator has to be set at +37°C (tolerance of +/-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.
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; (d) repeatability $\geq$ 1%. Blanking is carried out on the well identified in the section "Assay Procedure". 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 work station, all critical steps (dispensation, incubation, washing, reading, data handling) have to be carefully set, calibrated, controlled and regularly serviced in order to match the values reported in the section O "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 and for washing. This must be studied and controlled to minimize the possibility of contamination of adjacent wells. The use of ELISA automated work stations is recommended for blood screening when the number of samples to be tested exceed 20-30 units per run.
7. When using automatic devices, in case the vial holder of the instrument does not fit with the vials supplied in the kit, transfer the solution into appropriate containers and label them with the same label peeled out from the original vial. This operation is important in order to avoid mismatching contents of vials, when transferring them. When the test is over, return the secondary labeled containers to 2..8°C, firmly capped.
8. 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 compliance with the requirements described. Support is also provided for the installation of new instruments to be used with the kit.

L. PRE ASSAY CONTROLS AND OPERATIONS

1. Check the expiration date of the kit printed on the external label of the kit box. Do not use if expired.
2. 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.
3. Dilute all the content of the 20x concentrated Wash Solution as described above.
4. Dissolve the Calibrator as described above.
5. Allow all the other components to reach room temperature (about 1 hr) and then mix as described.
6. 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 as reported in the specific section.
7. Check that the ELISA reader has been turned on at least 20 minutes before reading.
8. If using an automated workstation, turn it on, check settings and be sure to use the right assay protocol.
9. Check that the micropipettes are set to the required volume.
10. Check that all the other equipment is available and ready to use.
11. 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.

Automated assay:

In case the test is carried out automatically with an ELISA system, we suggest to make the instrument aspirate 200 ul Sample Diluent and then 10 ul sample.
All the mixture is then carefully dispensed directly into the appropriate sample well of the microplate. Before the next sample is aspirated, needles have to be duly washed to avoid any cross-contamination among samples.
Do not dilute controls/calibrator as they are ready to use.
Dispense 200 ul controls/calibrator in the appropriate control/calibration wells.

Important Note: *Visually monitor that samples have been diluted and dispensed into appropriate wells. This is simply achieved by checking that the colour of dispensed samples has turned to dark bluish-green while the colour of the negative control has remained olive green.*

For the next operations follow the operative instructions reported below for the Manual Assay.
It is strongly recommended to check that the time lap between the dispensation of the first and the last sample will be calculated by the instrument and taken into consideration by delaying the first washing operation accordingly.

Manual assay:

1. Place the required number of Microwells in the microwell holder. Leave the 1st well empty for the operation of blanking.
2. Dispense 200 ul of Negative Control in triplicate, 200 ul Calibrator in duplicate and 200 ul Positive Control in single in proper wells. Do not dilute Controls and Calibrator as they are pre-diluted, ready to use !
3. Add 200 ul of Sample Diluent (DILSPE) to all the sample wells; then dispense 10 ul sample in each properly identified well. Mix gently the plate, avoiding overflowing and contaminating adjacent wells, in order to fully disperse the sample into its diluent.

Important note: *Check that the colour of the Sample Diluent, upon addition of the sample, changes from light green to dark bluish green, monitoring that the sample has been really added.*

4. Dispense 50 ul Assay Diluent (DILAS) into all the controls/calibrator and sample wells. Check that the color of samples has turned to dark blue.
5. Incubate the microplate for **45 min at +37°C**.

Important note: *Strips have to be sealed with the adhesive sealing foil, supplied, only when the test is carried out manually. Do not cover strips when using ELISA automatic instruments.*

6. Wash the microplate with an automatic washer by delivering and aspirating 350ul/well of diluted washing solution as reported previously (section I.3).
7. Pipette 100µl Enzyme Conjugate into each well, except the 1st blanking well, and cover with the sealer. Check that this pink/red coloured component has been dispensed in all the wells, except A1.

Important note: *Be careful not to touch the plastic inner surface of the well with the tip filled with the Enzyme Conjugate. Contamination might occur.*

8. Incubate the microplate for **45 min at +37°C**.
9. Wash microwells as in step 6.
10. Pipette 100µl Chromogen/Substrate mixture into each well, the blank well included. Then incubate the microplate at **room temperature (18-24°C) for 15 minutes**.

Important note: *Do not expose to strong direct illumination. High background might be generated.*

11. Pipette 100µl Sulphuric Acid into all the wells using the same pipetting sequence as in step 10 to stop the enzymatic reaction. Addition of acid will turn the positive control and positive samples from blue to yellow/brown.
12. Measure the colour intensity of the solution in each well, as described in section I.5, at 450nm filter (reading) and at 620-630nm (background subtraction), blanking the instrument on A1 (mandatory).

Important notes:

1. *Ensure that no finger prints are present on the bottom of the microwell before reading. Finger prints could generate false positive results on reading.*
2. *Reading has to be carried out just after the addition of the Stop Solution and anyway not any longer than 20 minutes after its addition. Some self oxidation of the chromogen can occur leading to high background.*
3. *Shaking at 350 ±150 rpm during incubation has been proved to increase the sensitivity of the assay of about 20%.*
4. *The Calibrator (CAL) does not affect the cut-off calculation and therefore the test results calculation. The Calibrator may be used only when a laboratory internal quality control is required by the management.*

N. ASSAY SCHEME

Method	Operations
Controls & Calibrator Samples Assay Diluent (DILAS)	200 ul 200ul dil.+10ul 50 ul
1st incubation	**45 min**
Temperature	+37°C
Wash step	n° 5 cycles with 20'' of soaking OR n° 6 cycles without soaking
Enzyme conjugate	100 ul
2nd incubation	**45 min**
Temperature	+37°C
Wash step	n° 5 cycles with 20'' of soaking OR n° 6 cycles without soaking
TMB/H2O2	100 ul
3rd incubation	**15 min**
Temperature	r.t.
Sulphuric Acid	100 ul
Reading OD	450nm / 620-630nm

An example of dispensation scheme is reported below:

Microplate

	1	2	3	4	5	6	7	8	9	10	11	12
A	BLK	S2										
B	NC	S3										
C	NC	S4										
D	NC	S5										
E	CAL	S6										
F	CAL	S7										
G	PC	S8										
H	S1	S9										

Legenda: BLK = Blank NC = Negative Control
CAL = Calibrator PC = Positive Control S = Sample

O. INTERNAL QUALITY CONTROL

A check is carried out on the controls and the calibrator any time the kit is used in order to verify whether their OD450nm values are as expected and reported in the table below.

Check	Requirements
Blank well	< 0.100 OD450nm value
Negative Control (NC)	< 0.050 mean OD450nm value after blanking
Calibrator	S/Co > 1.1
Positive Control	> 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 operate as follows:

Problem	Check
Blank well > 0.100 OD450nm	1. that the Chromogen/Sustrate solution has not got contaminated during the assay
Negative Control (NC) > 0.050 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 control instead of negative control; 4. that no contamination of the negative control or of their wells has occurred due to positive samples, to spills or to the enzyme conjugate; 5. that micropipettes haven't got contaminated with positive samples or with the enzyme conjugate 6. that the washer needles are not blocked or partially obstructed.
Calibrator S/Co < 1.1	1. that the procedure has been correctly executed; 2. that no mistake has been done in its distribution (ex.: dispensation of negative control instead of control serum) 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.
Positive Control < 1.000 OD450nm	1. that the procedure has been correctly executed; 2. that no mistake has been done in the distribution of controls (dispensation of negative control instead of positive control. In this case, the negative control will have an OD450nm value > 0.150, too. 3. that the washing procedure and the washer settings are as validated in the pre qualification study; 4. that no external contamination of the positive control has occurred.

Should these problems happen, after checking, report any residual problem to the supervisor for further actions.

P. CALCULATION OF THE CUT-OFF

The tests results are calculated by means of a cut-off value determined with the following formula on the mean OD450nm value of the Negative Control (NC):

NC + 0.350 = Cut-Off (Co)

The value found for the test is used for the interpretation of results as described in the next paragraph.

***Important note**: When the calculation of results is done by the operative system of an ELISA automated work station be sure that the proper formulation is used to calculate the cut-off value and generate the right interpretations of results.*

Q. INTERPRETATION OF RESULTS

Test results are interpreted as ratio of the sample OD450nm and the Cut-Off value (or S/Co) according to the following table:

S/Co	Interpretation
< 0.9	Negative
0.9 - 1.1	Equivocal
> 1.1	Positive

A negative result indicates that the patient has not been infected by HCV or that the blood unit may be transfused.
Any patient showing an equivocal result should be tested again on a second sample taken 1-2 weeks later from the patient and examined. The blood unit should not be transfused.
A positive result is indicative of HCV infection and therefore the patient should be treated accordingly or the blood unit should be discarded.

Important notes:

1. *Interpretation of results should be done under the supervision of the responsible of the laboratory to reduce the risk of judgment errors and misinterpretations.*
2. *Any positive result should be confirmed by an alternative method capable to detect IgG and IgM antibodies (confirmation test) before a diagnosis of viral hepatitis is formulated.*
3. *As proved in the Performance Evaluation of the product, the assay is able to detect seroconversion to anti HCV core antibodies* ***earlier*** *than some other commercial kits. Therefore a positive result, not confirmed with these commercial kits, does not have to be ruled out as a false positive result ! The sample has to be anyway submitted to a confirmation test (supplied upon request by DiaPro srl, code CCONF).*
4. *As long as the assay is able to detect also IgM antibodies some discrepant results with other commercial products for the detection of anti HCV antibodies - lacking anti hIgM conjugate in the formulation of the enzyme tracer and therefore missing IgM reactivity - may be present. The real positivity of the sample for antibodies to HCV should be then confirmed by examining also IgM reactivity, important for the diagnosis of HCV infection.*
5. *When test results are transmitted from the laboratory to an informatics centre, attention has to be done to avoid erroneous data transfer.*
6. *Diagnosis of viral hepatitis infection has to be done and released to the patient only by a qualified medical doctor.*

An example of calculation is reported below:

The following data must not be used instead or real figures obtained by the user.
Negative Control: 0.019 – 0.020 – 0.021 OD450nm
Mean Value: 0.020 OD450nm
Lower than 0.050 – Accepted
Positive Control: 2.189 OD450nm
Higher than 1.000 – Accepted
Cut-Off = 0.020+0.350 = 0.370
Calibrator: 0.550 - 0.530 OD450nm
Mean value: 0.540 OD450nm S/Co = 1.4
S/Co higher than 1.1 – Accepted
Sample 1: 0.070 OD450nm
Sample 2: 1.690 OD450nm
Sample 1 S/Co < 0.9 = negative
Sample 2 S/Co > 1.1 = positive

R. PERFORMANCES

Evaluation of Performances has been conducted in accordance to what reported in the Common Technical Specifications or CTS (art. 5, Chapter 3 of IVD Directive 98/79/EC).

1. LIMIT OF DETECTION

The limit of detection of the assay has been calculated by means of the British Working Standard for anti-HCV, NIBSC code 99/588-003-WI. The table below reports the mean OD450nm values of this standard when diluted in negative plasma and then examined.

Dilution	Lot # 1	Lot # 2
Factor	S/Co	S/Co
1 X	**2.0**	**2.0**
2 X	**1.1**	**1.2**
4 X	0.7	0.8
8 X	0.5	0.5
Negative plasma	0.3	0.3

In addition the sample coded Accurun 1 – series 3000 - supplied by Boston Biomedica Inc., USA, has been evaluated “in toto” showing the results below:

CVAB.CE Lot ID	Accurun 1 Series	S/Co
1201	3000	1.5
0602	3000	1.5
1202	3000	1.9

In addition, n° 7 samples, tested positive for HCV Ab with Ortho HCV 3.0 SAVe, code 930820, lot. # EXE065-1, were diluted in HCV Ab negative plasma in order to generate limiting dilutions and then tested again on CVAB.CE, lot. # 1202, and Ortho.
The following table reports the data obtained.

Sample n°	Limit Dilution	CVAB.CE S/Co	Ortho 3.0 S/Co
1	256 X	**1.9**	**1.3**
2	256 X	**1.9**	0.7
3	256 X	**2.4**	**1.0**
4	128 X	**2.5**	**3.2**
5	85 X	**3.3**	**1.4**
6	128 X	**2.2**	0.8
7	135 X	**3.2**	**2.2**

2. DIAGNOSTIC SPECIFICITY AND SENSITIVITY

The Performance Evaluation of the device was carried out in a trial conducted on more than total 5000 samples.

2.1 Diagnostic specificity:

It is defined as the probability of the assay of scoring negative in the absence of specific analyte. In addition to the first study, where a total of 5043 unselected blood donors,(including 1st time donors), 210 hospitalized patients and 162 potentially interfering specimens (other infectious diseases, E.coli antibody positive, patients affected by non viral hepatic diseases, dialysis patients, pregnant women, hemolized, lipemic, etc.) were examined, the diagnostic specificity was recently assessed by testing a total of 2876 negative blood donors on six different lots. A value of specificity of 100% was found.
No false reactivity due to the method of specimen preparation has been observed. Both plasma, derived with different standard techniques of preparation (citrate, EDTA and heparin), and sera have been used to determine the value of specificity.
Frozen specimens have been tested, as well, to check for interferences due to collection and storage.
No interference was observed.

2.2 Diagnostic Sensitivity

It defined as the probability of the assay of scoring positive in the presence of specific analyte.
The diagnostic sensitivity has been assessed externally on a total number of 359 specimens; a diagnostic sensitivity of 100% was found. Internally more than other 50 positive samples were tested, providing a value of diagnostic sensitivity of again 100%.
Positive samples from infections carried out by different genotypes of HCV were tested as well.
Furthermore, most of seroconversion panels available from Boston Biomedica Inc., USA, (PHV) and Zeptometrix, USA, (HCV) have been studied.
Results are reported below for some of them.

Panel	N° samples	DiaPro*	Ortho* **
PHV 901	11	9	9
PHV 904	7	2	4
PHV 905	9	3	4
PHV 906	7	7	7
PHV 907	7	3	2
PHV 908	13	10	8
PHV 909	3	2	2
PHV 910	5	3	3
PHV 911	5	3	3
PHV 912	3	1	1
PHV 913	4	2	2
PHV 914	9	5	5
PHV 915	4	3	0
PHV 916	8	4	3
PHV 917	10	6	6
PHV 918	8	2	0
PHV 919	7	3	3
PHV 920	10	6	6
HCV 10039	5	2	0
HCV 6212	9	6	7
HCV 10165	9	5	4

*Note: * Positive samples detected* *** HCV v.3.0*

Finally the Product has been tested on the panel EFS Ac HCV, lot n° 01/08.03.22C/01/A, supplied by the Etablissement Francais Du Sang (EFS), France, with the following results:

EFS Panel Ac HCV

Sample	Lot # 1 S/Co	Lot # 2 S/Co	Lot # 2 S/Co	Results expected
HCV 1	**2.2**	**2.4**	**2.6**	**positive**
HCV 2	**1.6**	**2.0**	**2.1**	**positive**
HCV 3	**1.5**	**1.7**	**1.6**	**positive**
HCV 4	**5.2**	**6.5**	**5.5**	**positive**
HCV 5	**1.6**	**1.8**	**1.6**	**positive**
HCV 6	0.4	0.4	0.4	negative

3. PRECISION:
It has been calculated on two samples, one negative and one low positive, examined in 16 replicates in three separate runs. Results are reported as follows:

Lot # 1202

Negative Sample (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.094	0.099	0.096	0.096
Std.Deviation	0.008	0.007	0.008	0.007
CV %	8.7	6.6	7.9	7.7

Cal # 2 – 7K (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.396	0.403	0.418	0.406
Std.Deviation	0.023	0.029	0.027	0.026
CV %	5.9	7.1	6.4	6.5
S/Co	1.1	1.1	1.2	1.1

Lot # 0602

Negative Sample (N = 16)

Mean values	1st run	2nd run	3rd run	Average
OD 450nm	0.097	0.096	0.094	0.096
Std.Deviation	0.009	0.010	0.008	0.009
CV %	8.9	10.1	8.4	9.1

Cal # 2 – 7K (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.400	0.395	0.393	0.396
Std.Deviation	0.021	0.025	0.026	0.024
CV %	5.4	6.2	6.6	6.1
S/Co	1.2	1.2	1.1	1.2

Lot # 0602/2

Negative Sample (N = 16)

Mean values	1st run	2nd run	3rd run	Average
OD 450nm	0.087	0.091	0.088	0.089
Std.Deviation	0.009	0.007	0.008	0.008
CV %	10.0	8.2	8.6	8.9

Cal # 2 – 7K (N = 16)

Mean values	1st run	2nd run	3rd run	Average
OD 450nm	0.386	0.390	0.391	0.389
Std.Deviation	0.023	0.021	0.023	0.022
CV %	6.0	5.3	5.8	5.7
S/Co	1.1	1.2	1.2	1.2

The variability shown in the tables above did not result in sample misclassification.

S. LIMITATIONS
Repeatable false positive results, not confirmed by RIBA or similar confirmation techniques, were assessed as less than 0.1% of the normal population.
Frozen samples containing fibrin particles or aggregates after thawing have been observed to generate some false results.

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All the IVD Products manufactured by the company are under the control of a certified Quality Management System approved by an EC Notified Body. Each lot is submitted to a quality control and released into the market only if conforming with the EC technical specifications and acceptance criteria.

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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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Ed. 09/2018 **DCM114-7**

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CE

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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Ed. 09/2018 **DCM114-7**

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CE

DiaMetra
care for quality

DCM114-7
Ed. 09/2018

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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Ed. 09/2018 **DCM114-7**

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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