Anti-LC-1 ELISA (IgG)
Test instruction

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

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

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

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

Contents of the test kit:

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

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

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

Preparation and stability of the reagents

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

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

- **Calibrator and controls:** Ready for use. The reagents must be mixed thoroughly before use.

- **Enzyme conjugate:** Ready for use. The enzyme conjugate must be mixed thoroughly before use.

- **Sample buffer:** Ready for use.

- **Wash buffer:** The wash buffer is a 10x concentrate. If crystallisation occurs in the concentrated buffer, warm it to +37°C and mix well before diluting. The quantity required should be removed from the bottle using a clean pipette and diluted with deionised or distilled water (1 part reagent plus 9 parts distilled water).
  For example: For 1 microplate strip, 5 ml concentrate plus 45 ml water.
  The working strength wash buffer is stable for 4 weeks when stored at +2°C to +8°C and handled properly.

- **Chromogen/substrate solution:** Ready for use. Close the bottle immediately after use, as the contents are sensitive to light ✳. The chromogen/substrate solution must be clear on use. Do not use the solution if it is blue coloured.

- **Stop solution:** Ready for use.

Warning: The calibrator and controls of human origin have tested negative for HBsAg, anti-HCV, anti-HIV-1 and anti-HIV-2. Nonetheless, all materials should be treated as being a potential infection hazard and should be handled with care. Some of the reagents contain the agent sodium azide in a non-declarable concentration. Avoid skin contact.

Preparation and stability of the patient samples

Samples: Human serum or EDTA, heparin or citrate plasma.

Stability: Patient samples to be investigated can generally be stored at +2°C to +8°C for up to 14 days. Diluted samples should be incubated within one working day.

Sample dilution: Patient samples are diluted **1:101** in sample buffer. For example: dilute 10 µl sample in 1.0 ml sample buffer and mix well by vortexing (sample pipettes are not suitable for mixing).

NOTE: The calibrator and controls are prediluted and ready for use, do not dilute them.

Incubation

Sample incubation: (1st step)
Transfer 100 µl of the calibrator, positive or negative control or diluted patient samples into the individual microplate wells according to the pipetting protocol. Incubate for **30 minutes** at room temperature (+18°C to +25°C).

Washing:
Manual: Empty the wells and subsequently wash 3 times using 300 µl of working strength wash buffer for each wash.
Automatic: Wash the reagent wells 3 times with 450 µl of working strength wash buffer (program setting: e.g. TECAN Columbus Washer "Overflow Mode").

Leave the wash buffer in each well for 30 to 60 seconds per washing cycle, then empty the wells. After washing (manual and automated tests), thoroughly dispose of all liquid from the microplate by tapping it on absorbent paper with the openings facing downwards to remove all residual wash buffer.

Note: Residual liquid (> 10 µl) remaining in the reagent wells after washing can interfere with the substrate and lead to false low extinction values. Insufficient washing (e.g., less than 3 wash cycles, too small wash buffer volumes, or too short residence times) can lead to false high extinction values.
Free positions on the microplate strip should be filled with blank wells of the same plate format as that of the parameter to be investigated.

Conjugate incubation: (2nd step)
Pipette 100 µl of enzyme conjugate (peroxidase-labelled anti-human IgG) into each of the microplate wells. Incubate for **30 minutes** at room temperature (+18°C to +25°C).

Washing:
Empty the wells. Wash as described above.

Substrate incubation: (3rd step)
Pipette 100 µl of chromogen/substrate solution into each of the microplate wells. Incubate for **15 minutes** at room temperature (+18°C to +25°C) protect from direct sunlight.

Stopping:
Pipette 100 µl of stop solution into each of the microplate wells in the same order and at the same speed as the chromogen/substrate solution was introduced.

Measurement:
Photometric measurement of the colour intensity should be made at a wavelength of 450 nm and a reference wavelength between 620 nm and 650 nm **within 30 minutes of adding the stop solution**. Prior to measuring, slightly shake the microplate to ensure a homogeneous distribution of the solution.

Pipetting protocol

	1	2	3	4	5	6	7	8	9	10	11	12
A	**C**	**P 6**	**P 14**	**P 22**								
B	**pos.**	**P 7**	**P 15**	**P 23**								
C	**neg.**	**P 8**	**P 16**	**P 24**								
D	**P 1**	**P 9**	**P 17**									
E	**P 2**	**P 10**	**P 18**									
F	**P 3**	**P 11**	**P 19**									
G	**P 4**	**P 12**	**P 20**									
H	**P 5**	**P 13**	**P 21**									

The above pipetting protocol is an example of the semiquantitative determination of antibodies in 24 patient samples (P 1 to P 24).

Calibrator (C), positive (pos.) and negative (neg.) control as well as the patient samples have been incubated in one well each. The reliability of the ELISA test can be improved by duplicate determinations of each sample.
The wells can be broken off individually from the strips. This makes it possible to adjust the number of test substrates used to the number of samples to be examined and minimises reagent wastage.
Both positive and negative controls serve as internal controls for the reliability of the test procedure. They should be assayed with each test run.

Calculation of results

Semiquantitative: Results can be evaluated semiquantitatively by calculating a ratio of the extinction value of the control or patient sample over the extinction value of calibrator. Use the following formula to calculate the ratio:

$$\frac{\textbf{Extinction of the control or patient sample}}{\textbf{Extinction of calibrator}} = \textbf{Ratio}$$

EUROIMMUN recommends interpreting results as follows:

Ratio <1.0: **negative**
Ratio ≥1.0: **positive**

For duplicate determinations the mean of the two values should be taken. If the two values deviate substantially from one another, EUROIMMUN recommends retesting the samples.

For diagnosis, the clinical picture of the patient patient always needs to be taken into account along with the serological findings.

Test characteristics

Calibration: As no international reference serum exists for antibodies against LC-1, the results are given as a ratio. This represents a relative measurement for the concentration of antibodies in serum or plasma.

For every group of tests performed, the extinction values of the calibrator and the ratio of the positive and negative control sera must lie within the limits stated for the relevant test kit lot. A quality control certificate containing these reference values is included. If the values specified for the control sera are not achieved, the test results may be inaccurate and the test should be repeated.

The binding activity of the antibodies and the activity of the enzyme used are temperature-dependent. It is therefore recommended using a thermostat in all three incubation steps. The higher the room temperature (+18°C to +25°C) during the incubation steps, the greater the extinction values will be. Corresponding variations apply also to the incubation times. However, the calibrator is subject to the same influences, so that such variations will be largely compensated in the calculation of the result.

Antigen: The microplate wells were coated with recombinant LC-1. The corresponding human cDNA was expressed in insect cells using a baculovirus vector. The specific target antigen of anti-LC-1 antibodies was identified in 1999 as the enzyme formiminotransferase cyclodeaminase (Lapierre et al.).

Detection limit: The lower detection limit is defined as the mean value of an analyte-free sample plus three times the standard deviation and is the smallest detectable antibody titer. The lower detection limit of the Anti-LC-1 ELISA (IgG) is ratio 0.05.

Cross reactivity: This ELISA specifically detects autoantibodies of class IgG against LC-1. When investigating patient sera for autoantibodies against SLA (n = 2) and LKM (n = 8) no cross reactions were found.

Interference: Haemolytic, lipaemic and icteric samples showed no influence on the result up to a concentration of 10 mg/ml for haemoglobin, 20 mg/ml for triglycerides and 0.4 mg/ml for bilirubin in this ELISA.

Reproducibility: The reproducibility of the test was investigated by determining the intra- and inter-assay coefficients of variation (CV) using 3 samples. The intra-assay CVs are based on 20 determinations and the inter-assay CVs on 4 determinations performed in 6 different test runs.

Intra-assay variation, n = 20		
Sample	**Mean value (Ratio)**	**CV (%)**
1	3.1	4.9
2	4.7	2.7
3	8.0	2.4

Inter-assay variation, n = 4 x 6		
Sample	**Mean value (Ratio)**	**CV (%)**
1	2.5	10.8
2	3.8	10.2
3	6.7	9.4

Prevalence and specificity: Sera of 93 patients with autoimmune hepatitis, 183 patients with hepatitis A, hepatitis B, toxic liver diseases, steatohepatitis or primary biliary cirrhosis (PBC) and 200 healthy blood donors were examined with the EUROIMMUN Anti-LC-1 ELISA (IgG). The prevalence of antibodies against LC-1 in autoimmune hepatitis was 5.4% with a specificity of 100%.

Reference range: The levels of the anti-LC-1 antibodies (IgG) were analysed with this EUROIMMUN ELISA in a panel of 200 healthy blood donors. With a cut-off ratio of 1.0, all blood donors were anti-LC-1 negative.

Literature references

1. Martini E, Abauf N, Cavalli F, Durand V, Johanet C, Homberg JC. **Antibody to liver cytosol (anti-LC-1) in patients with autoimmune chronic active hepatitis type 2.** Hepatology 8: 1662-1666 (1988).

2. Lapierre P, Hajoui O, Homberg JC, Alvarez F. **Formiminotransferase cyclodeaminase is an organ-specific autoantigen recognized by sera of patients with autoimmune hepatitis.** Gastroenterology 116: 643-649 (1999).

ORGENTEC Diagnostika GmbH
Carl-Zeiss-Straße 49-51
55129 Mainz - Germany
Phone: +49 (0) 61 31 / 92 58-0
Fax: +49 (0) 61 31 / 92 58-58
Internet: www.orgentec.com

sebia
The new language of life

CE
IVD

548_6
www.orgentec.com

REF ORG 548 Anti-MCV

INTENDED PURPOSE

Anti-MCV is an ELISA test system for the quantitative measurement of IgG class autoantibodies against mutated citrullinated vimentin (MCV) in human serum or plasma. This product is intended for professional in vitro diagnostic use only.
Measurement of anti-MCV antibodies contributes to early diagnosis of rheumatoid arthritis (RA), where anti-MCV antibody levels represent one parameter of a multi-criterion diagnostic process, encompassing both clinical and laboratory-based assessments.

SYMBOLS USED ON LABELS

IVD	In vitro diagnostic medical device
	Manufacturer
REF	Catalogue number
	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
548_6	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

Rx only — Applicable for U.S.A.: Prescription in vitro diagnostic product

For In Vitro Diagnostic Use — Applicable for U.S.A.: in vitro diagnostic product

PRINCIPLE OF THE TEST

Mutated citrullinated vimentin (MCV) 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 548	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: ***MCV***
CALIBRATOR A	1x 1.5 ml	Calibrator A 0 U/ml, containing serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use.
CALIBRATOR B	1x 1.5 ml	Calibrator B 20 U/ml, containing MCV antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use.
CALIBRATOR C	1x 1.5 ml	Calibrator C 40 U/ml, containing MCV antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use.
CALIBRATOR D	1x 1.5 ml	Calibrator D 100 U/ml, containing MCV antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use.
CALIBRATOR E	1x 1.5 ml	Calibrator E 300 U/ml, containing MCV antibodies in a serum/buffer matrix (PBS, BSA, NaN3 0.09%), yellow. Ready to use.
CALIBRATOR F	1x 1.5 ml	Calibrator F 1000 U/ml, containing MCV antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use.
CONTROL +	1x 1.5 ml	Control positive, containing MCV 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 MCV 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

This assay system is calibrated in relative arbitrary units, since no international reference preparation is available for this assay.

Measuring range

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

Expected values

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

Interpretation of results

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

Linearity

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

Sample	Dilution	Observed U/ml	Expected U/ml	O/E [%]
1	1:100	882.8	882.8	100
.	1:200	386.0	441.4	87
.	1:400	205.2	220.7	93
.	1:800	110.7	110.4	100
.	1:1600	52.2	55.2	95
.	1:3200	23.4	27.6	85
2	1:100	932.1	932.1	100
.	1:200	486.0	466.1	104
.	1:400	250.1	233.0	107
.	1:800	126.6	116.5	109
.	1:1600	61.7	58.3	106
.	1:3200	28.2	29.1	97
3	1:100	727.9	727.9	100
.	1:200	362.4	364.0	100
.	1:400	178.2	182.0	98
.	1:800	85.7	91.0	94
.	1:1600	47.1	45.5	104
.	1:3200	19.2	22.7	85

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	22.7	6.2
2	118.8	6.4
3	548.1	4.6

Inter-Assay		
Sample	Mean U/ml	CV %
1	20.2	5.3
2	111.0	9.2
3	451.6	7.7

Interfering substances

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

Study results

Study population	n	n Pos	%
Rheumatoid arthritis	490	398	81.2
Other diseases	522	14	2.7
Normal human sera	234	1	0.4

		Clinical Diagnosis		
		POS	NEG	
ORG 548	POS	398	15	
	NEG	92	741	
		490	756	1246

Sensitivity: 81.2 %
Specificity: 98.0 %
Overall agreement: 91.4 %

LIMITATIONS OF THE PROCEDURE

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

REFERENCES

1. F.Bobbio-Pallavicini, C.Alpini, R.Caporali, S.Avalle, S.Bugatti, C.Montecuccio. Autoantibody profile in rheumatoid arthritis during long-term infliximab treatment. Arthritis Res Ther 2004, 6:R264-R272 (DOI 10.11 86/ar1173)
2. E.R.Vossenaar, N.Deprés, E.Lapointe,A.van der Heijden, M.Lora,T.Senshu,W.J.van Venfooij, H.A.Ménard. Rheumatoid arthritis specific anti Sa antibodies target citrullinated vimentin. Arthritis Research & Therapie Vol.6 No.2
3. O.Vittecoq, S.Poplin, K.Krzanowska, F.Jouen-Beades, J.F.Ménard, A.Daragon, F.Tron, X.Loet. Rheumatoid Factor is the strongest predictor of radiological progression of rheumatoid arthritis in a three-year prospective study in community-recruited patients. Rheumatology 2003; 42:939-946
4. W.J. van Venrooij, J.M.Hazes, H.Visser. Anticitrullinated protein/peptide antibody and its role in the diagnosis and prognosis of early rheumatoid arthritis. The Netherland Journal of Medicine.
5. M.Escalon, F.J.Lópees-Longo, C.M. González, I.Monteagudo, M.Rodriguez-Mahou, R.Grau, L.Carreno. Anti-Sa Sera from patients with Rheumatoid Arthritis contain at least 2 different subpopulations of Anti-Sa antibodies. The Journal of Rheumatology 2002; 29:10 2053-60
6. Ch.Vincent, L.Nogueira, M.Sebba, S.Chapuy-Regaud, M.Arnaud, O.Letourneur, D.Rolland, B.Rounie, A. Cantagrel, M.Jolivet, G.Serre. Detecion of antibodies to dertermined recombinant tat filaggrin by Enzyme-Linked Immunosorbent Assay. Arthritis & Rheumatism Vo. 46, No.8, August 2002, pp. 2051 -58
7. G.Steiner, J.Smolen. Antibodies in rheumatoid arthritis and their clinical significance. Arthritis Res 2002,4 (suppl 2):S1-S5
8. R.Goldbach-Mansky, J.Lee, A.McCoy, J.Hoxworth, C.Yarboro, J.S.Smolen, G.Steiner, A.Rosen, C.Zhang, H.A. Ménard, Z.J.Zhou, T.Palosuo, W.J.Van Venrooij, R.L.Wilder, J.H.Klippel, H.R.Schumacher Jr., H.S.El-Gabalawy. Rheumatoid arthritis associated antibodies in pati-ents with synovitis of recent onset. Arthritis Res 2000,2:236–243
9. H.Ménard, E.Lapointe, M.D.Rochdi, Z.J.Zhou. Insights into rheumatoid arthritis derived from the Sa immune system. Arthritis Research 2000,2:429-432
10. G.Hayem, P.Chazerain, B.Combe, A.Elias, T.Haim, P.Nicaise, K.Benali, J-F Eliau, M-F Kahn, J.Sany, O.Meyer. Anti-Sa antibody is an accurate diagnostic and prognostic Marker in adult Rheumatoid Arthritis.The Journal of Rheumatology 1 999;26:7-1 3
11. N. Deprés, G.Boire, F.J. Lopez-Longo, H.A. Ménard. The Sa System : A novel antigen antibo-dy system specific for rheumatoid arthritis. The Journal of Rheumatology 1994; 21 -:1 027- 33
12. E.R.Vossenaar,T.R.D. Radstake,A. van der Heijden, M.A.M. van Mansum, C. Dieteren, D.-J. de Rooij, P. Barrera, A.J.W. Zendman,W.J. van Venrooij. Expression and activity of citrullinatin peptidylarginine deiminase enzymes in monocytes and macrophages. Ann Rheum Dis 2004; 63:373-381
13. H. Burkhardt, B. Sehnert, R. Bockermann, A. Engström, J.R. Kalden, R. Holmdahl. Humoral immune response to citrullinated collagen type II determinants in early rheumatoid arthritis. Eur. J. Immunol. 2005.35: 1643-1652

14. Ch.Vincent, L. Nogueira, C. Clavel, M. Sebbag, G. Serre. Autoantibodies to citrullinated pro-teins: ACPA. Autoimmunity, February 2005; 38 (1): 17-24
15. M. Sebbag, S. Chapuy-Regaud, I. Auger, E. Petit-Texeira, C. Clavel, L. Nogueira, Ch.Vincent, F. Cornélis,J. Roudier, G. Serre.Clinical and pathophysiological significance of the autoimmu¬ne response to citrullinated proteins in rheumatoid arthritis. Joint bone Spine 71 (2004) 493-502

References Concerning Classification of RA

16. Liao KP, Batra KL, Chibnik L, Schur PH, Costenbader KH. Anti-CCP revised criteria for the classification of rheumatoid arthritis. Ann Rheum Dis 2008.
17. Arnett FC, Edworthy SM, Bloch DA, McShane DJ, Fries JF, Cooper NS et al. The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. Arthritis Rheum 1988; 31(3):315 -24.Further Reading on Diagnostic Performance of Anti-MCV vs. Predicate Assay:
18. Gyetvai A, Szekanecz Z, Soos L, Szabo Z, Fekete A, Kapitany A et al. New classification of the shared epitope in rheumatoid arthritis: impact on the production of various anti-citrullinated protein antibodies. Rheumatology (Oxford) 2010; 49(1):25-33.
19. Pruijn G, Wiik A, van Venrooij W. The use of citrullinated peptides and proteins for the diagnosis of rheumatoid arthritis. Arthritis Research & Therapy 2010; 12(1):203.
20. Aggarwal R, Liao K, Nair R, Ringold S, Costenbander KH. Anti-citrullinated peptide antibody assays and their role in the diagnosis of rheumatoid arthritis. Athritis Rheum 2009; 61(11):1472-83.
21. Feitsma AL, van der Voort EI, Franken KL, El BH, Elferink BG, Drijfhout JW et al. Identification of citrullinated vimentin peptides as T cell epitopes in HLA-DR4-positive patients with rheumatoid arthritis. Arthritis Rheum 2009; 62(1):117-25.
22. Klareskog L, Catrina AI, Paget S. Rheumatoid arthritis. Lancet 2009; 373(9664):659-72.
23. Liu X, Jia R, Zhao J, Li Z. The Role of Anti-Mutated Citrullinated Vimentin Antibodies in the Diagnosis of Early Rheumatoid Arthritis. J Rheumatol 2009.
24. Luime JJ, Colin EM, Hazes JM, Lubberts E. Does anti-MCV has additional value as serological marker in the diagnostic and prognostic work-up of patients with rheumatoid arthritis? A systematic review. Ann Rheum Dis 2009.
25. Mjaavatten MD, Uhlig T, Haugen AJ, Nygaard H, Sidenvall G, Helgetveit K et al. Positive anti-citrullinated protein antibody status and small joint arthritis are consistent predictors of chronic disease in patients with very early arthritis: results from the NOR-VEAC cohort. Arthritis Res Ther 2009; 11(5):R146.
26. Engelmann R, Brandt J, Eggert M, Karberg K, Krause A, Neeck G et al. The Anti-mutated Citrullinated Vimentin Response Classifies Patients with Rheumatoid Arthritis into Broad and Narrow Responders. J Rheumatol 2009.
27. Raza K, Mathsson L, Buckley CD, Filer A, Ronnelid J. Anti-modified citrullinated vimentin (MCV) antibodies in patients with very early synovitis. Ann Rheum Dis 2009.
28. Snir O, Widhe M, Hermansson M, von SC, Lindberg J, Hensen S et al. Antibodies to several citrullinated antigens are enriched in the joints of rheumatoid arthritis patients. Arthritis Rheum 2009; 62(1):44-52.
29. Syversen SW, Goll GL, van der Heijde D, Landewe R, Lie BA, Odegard S et al. Prediction of radiographic progression in rheumatoid arthritis and the role of antibodies against mutated citrullinated vimentin: results from a ten-year prospective study. Ann Rheum Dis 2009.
30. van der Linden MP, van der Woude D, Ioan-Facsinay A, Levarht EW, Stoeken-Rijsbergen G, Huizinga TW et al. Value of anti-modified citrullinated vimentin and third-generation anti-cyclic citrullinated peptide compared with second-generation anti-cyclic citrullinated peptide and rheumatoid factor in predicting disease outcome in undifferentiated arthritis and rheumatoid arthritis. Arthritis Rheum 2009; 60(8):2232-41.
31. Wagner E, Skoumal M, Bayer PM, Klaushofer K. Antibody against mutated citrullinated vimentin: a new sensitive marker in the diagnosis of rheumatoid arthritis. Rheumatol Int 2009.
32. Keskin G, Inal A, Keskin D, Pekel A, Baysal O, Dizer U et al. Diagnostic Utility of Anti-Cyclic Citrullinated Peptide and Anti-Modified Citrullinated Vimentin Antibodies in Rheumatoid Arthritis. Protein Pept Lett 2008; 15 (3):314-7.
33. Szekanecz Z, Lakos G. Rheumatoid arthritis diagnosis with antimutated citrullinated vimentin ELISA by Orgentec Diagnostika. Exp Opin Med Diagnostics 2008; 2(9):1083-90.
34. Szekanecz Z, Soos L, Szabo Z, Fekete A, Kapitany A, Vegvari A et al. Anti-Citrullinated Protein Antibodies in Rheumatoid Arthritis: As Good as it Gets? Clin Rev Allergy Immunol 2008; 34(1):26-31.

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 548_IFU_EN_QM113183_2023-08-02_5* 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

Anti-SLA/LP ELISA (IgG)
Test instruction

ORDER NO.	ANTIBODIES AGAINST	IG-CLASS	SUBSTRATE	FORMAT
EA 1302-9601 G	SLA/LP (soluble liver antigen/ liver-pancreas antigen)	IgG	Ag-coated microplate wells	96 x 01 (96)

Indications: Increase in transaminases for unclear reasons, suspected autoimmune hepatitis.

Principles of the test: The ELISA test kit provides a semiquantitative or quantitative in vitro assay for human autoantibodies of the IgG class against SLA/LP (soluble liver antigen/liver-pancreas antigen) in serum or plasma. The test kit contains microtiter strips each with 8 break-off reagent wells coated with SLA/LP. In the first reaction step, diluted patient samples are incubated in the wells. In the case of positive samples, specific IgG antibodies (also IgA and IgM) will bind to the antigens. To detect the bound antibodies, a second incubation is carried out using an enzyme-labelled anti-human IgG (enzyme conjugate) which is capable of promoting a colour reaction.

Contents of the test kit:

Component	Colour	Format	Symbol
1. Microplate wells, coated with antigens: 12 microplate strips each containing 8 individual break-off wells in a frame, ready for use	---	12 x 8	STRIPS
2. Calibrator 1 200 RU/ml (IgG, human), ready for use	dark red	1 x 2.0 ml	CAL 1
3. Calibrator 2 20 RU/ml (IgG, human), ready for use	red	1 x 2.0 ml	CAL 2
4. Calibrator 3 2 RU/ml (IgG, human), ready for use	light red	1 x 2.0 ml	CAL 3
5. Positive control (IgG, human), ready for use	blue	1 x 2.0 ml	POS CONTROL
6. Negative control (IgG, human), ready for use	green	1 x 2.0 ml	NEG CONTROL
7. Enzyme conjugate peroxidase-labelled anti-human IgG (rabbit), ready for use	green	1 x 12 ml	CONJUGATE
8. Sample buffer ready for use	light blue	1 x 100 ml	SAMPLEBUFFER
9. Wash buffer 10x concentrate	colourless	1 x 100 ml	WASHBUFFER 10x
10. Chromogen/substrate solution TMB/H_2O_2, ready for use	colourless	1 x 12 ml	SUBSTRATE
11. Stop solution 0.5 M sulphuric acid, ready for use	colourless	1 x 12 ml	STOP SOLUTION
12. Test instruction	---	1 booklet	
13. Protocol with target values	---	1 protocol	

LOT	Lot		storage temperature
IVD	In vitro determination	CE	unopened usable until

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

Waste disposal: Patient samples, calibrators, controls and incubated microplate strips shoud be handled as infectious waste. All reagents should be disposed of according to official regulations.

Preparation and stability of the reagents

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

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

- **Calibrators and controls:** Ready for use. The reagents must be mixed thoroughly before use.

- **Enzyme conjugate:** Ready for use. The enzyme conjugate must be mixed thoroughly before use.

- **Sample buffer:** Ready for use.

- **Wash buffer:** The wash buffer is a 10x concentrate. If crystallization occurs in the concentrated buffer, warm it to 37°C and mix well before diluting. The quantity required should be removed from the bottle using a clean pipette and diluted with deionized or distilled water (1 part reagent plus 9 parts distilled water).
  For example, for 1 microplate: 5 ml concentrate plus 45 ml water.
  The ready-to-use diluted wash buffer is stable for 4 weeks when stored at +2°C to +8°C and handled properly.

- **Chromogen/substrate solution:** Ready for use. Close the bottle immediately after use, as the contents are sensitive to light. The chromogen/substrate solution must be clear on use. Do not use the solution if it is blue coloured.

- **Stop solution:** Ready for use.

Warning: Calibrators and controls used have tested negative for HBsAg, anti-HCV, anti-HIV-1 and anti-HIV-2 using enzyme immunoassays and indirect immunofluorescence methods. Nonetheless, all materials should be treated as being a potential infection hazard and should be handled with care. Some of the reagents contain the toxic agent sodium azide. Avoid contact with the skin.

Preparation and stability of the patient samples

Sample material: Human serum or EDTA, heparin or citrate plasma.

Stability: Patient samples to be investigated can generally be stored at +2°C to +8°C for up to 14 days. Diluted samples should be incubated within one working day.

Sample dilution: Patient samples are diluted **1:101** sample buffer. For example: dilute 10 µl serum in 1.0 ml sample buffer and mix well by vortexing (sample pipettes are not suitable for mixing).

NOTE: Calibrators and controls are prediluted and ready for use, do not dilute them.

Incubation

For **semiquantative analysis** incubate **calibrator 2** along with the positive and negative controls and patient samples. For **quantitative analysis** incubate **calibrators 1, 2 and 3** along with the positive and negative controls and patient samples.

Sample incubation: (1. step)
Transfer 100 µl calibrators, positive and negative controls or diluted patient samples into the individual microplate wells according to the pipetting protocol. Incubate for **30 minutes** at room temperature (+18°C to +25°C).

Washing:
Manual: Empty the wells and subsequently wash 3 times using 300 µl of working strength wash buffer for each wash.
Automatic: Wash reagent wells 3 times with 400 µl working strength wash buffer (program setting: e.g. TECAN Columbus Washer “Overflow Modus”).

Leave the wash buffer in each well for 30 to 60 seconds per washing cycle, then empty the wells. After washing (manual and automated tests), thoroughly dispose of all liquid from the microplate by tapping it on absorbent paper with the openings facing downwards to remove all residual wash buffer.

Note: Residual liquid (> 10 µl) remaining in the reagent wells after washing can interfere with the substrate and lead to falsely low extinction values. Insufficient washing (e.g., less than 3 wash cycles, too small wash buffer volumes, or too short reaction times) can lead to falsely high extinction values.
Free positions on the microplate strip should be filled with blank wells of the same plate format as that of the parameter to be investigated.

Conjugate incubation: (2. step)
Pipette 100 µl of enzyme conjugate (peroxidase-labelled anti-human IgG) into each of the microplate wells. Incubate for **30 minutes** at room temperature (+18°C bis 25°C).

Washing:
Empty the wells. Wash as described above.

Substrate incubation: (3. step)
Pipette 100 µl of chromogen/substrate solution into each of the microplate wells. Incubate for **15 minutes** at room temperature (+18°C bis 25°C) protect from direct sunlight.

Stopping the reaction:
Pipette 100 µl of stop solution into each of the microplate wells in the same order and at the same speed as the chromogen/substrate solution was introduced.

Measurement:
Photometric measurement of the colour intensity should be made at a wavelength of 450 nm and a reference wavelength of between 620 nm and 650 nm **within 30 minutes of adding the stop solution.** Prior to measuring, slightly shake the micro-plate to ensure a homogeneous distribution of the solution.

Pipetting protocol

	1	2	3	4	5	6	7	8	9	10	11	12
A	**C 2**	**P 6**	**P 14**	**P 22**			**C 1**	**P 4**	**P 12**	**P 20**		
B	**pos.**	**P 7**	**P 15**	**P 23**			**C 2**	**P 5**	**P 13**	**P 21**		
C	**neg.**	**P 8**	**P 16**	**P 24**			**C 3**	**P 6**	**P 14**	**P 22**		
D	**P 1**	**P 9**	**P 17**				**pos.**	**P 7**	**P 15**	**P 23**		
E	**P 2**	**P 10**	**P 18**				**neg.**	**P 8**	**P 16**	**P 24**		
F	**P 3**	**P 11**	**P 19**				**P 1**	**P 9**	**P 17**			
G	**P 4**	**P 12**	**P 20**				**P 2**	**P 10**	**P 18**			
H	**P 5**	**P 13**	**P 21**				**P 3**	**P 11**	**P 19**			

The pipetting protocol for microtiter strips 1-4 is an example for the **semiquantitative analysis** of 24 patient samples (P 1 to P 24).
The pipetting protocol for microtiter strips 7-10 is an example for the **quantitative analysis** of 24 patient samples (P 1 to P 24).

The calibrators (C 1 to C 3), the positive (pos.) and negative (neg.) controls, and the patient samples have each been incubated in one well. The reliability of the ELISA test can be improved by duplicate determinations for each sample.
The wells can be broken off individually from the strips. This makes it possible to adjust the number of test substrates used to the number of samples to be examined and minimizes reagent wastage.
Both positive and negative controls serve as internal controls for the reliability of the test procedure. They should be assayed with each test run.

Calculation of results

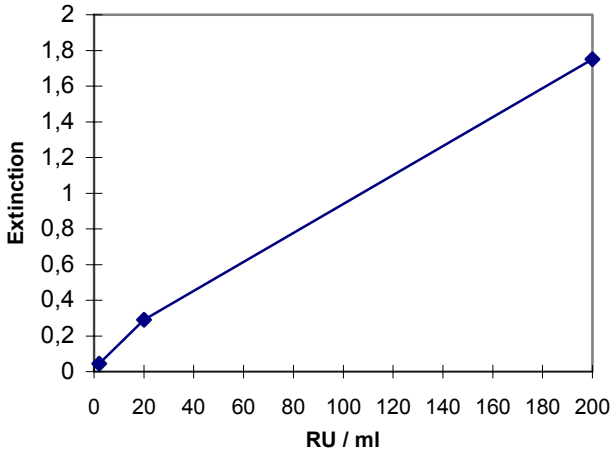
Semiquantitative: Results can be evaluated semiquantitatively by calculating a ratio of the extinction value of the control or patient sample over the extinction value of calibrator 2. Calculate the ratio according the following formula:

$$\frac{\text{Extinction of the control or patient sample}}{\text{Extinction of calibrator 2}} = \text{Ratio}$$

EUROIMMUN recommends interpreting results as follows:

Ratio <1.0:	**negative**
Ratio ≥1.0:	**positive**

Quantitative: The standard curve from which the concentration of antibodies in the serum samples can be taken is obtained by point-to-point plotting of the extinction values measured for the 3 calibration sera against the corresponding units (linear/linear). Use “point-to-point” plotting for calculation of the standard curve by computer. The following plot is an example of a typical calibration curve. Please do not use this curve for the determination of antibody concentrations in patient samples.



If the extinction of a serum sample lies above the value of calibrator 1 (200 RU/ml). The result shold be given as ">200 RU/ml". It is recommended that the sample be re-tested at a dilution of 1:400. The result in IU/ml read from the calibration curve for this sample must then be multiplied by a factor of 4.

The upper limit of the normal range **(cut-off)** recommended by EUROIMMUN is 20 relative units (RU) /ml. EUROIMMUN recommends interpreting results as follows:

<20 RU/ml:	**negative**
≥20 RU/ml:	**positive**

For duplicate determinations the mean of the two values should be taken. If the two values deviate substantially from one another the sample should be retested.

For diagnosis, the clinical symptoms of the patient should always be taken into account alongside the serelogical results.

Test characteristics

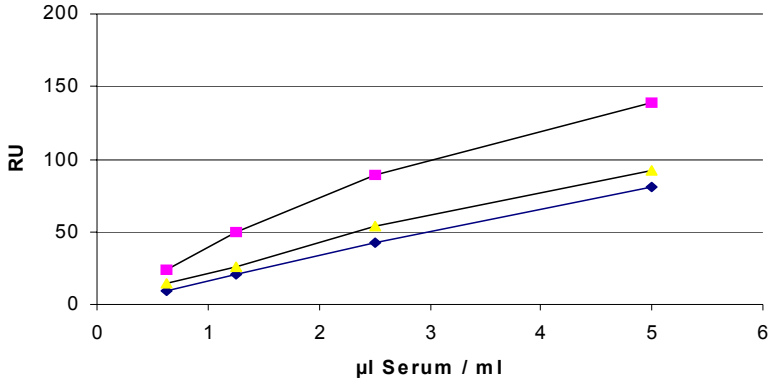
Calibration: As no international reference serum exists for antibodies against SLA/LP, the calibration is performed in relative units (RU).

For every group of tests performed, the extinction values of the calibrators and the relative units and/or ratios determined for the positive and negative controls must lie within the limits stated for the relevant test kit lot. A protocol containing these target values is included. If the values specified for the controls are not achieved, the test results may be inaccurate and the test should be repeated.

The activity of the enzyme used is temperature-dependent and the extinction values may vary if a thermostat is not used. The higher the room temperature (+18°C to +25°C) during substrate incubation, the greater will be the extinction values. Corresponding variations apply also to the incubation times. However, the calibrators are subject to the same influences, with the result that such variations will be largely compensated in the calculation of the result.

Antigen: The microplate wells were coated with recombinant SLA/LP. The corresponding human cDNA was expressed in E. coli. Identification of SLA/LP at the DNA level succeeded in 1998 by cloning the target antigen (A. W. Lohse, University Hospital of Mainz). SLA/LP is probably a cytoplasmic molecule with a molecular weight of 50 kDa which is involved in the regulation of the protein biosynthesis (an UGA-suppressor-tRNA associated protein). Previous descriptions of SLA as being the liver cytokeratins 8 and 18 or glutathione-S-transferase were apparently wrong.

Linearity: The linearity of the test was investigated by assaying serial dilutions of patient samples with high antibody concentrations. The chart below shows the typical linearity of samples on the basis of 3 patient sera. The Anti-SLA/LP ELISA (IgG) is linear in the measurement range 2 - 200 RU/ml.



Detection limit: The detection limit is defined as a value of three times the standard deviation of an analyte-free sample and is the smallest detectable antibody titer. The detection limit of the Anti-SLA/LP ELISA (IgG) is approximately 1 RU/ml.

Cross reactivity: This ELISA showed no cross reactivity.

Interference: Haemolytic, lipaemic and icteric samples showed no influence at the result up to a concentration of 10 mg/ml for hemoglobin, 20 mg/ml for triglycerides and 0.4 mg/ml for bilirubin in this ELISA.

Reproducibility: The reproducibility of the test was investigated by determining the intra- and inter-assay coefficients of variation (CV) using 3 sera with values at different points on the calibration curve. The intra-assay CVs are based on 20 determinations and the inter-assay CVs on 4 determinations performed on 6 different days.

Intra-Assay Variation, n = 20		
Serum	**Mean value (RU/ml)**	**CV (%)**
1	41	2.6
2	110	2.3
3	154	3.7

Inter-Assay Variation, n = 4 x 6		
Serum	**Mean value (RU/ml)**	**CV (%)**
1	44	4.4
2	118	3.6
3	169	3.8

Prevalence and specificity: Sera from 454 patients with autoimmune hepatitis, 165 patients with other liver diseases and 200 healthy blood donors were examined with the Anti-SLA/LP ELISA. The prevalence anti-SLA/LP antibodies in non-Japanese AIH patients was in the range between 15% and 19%. The test showed a specificity of 100%.

Patient group (n=819)	Origin of sera	n	Anti-SLA/LP-positive
Autoimmune hepatitis	Prof. Lohse, Univ. Mainz	108	21 (19%)
	Brasilian panel	154	25 (16%)
	Japanese panel	43	2 (5%)
	American panel	149	23 (15%)
AIH (anti-LKM-1 positive)	Dr. Gruber, Univ. München	18	0
Primary biliary cirrhosis	Dr. Gruber, Univ. München PD Dr. Wick, Klinikum Großhadern	30	0
Hepatitis-B infection	Labor Dr. Stöcker, Lübeck	40	0
Hepatitis-C infection	Prof. Lohse, Univ. Mainz Labor Dr. Stöcker, Lübeck	39	0
Steatohepatitis	Prof. Lohse, Univ. Mainz	24	0
Toxic liver damage	Prof. Lohse, Univ. Mainz	14	0
Blood donors	Med. Univ. Lübeck	200	0

Reference range: The levels of the anti-SLA/LP antibodies (IgG) were analyzed with this EUROIMMUN ELISA in a panel of 200 healthy blood donors. With a cut-off of 20 RU/ml, all blood donors were anti-SLA/LP negative.

Clinical significance

The determination of autoantibodies against soluble liver antigen/liver-pancreas antigen (SLA/LP) is a new and important component in the diagnostics of autoimmune diseases of the liver.

Autoimmune diseases of the liver include
- autoimmune hepatitis (AIH),
- primary biliary liver cirrhosis (PBC) and
- primary sclerosing cholangiitis (PSC).

Mainly women are affected by **autoimmune hepatitis** (**AIH**, earlier designations: lupoid hepatitis, chronically active hepatitis). The disease manifests itself through increased levels of bilirubin, liver enzymes and immunoglobulins, through typical histological changes (liver biopsies show necrosis of the parenchymal cells with lymphocyte and plasma cell infiltrates) and through the occurrence of various autoantibodies. The disease can occur from infancy until the old age, however, it affects most frequently young adults.
The incidence of AIH in western Europe is 1.9 cases per 100,000 inhabitants per year. Untreated, autoimmune hepatitis soon develops into liver cirrhosis. However, if immunosuppressive low-dosed therapy is commenced in good time and continued consistently throughout the patient's life, the patient has a normal life expectancy. For differential diagnosis, a current infection with hepatitis viruses must be ruled out by investigation of the appropriate serological parameters.

Circulating autoantibodies have gained great significance for the diagnosis of AIH. They occur in the majority of patients, but their role in the pathogenesis is questionable. Also, there is no clear correlation between the activity or the prognosis of the disease and the antibody titre. Besides antibodies against SLA/LP the following autoantibodies are associated with AIH: antibodies against cell nuclei (**ANA**), **nDNA**, smooth muscle (**SMA**, with the most important target antigen being **F-actin**), liver-kidney microsomes (**LKM-1**, target antigen: cytochrome P450 IID6) and granulocytes (**pANCA**). The **autoantibodies against SLA/LP** that can today be measured by EUROIMMUN enzyme immunoassays probably have the highest diagnostic accuracy of all antibodies involved in autoimmune hepatitis. Anti-SLA/LP occur in AIH either singly or together with other autoantibodies. Although their prevalence is only between 10% and 30%, the predictive value is almost 100%: Every positive result essentially provides evidence of autoimmune hepatitis (provided that the corresponding clinical symptoms are present).

Investigations of antibodies against SLA/LP have up until now only been possible in a few special laboratories. It was earlier suspected that the SLA antigen is identical with the liver cytokeratins 8 and 18 or with the enzyme glutathione-S-transferase. It has only recently been found that this is apparently not the case. The newly discovered target antigen SLA/LP has presently only been identified reliably at DNA level. It is probably a cytoplasmic molecule which is involved in the regulation of the protein biosynthesis (an UGA-suppressor-tRNA associated protein). The great diagnostic value of autoantibodies against SLA/LP has now been clearly proved.

Autoantibodies against cell nuclei (ANA) and against smooth muscle (SMA) are frequent in AIH, but also occur in 10% to 20% of patients with chronic viral hepatitis and in other diseases. Autoantibodies against LKM-1 can only be demonstrated in about 1% of adult AIH patients, but they are more frequent in children. Antibodies against LKM-1 can also be found 1% to 2% of patients with a positive hepatitis C serology. As opposed to all other autoantibodies, antibodies against SLA/LP are highly specific for AIH and have not been described in viral hepatitis.

Some authors classify autoimmune hepatitis in accordance with the autoantibody status: Subtype I (ANA, SMA), Subtype II (antibodies against LKM-1) and Subtype III (antibodies against SLA/LP). This classification has probably neither a clinical nor a therapeutic and prognostic significance.

The diagnosis of AIH is based on the clinical picture, the biochemical and serological test results and the histological evidence of an inflammation reaction. It requires the exclusion of other causes of chronic hepatitis, such as viruses, alcohol and drugs, and a delimitation to the other autoimmune liver diseases (primary biliary liver cirrhosis and primary sclerosing cholangiitis). The diagnosis AIH is appropriate when 4 of the following 5 main criteria are fulfilled. If all 5 criteria are present, the diagnosis is definite. The diagnosis is confirmed if the patient responds to immunosuppressive therapy.

Main criteria for the diagnosis of autoimmune hepatitis:
1. Histological evidence of hepatitis
2. Detection of autoantibodies (ANA, SMA, LKM, SLA/LP)
3. Hypergammaglobulinaemia
4. Negative HBV and HCV serology
5. Detection of the HLA antigens B8, DR3 or DR4

The serological determination of **autoantibodies against SLA/LP** provides an exact delimitation to viral hepatitis in many patients with AIH. The autoimmune serology of hepatitis has thus been enriched by a further parameter whose significance must be rated more highly than that of antibodies against cell nuclei, DNA, smooth muscle and liver-kidney microsomes (**ANA, nDNA, ASMA, LKM**). Testing for these antibodies has considerable consequences for the hepatology clinic: the consequence of incorrect treatment of AIH with interferon can be just as fatal as the immunosuppressive therapy of the virus infection.
Primary biliary cirrhosis (PBC) is a liver cirrhosis which takes the form of a non-suppurative, destructive inflammation of the biliferous ducts. It occurs in woman ten times more frequently than in men. In the foreground of the clinical picture is cholestasis. In Europe, 13 out of every 100,000 persons develop this disease each year. As a cirrhosis does not occur in every case, and mostly not until the late stage, this disease is better described as **"chronic non-suppurative destructive cholangiitis"**. Pathognomonic is the serological detection of autoantibodies against the mitochondrial fraction M2 (**AMA M2**) and against **Nuclear Dots** (acid protein SP100). Approximately 10% to 20% of PBC patients develop a secondary autoimmune hepatitis (also called **overlap syndrome**). In these cases, as in AIH, autoantibodies can frequently be detected. Antibodies against SLA/LP indicate a secondary AIH (overlap syndrome) which requires an immunosuppressive therapy.

The incidence of **primary sclerosing cholangiitis (PSC)** is quoted as being 4 cases in every 100,000 inhabitants per year. The clinical picture is also characterised by cholestasis. Diagnostic pointers are the relevant laboratory findings, the histology and ERCP (endoscopic retrograde cholangiopancreaticography). Men are mainly affected, and in half of the patients a concomitant Colitis ulcerosa is present (inversely, the prevalence of PSC in cases of Colitis ulcerosa is 4%). Most patients with PSC display autoantibodies against granulocytes (**pANCA**). Occasionally, pANCA can also occur in AIH and PBC, and their value for the differential diagnosis is limited in this respect - however, pANCA are an indication

of an autoimmune disease of the liver and can possibly be used as a means of delimitation to infectious forms of hepatitis. Even though overlaps between PSC and AIH have been reported, antibodies against SLA/LP have not been found in such patients.

Literature references

1. Baeres M, Herkel J, Czaja AJ, Wies I, Kanzler S, Cancado ELR, Porta G, Nishioka M, Simon T, Daehnrich C, Schlumberger W, Galle PR, Lohse AW. **Establishment of standardized SLA/LP immunoassays: specificity for autoimmune hepatitis, worldwide occurrence, and clinical characteristics.** Gut 51: 259-264 (2002).
2. Berg PA, Stechemesser E, Strienz J. **Hypergammaglobulinämische chronisch aktive Hepatitis mit Nachweis von Leber-Pankreas-spezifischen komplementbindenden Autoantikörpern.** Verhandlungen Deutsche Gesellschaft für Innere Medizin 87: 921-927 (1981).
3. Boberg KM, Aadland E, Jahnsen J, Raknerud N, Stiris M, Bell H. **Incidence and prevalence of primary biliary cirrhosis, primary sclerosing cholangitis, and autoimmune hepatitis in a Norwegian population.** Scandinavian Journal of Gastroenterology 33: 99-103 (1998).
4. Costa,M, Rodriguez-Sanchez JL, Czaja AJ, Gelpi C. **Isolation and characterization of cDNA encoding the antigenic protein of the human tRNP$^{(Ser)Sec}$ complex by autoantibodies from patients with type-1 autoimmune hepatitis.** Clinical and Experimental Immunology 121: 364-374 (2000).
5. Kanzler S, Weidemann C, Gerken G, Löhr HF, Galle PR, Meyer zum Büschenfelde K-H, Lohse AW. **Clinical significance of autoantibodies to soluble liver antigen in autoimmune hepatitis.** Journal of Hepatology 31: 635-640 (1999).
6. Kanzler S, Löhr HF, Gerken G, Galle PR, Lohse AW. **Long-term management and prognosis of autoimmune hepatitis (AIH): A single center experience.** Zeitschrift für Gastroenterologie 39: 339-348 (2001).
7. Kanzler S, Bozkurt S, Herkel J, Galle PR, Dienes HP, Lohse AW. **Nachweis von SLA/LP-Autoantikörpern bei Patienten mit primär biliärer Zirrhose als Marker für eine sekundäre autoimmune Hepatitis (Overlapsyndrom).** Deutsche Medizinische Wochenschrift 126: 450-456 (2001).
8. Krawitt EL. **Autoimmune hepatitis.** New England Journal of Medicine 334: 897-903 (1996).
9. Lohse AW, Gerken G, Mohr H, Löhr HF, Treichel U, Dienes HP, et al. **Relation between autoimmune liver diseases and viral hepatitis: Clinical and serological characteristics in 859 patients.** Zeitschrift für Gastroenterologie 33: 571-578 (1995).
10. Lohse AW. **Autoimmune liver disease.** In: Gastroenterology and Hepatology (Hrsg: G. Bianchi Porro), Maidenhead, England: 499-509 (1999).
11. Manns MP, Gerken G, Kyriatsoulis A, Staritz M, Meyer zum Büschenfelde KH. **Characterisation of a new subgroup of autoimmune chronic active hepatitis by autoantibodies against a soluble liver antigen.** Lancet i: 292-294 (1987).
12. McFarlane IG. **The relationship between autoimmune markers and different clinical syndromes in autoimmune hepatitis.** Gut 42: 599-602 (1998).
13. Meyer zum Büschenfelde KH, Lohse AW. **Autoimmune hepatitis.** New England Journal of Medicine 333: 1004-1005 (1995).
14. Miyakawa H, Kawashima Y, Kitazawa E, Kawaguchi N, Kato T, Kikuchi K, Imai E, Fujikawa H, Hashimoto E, Schlumberger W. **Low frequency of anti-SLA/LP autoantibody in Japanese adult patients with autoimmune liver diseases: analysis with recombinant antigen assay.** Journal of Autoimmunity 21: 77-82 (2003).

15. Obermayer-Straub P, Strassburg CP, Manns MP. **Autoimmune hepatitis.** Journal of Hepatology 32 (1 Suppl.): 181-197 (2000).

16. Schramm C, Kanzler S, Meyer zum Büschenfelde K-H, Galle PR, Lohse AW: **Autoimmune hepatitis in the elderly.** American Journal of Gastroenterology 96: 99-103 (1998).

17. Stechemesser E, Strienz J, Berg PA. **Serological definition of new subgroup of patients with autoimmune chronic active hepatitis (letter).** Lancet i: 683 (1987).

18. Wächter B, Kyriatsoulis A, Lohse AW, Gerken G, Meyer zum Büschenfelde K-H, Manns M. **Characterization of liver cytokeratin as a major target antigen of anti-SLA antibodies.** Journal of Hepatology 11: 232-239 (1990).

19. Wesierska-Gadek J, Grimm R, Hitchman E, Penner E. **Members of the glutathione S-transferase gene family are antigens in autoimmune hepatitis.** Gastroenterology 114: 329-335 (1998).

20. Wies I, Denzer U, Brunner S, Meyer zum Büschenfelde K-H, Manns MP, Arand M, et al. **Neither cytokeratins nor glutathione-S-transferase is major target antigen of autoantibodies to soluble liver antigen (SLA) in autoimmune hepatitis (abstract).** Hepatology 548A (1998).

21. Wies I, Brunner S, Henninger J, Herkel J, Kanzler S, Meyer zum Büschenfelde K-H, Lohse AW. **Identification of target antigen for SLA/LP autoantibodies in autoimmune hepatitis.** Lancet 355: 1510-1515 (2000).

ORGENTEC Diagnostika GmbH
Carl-Zeiss-Straße 49-51
55129 Mainz - Germany
Phone: +49 (0) 61 31 / 92 58-0
Fax: +49 (0) 61 31 / 92 58-58
Internet: www.orgentec.com

sebia
The new language of life

CE

IVD

510_4

www.orgentec.com

REF ORG 510 Anti-Sm

INTENDED PURPOSE

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

The detection of autoantibodies against Sm proteins is a component of the multi-parametric ACR criteria for the diagnosis of systemic lupus erythematosus (SLE). The detection of Sm antibodies serves as a prognostic marker for SLE, there is a relationship between the appearance of Sm antibodies and severe organ manifestations of the disease. 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
510_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 Sm is bound to microwells.
The determination is based on an indirect enzyme linked immune reaction with the following steps:
Specific antibodies in the patient sample bind to the antigen coated on the surface of the reaction wells. After incubation, a washing step removes unbound and unspecifically bound serum or plasma components. Subesquently added enzyme conjugate binds to the immobilized antibody-antigen-complexes. After incubation, a second washing step removes unbound enzyme conjugate. After addition of substrate solution the bound enzyme conjugate hydrolyses the substrate forming a blue coloured product. Addition of an acid stopps the reaction generating a yellow end-product. The intensity of the yellow color
correlates with the concentration of the antibody-antigen-complex and can be measured photometrically at 450 nm.

WARNINGS AND PRECAUTIONS

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

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

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

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

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

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

CONTENTS OF THE KIT

ORG 510	▽ 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 Sm antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use.
CALIBRATOR C	1x 1.5 ml	Calibrator C 25 U/ml, containing Sm antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use.
CALIBRATOR D	1x 1.5 ml	Calibrator D 50 U/ml, containing Sm antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use.
CALIBRATOR E	1x 1.5 ml	Calibrator E 100 U/ml, containing Sm antibodies in a serum/buffer matrix (PBS, BSA, NaN3 0.09%), yellow. Ready to use.
CALIBRATOR F	1x 1.5 ml	Calibrator F 200 U/ml, containing Sm antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use.
CONTROL +	1x 1.5 ml	Control positive, containing Sm antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use. The concentration is specified on the certificate of analysis.
CONTROL −	1x 1.5 ml	Control negative, containing Sm antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use. The concentration is specified on the certificate of analysis.
DILUENT	20 ml	Sample Buffer P, containing PBS, BSA, detergent, preservative sodium azide 0.09%, yellow, concentrate (5 x).
CONJUGATE	15 ml	Enzyme Conjugate containing anti-human IgG antibodies, HRP labelled; PBS, BSA, detergent, preservative PROCLIN 0.05%, light red. Ready to use.
TMB	15 ml	TMB Substrate; containing 3,3', 5,5'- Tetramethylbenzidin, colorless. Ready to use.
STOP	15 ml	Stop solution; contains acid. Ready to use.
WASH	20 ml	Wash Buffer, containing Tris, detergent, preservative sodium azide 0.09%; 50 x conc.

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	161.4	161.4	100
	1:200	81.0	80.7	100
	1:400	39.1	40.4	97
	1:800	19.3	20.2	96
2	1:100	292.6	292.6	100
	1:200	146.9	146.3	100
	1:400	73.3	73.2	100
	1:800	35.3	36.6	97

Limit of detection

Functional sensitivity was determined to be: 1 U/ml

Reproducibility

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

Intra-Assay		
Sample	Mean U/ml	CV %
1	51.4	4.1
2	84.8	2.0
3	157.2	2.6

Inter-Assay		
Sample	Mean U/ml	CV %
1	53.3	5.3
2	83.7	2.6
3	153.9	1.3

Interfering substances

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

Study results

Study population	n	n Pos	%
SLE	70	36	51.4
Rheumatoid arthritis	20	3	15.0
Normal human sera	100	2	2.0

		Clinical Diagnosis POS	NEG	
ORG 510	POS	36	5	
	NEG	34	115	
		70	120	190

Sensitivity: 51.4 %
Specificity: 95.8 %
Overall agreement: 79.5 %

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

1. Alba P, Bento L, Cuadrado MJ, Karim Y, Tungekar MF, Abbs I et al. Anti-dsDNA, anti-Sm antibodies, and the lupus anticoagulant: significant factors associated with lupus nephritis. Ann Rheum Dis 2003; 62(6):556-560.
2. Antico A, Platzgummer S, Bassetti D, Bizzaro N, Tozzoli R, Villalta D. Diagnosing systemic lupus erythematosus: new-generation immunoassays for measurement of anti-dsDNA antibodies are an effective alternative to the Farr technique and the Crithidia luciliae immunofluorescence test. Lupus 2010; 19(8):906-912.
3. Brouwer R, Hengstman GJ, Vree EW, Ehrfeld H, Bozic B, Ghirardello A et al. Autoantibody profiles in the sera of European patients with myositis. Ann Rheum Dis 2001; 60(2):116-123.
4. Castro C, Gourley M. Diagnostic testing and interpretation of tests for autoimmunity. J Allergy Clin Immunol 2010; 125(2 Suppl 2):S238-S247.
5. Defendenti C, Atzeni F, Spina MF, Grosso S, Cereda A, Guercilena G et al. Clinical and laboratory aspects of Ro/SSA-52 autoantibodies. Autoimmun Rev 2011; 10(3):150-154.
6. Eriksson C, Kokkonen H, Johansson M, Hallmans G, Wadell G, Rantapaa-Dahlqvist S. Autoantibodies predate the onset of Systemic Lupus Erythematosus in northern Sweden. Arthritis Research & Therapy 2011; 13(1):R30.
7. Haugbro K, Nossent JC, Winkler T, Figenschau Y, Rekvig OP. Anti-dsDNA antibodies and disease classification in antinuclear antibody positive patients: the role of analytical diversity. Ann Rheum Dis JID - 0372355 2004; 63 (4):386-394.
8. Ippolito A, Wallace DJ, Gladman D, Fortin PR, Urowitz M, Werth V et al. Autoantibodies in systemic lupus erythematosus: comparison of historical and current assessment of seropositivity. Lupus 2011; 20(3):250-255.
9. Isenberg DA, Manson JJ, Ehrenstein MR, Rahman A. Fifty years of anti-ds DNA antibodies: are we approaching journey's end? Rheumatology (Oxford) 2007; 46(7):1052-1056.
10. Kattah NH, Kattah MG, Utz PJ. The U1-snRNP complex: structural properties relating to autoimmune pathogenesis in rheumatic diseases. Immunol Rev 2010; 233(1):126-145.
11. Kumar Y, Bhatia A, Minz RW. Antinuclear antibodies and their detection methods in diagnosis of connective tissue diseases: a journey revisited. Diagn Pathol 2009; 4:1.
12. Meroni PL, Schur PH. ANA screening: an old test with new recommendations. Ann Rheum Dis 2010; 69:1420 -1422.
13. Petri M, Magder L. Classification criteria for systemic lupus erythematosus: a review. Lupus 2004; 13(11):829 -837.
14. Poole BD, Schneider RI, Guthridge JM, Velte CA, Reichlin M, Harley JB et al. Early targets of nuclear RNP humoral autoimmunity in human systemic lupus erythematosus. Arthritis Rheum 2009; 60(3):848-859.
15. Putova I, Dostal C, Becvar R. Prevalence of antinucleosome antibodies by enzyme-linked immunosorbent assays in patients with systemic lupus erythematosus and other autoimmune systemic diseases. Ann N Y Acad Sci 2007; 1109:275-286.
16. Reveille JD. Predictive value of autoantibodies for activity of systemic lupus erythematosus. Lupus JID - 9204265 2004; 13(5):290-297.
17. Simon JA, Cabiedes J, Ortiz E, Alcocer-Varela J, Sanchez-Guerrero J. Anti-nucleosome antibodies in patients with systemic lupus erythematosus of recent onset. Potential utility as a diagnostic tool and disease activity marker. Rheumatology (Oxford) 2004; 43(2):220-224.
18. Sinclair D, Saas M, Williams D, Hart M, Goswami R. Can an ELISA replace immunofluorescence for the detection of anti-nuclear antibodies?--The routine use of anti-nuclear antibody screening ELISAs. Clin Lab 2007; 53(3-4):183-191.
19. Tozzoli R, Bizzaro N, Tonutti E, Villalta D, Bassetti D, Manoni F et al. Guidelines for the laboratory use of autoantibody tests in the diagnosis and monitoring of autoimmune rheumatic diseases. Am J Clin Pathol 2002; 117(2):316-324.
20. Maidhof W., Hilias O. Lupus: an pverview of the disease and management options. P T 2012; 37(4):240-9.

21. Hahn BH, McMahon MA, Wilkinson A, Wallace WD, Daikh DI, Fitzgerald JD et al. American College of Rheumatology guidelines for screening, treatment, and management of lupus nephritis. Arthritis Care Res (Hoboken) 2012; 64(6):797-808.

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 510_IFU_EN_QM113137_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

sifin diagnostics gmbh | Berliner Allee 317-321 | 13088 Berlin | Germany | T: +49 30 700 144-0 | F: +49 30 700 144-30 | info@sifin.de | www.sifin.de

Anti-Salmonella O-Gruppen-Pools

Testreagenz für die Objektträgeragglutination

INFORMATION ZUM GEBRAUCH DURCH FACHPERSONAL

CE IVD

de

	O-Gruppen-Pools	Vol.
Anti-Salmonella OMA	A, B, D, E, L	1 ml, 3 ml
Anti-Salmonella OMB	C, F, G, H	1 ml, 3 ml
Anti-Salmonella OMC	I, J, K, M, N, O, P	1 ml
Anti-Salmonella OMD	Q, R, S, T, U, V, W	1 ml

	O-Gruppen-Pools	Vol.
Anti-Salmonella OME	X, Y, Z, 51, 52, 53	1 ml
Anti-Salmonella OMF	54-59	1 ml
Anti-Salmonella OMG	60-63, 65-67	1 ml

Zweckbestimmung
Die Testreagenzien dienen dem serologischen Nachweis von isolierten *Salmonella*-Stämmen aus Untersuchungsmaterial humaner und anderer Herkunft mit Hilfe der Objektträgeragglutination.
Die weitere serologische Differenzierung ist mit den gruppenspezifischen Testreagenzien sowie den Testreagenzien Anti-Salmonella O, Vi entsprechend dem White-Kauffmann-Le-Minor-Schema (Kauffmann-White-Schema) durchzuführen.

Testprinzip
Besitzt der isolierte Stamm ein dem Erfassungsbereich des Testreagenzes entsprechendes *Salmonella* Antigen, wird dieses beim Mischen mit dem spezifischen Antikörper gebunden. Infolge der Antigen-Antikörper-Reaktion wird der Stamm deutlich sichtbar agglutiniert.

Zusammensetzung
Die Testreagenzien sind Mischungen von monoklonalen Antikörpern. Sie werden aus Zellkulturüberständen von Hybridomzelllinien hergestellt, die Antikörper gegen die entsprechenden gruppenspezifischen *Salmonella* O-Antigene sezernieren.
Das Testreagenz Anti-Salmonella OMC enthält zur Erfassung der O-Gruppe K Serum von immunisierten Kaninchen, das durch Absorption von unspezifischen Agglutininen befreit wurde.
Konservierungsmittel: Natriumazid (NaN_3) 0,9 mg/ml

Darreichungsform, Haltbarkeit und Lagerungsbedingungen
Die Testreagenzien liegen in flüssiger, gebrauchsfertiger Form vor.
Sie sind original verschlossen und nach erstmaligem Öffnen bei Lagerung bei 2...8 °C bis zu dem auf dem Etikett angegebenen Datum verwendbar. Nach Benutzung sind die Fläschchen wieder gut zu verschließen.
Gelegentlich können in den Testreagenzien nicht mikrobiell verursachte Trübungen auftreten. Sie beeinträchtigen nicht die Wirksamkeit der Testreagenzien und können durch Zentrifugation oder Filtration beseitigt werden.
Vor Gebrauch müssen die Testreagenzien auf Raumtemperatur (18...26 °C) temperiert werden.

Warnhinweise und Vorsichtsmaßnahmen
Durch die biotechnologische Herstellung der Testreagenzien ist das Risiko einer Kontamination durch infektiöse Krankheitserreger nahezu ausgeschlossen. Wegen des Gehalts an tierischen Materialien (fötales Kälberserum, Stabilisator) sowie durch den Zusatz von Kaninchenserum sollten sie als potentiell infektiös gehandhabt werden.
Wegen des Gehalts an Natriumazid Kontakt mit der Haut und Schleimhäuten vermeiden; gegebenenfalls mit reichlich Wasser spülen.
Da bei der Durchführung der Objektträgeragglutination mit nativem Erregermaterial gearbeitet wird, müssen die notwendigen Arbeitsschutzmaßnahmen eingehalten werden (Infektionsgefahr)!

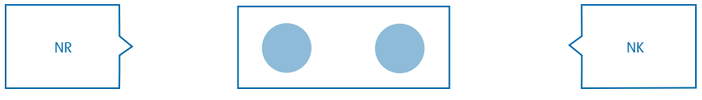
Nicht mitgelieferte Arbeitsmaterialien und Ausrüstungen
Glasobjektträger, Rührstäbchen, physiologische Kochsalzlösung, Abwurfbehälter für infektiöses Material, White-Kauffmann-Le-Minor-Schema.

Untersuchungsmaterial und Methodik
Das Untersuchungsmaterial wird auf nicht selektiven Nährböden wie Nähr- oder Blutagar bzw. auf Kligler-Nährboden ausgestrichen und 16-20 Stunden bei 35...37 °C inkubiert.
Von einer verdächtigen Kolonie wird etwas Bakterienmasse auf einem Objektträger in einen Tropfen Testreagenz (ca. 25 µl) zu einer homogenen, leicht milchigen Suspension eingerieben. Der Objektträger sollte sich auf einer dunklen Unterlage befinden.
Der Objektträger wird vor eine Lichtquelle gegen einen schwarzen Hintergrund gehalten, geschwenkt und das Ergebnis mit bloßem Auge abgelesen.
Zum Ausschluss von Spontanagglutinationen ist eine Negativkontrolle (NK) mit physiologischer Kochsalzlösung anstelle des Testreagenzes mitzuführen.

Ablauf der Prüfung
Zunächst erfolgt die Prüfung mit den Testreagenzien Anti-Salmonella OMA und Anti-Salmonella OMB mit denen ca. 98 % der Salmonellen erfasst werden. Agglutiniert ein Stamm nicht mit diesen beiden Testreagenzien sollte er mit Anti-Salmonella Vi (REF TR 1316) geprüft werden. Fällt die Reaktion bei dieser Prüfung negativ aus, sollte der Stamm mit den Testreagenzien Anti-Salmonella OMC, Anti-Salmonella OMD, Anti-Salmonella OME, Anti-Salmonella OMF, Anti-Salmonella OMG agglutiniert werden.

Bewertung der Ergebnisse
Eine Auswertung ist nur möglich, wenn die Negativkontrolle (NK) milchig trüb bleibt.
Positiv: Sichtbare Agglutination nach 1 bis 20 Schwenkungen. Bei stark positiver Reaktion (PR) tritt eine Agglutination (grob- oder feinflockig) bereits beim Einreiben der Bakterienmasse auf, bei schwach positivem Ergebnis erst nach 10 bis 20 Schwenkungen.

Negativ: Eine milchig-trüb bleibende Suspension oder eine nach mehr als 20 Schwenkungen auftretende Reaktion ist negativ (NR).



Qualitätssicherung bei der Testdurchführung
Für die Qualitätskontrolle des serologischen Nachweises der O-Gruppen-Antigene von *Salmonella*-Stämmen mit der Objektträgeragglutination ist es wichtig, dass die dazu verwendeten Stämme ihre Antigene an der Zelloberfläche gut exprimieren. Es empfiehlt sich daher, Stämme aus Ringversuchen, extern vollständig charakterisierte Feldstämme definierter Herkunft oder geeignete kommerziell erhältliche *Salmonella*-Testantigene zur Qualitätskontrolle einzusetzen.

Grenzen der Methode
Die Testreagenzien reagieren mit *Salmonella*-Stämmen spezifisch, d. h. sie agglutinieren nur Salmonellen der deklarierten O-Gruppen-Pools. Aufgrund von Antigenidentitäten bzw. –verwandtschaften können in Einzelfällen Kreuzreaktionen mit anderen Genera der *Enterobacteriaceae* (z. B. mit Stämmen von *Citrobacter* spp., *Hafnia alvei*, *Proteus* spp. oder *E. coli*) auftreten. Deshalb ist biochemisch die Zuordnung zum Genus *Salmonella* erforderlich.

Erklärung der benutzten Symbole

LOT	Chargennummer (Chargenbezeichnung)
REF	Bestellnummer
IVD	In-vitro-Diagnostikum
TR	Testreagenz
	Verwendbar bis JJJJ-MM (MM = Monatsende)
	Temperaturbegrenzung
	Gebrauchsanweisung beachten
	Objektträgeragglutination

Datum der Fassung: 28/01/2021

sifin diagnostics gmbh | Berliner Allee 317-321 | 13088 Berlin | Germany | T: +49 30 700 144-0 | F: +49 30 700 144-30 | info@sifin.de | www.sifin.de

Anti-Salmonella Pools de groupes O

Réactif d'essai pour l'agglutination sur lame

INFORMATION À L'USAGE DES PROFESSIONNELS

CE IVD

fr

	Pool de groupes O	Vol.
Anti-Salmonella OMA	A, B, D, E, L	1 ml, 3 ml
Anti-Salmonella OMB	C, F, G, H	1 ml, 3 ml
Anti-Salmonella OMC	I, J, K, M, N, O, P	1 ml
Anti-Salmonella OMD	Q, R, S, T, U, V, W	1 ml

	Pool de groupes O	Vol.
Anti-Salmonella OME	X, Y, Z, 51, 52, 53	1 ml
Anti-Salmonella OMF	54-59	1 ml
Anti-Salmonella OMG	60-63, 65-67	1 ml

Usage prévu
Les réactifs d'essai servent à la détection sérologique de souches de *Salmonella* isolées à partir d'un matériau d'essai d'origine humaine ou autre, au moyen de l'agglutination sur lame.
La différenciation sérologique suivante doit être effectuée avec les réactifs d'essai spécifiques de groupe ainsi que les réactifs d'essai Anti-Salmonella O, Vi, conformément au schéma de White-Kauffmann-Le Minor (schéma Kauffmann-White).

Principe de l'essai
Si la souche isolée possède un antigène *Salmonella* correspondant à la plage de détection du réactif d'essai, celui-ci se lie à l'anticorps spécifique lors du mélange. En conséquence de la réaction de l'antigène et de l'anticorps, on constate que la souche est nettement agglutinée.

Composition
Les réactifs d'essai sont des mélanges d'anticorps monoclonaux . Ils sont préparés à partir de surnageants de culture cellulaire de lignées cellulaires d'hybridomes qui secrètent des anticorps contre les antigènes O spécifiques de groupe *Salmonella* correspondants.
Le réactif d'essai Anti-Salmonella OMC comprend pour la détection du groupe O du sérum K de lapins immunisés qui a été libéré par absorption d'agglutinines non spécifiques.
Conservateur : azoture de sodium (NaN_3) 0,9 mg/ml

Présentation, conservation et conditions de stockage
Les réactifs d'essai se trouvent sous forme liquide, prête à l'emploi.
S'ils sont conservés dans leur emballage d'origine et une fois ouverts, conservés à une température de 2...8 °C, ils peuvent être utilisés jusqu'à la date indiquée sur l'étiquette. Après utilisation, les flacons doivent être bien refermés.
Des turbidités qui ne sont pas d'origine microbienne peuvent apparaître occasionnellement dans les réactifs d'essai. Elles n'affectent pas l'efficacité des réactifs d'essai et peuvent être éliminées par centrifugation ou filtration.
Avant emploi, les réactifs d'essai doivent être ajustés à température ambiante (18...26 °C).

Mises en garde et précautions d'emploi
En raison de la préparation biotechnologique des réactifs d'essai, le risque d'une contamination par des agents pathogènes infectieux est pratiquement exclu. En raison de la teneur en matériaux d'origine animale (sérum de veau fœtal, stabilisateur) et de l'ajout de sérum de lapin, ils devraient être manipulés comme des produits potentiellement infectieux.
A cause de la teneur en azoture de sodium, il faut éviter le contact avec la peau et les muqueuses et le cas échéant, rincer abondamment à l'eau.
Puisque l'on travaille avec des matériaux pathogènes natifs lors de la réalisation de l'agglutination sur lame, il faut respecter les mesures de sécurité au travail (risque d'infection) !

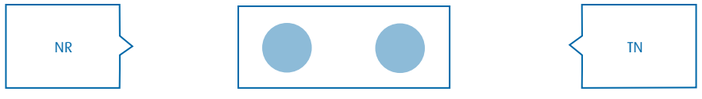
Matériaux de travail et équipements non fournis
Lame de verre, agitateur, solution physiologique de sel de cuisine, collecteur de déchets pour les matériaux infectieux, schéma de White-Kauffmann-Le Minor.

Matériaux d'essai et méthodologie
On étale le matériau d'essai sur des milieux de culture non sélectifs comme la gélose nutritive ou la gélose au sang ou sur des milieux de culture Kligler et on le laisse incuber durant 16 à 20 heures à 35...37 °C.
On enduit un peu de substance bactérienne issue d'une colonie suspecte sur une lame dans une goutte de réactif d'essai (environ 25 µl) de manière à obtenir une suspension homogène, légèrement laiteuse. La lame devrait se trouver sur une base sombre.
On maintient la lame devant une source de lumière, contre un fond noir, on la fait tourner et on lit le résultat à l'œil nu.
Pour exclure des agglutinations spontanées, il faut réaliser un témoin négatif (TN) avec une solution physiologique de sel de cuisine au lieu du réactif d'essai.

Déroulement du contrôle
Le contrôle s'effectue d'abord avec les réactifs d'essai Anti-Salmonella OMA et Anti-Salmonella OMB avec lesquels on détecte environ 98 % des salmonelles. Si une souche ne s'agglutine pas avec ces deux réactifs d'essai, il devrait être contrôlé avec Anti-Salmonella Vi, REF TR 1316. Si la réaction est négative lors du contrôle, la souche devrait s'agglutiner avec les réactifs d'essai Anti-Salmonella OMC, Anti-Salmonella OMD, Anti-Salmonella OME, Anti-Salmonella OMF, Anti-Salmonella OMG.

Evaluation des résultats
Une évaluation n'est possible que si le témoin négatif (TN) reste trouble et laiteux.
Positif: agglutination visible au bout de 1 à 20 rotations. En cas de réaction fortement positive (RP), on constate une agglutination (de flocons grossiers ou fins) dès la substance bactérienne enduite, en cas de résultat faiblement positif, uniquement au bout de 10 à 20 rotations.

Négatif : une suspension trouble et laiteuse ou une réaction qui survient au bout de plus de 20 rotations est négative (RN).



Assurance qualité lors de la réalisation du test
Pour le contrôle qualité de la détection sérologique des antigènes du groupe O de souches de *Salmonella*, il est important que les souches utilisées expriment bien leurs antigènes à la surface de la cellule. Pour effectuer le contrôle qualité, il est donc recommandé d'utiliser des souches issues de tests interlaboratoires,des souches sauvages d'origine définie entièrement caractérisées en externe ou des antigènes de *Salmonella* à tester disponibles dans le commerce.

Limites de la méthode
Les réactifs d'essai réagissent spécifiquement avec les souches de *Salmonella*, c'est-à-dire qu'ils s'agglutinent seulement avec les salmonelles des pools de groupes O déclarés. En raison des identités et des parentés de l'antigène, il peut se produire dans des cas isolés des réactions croisées avec d'autres genres d'*Enterobacteriaceae* (par ex. avec des souches de *Citrobacter* spp., *Hafnia alvei*, *Proteus* spp. ou *E. coli*). Par conséquent, le classement dans le genre *Salmonella* a lieu au moyen d'une analyse biochimique.

Explication des symboles utilisés

LOT	Code du lot
REF	Référence du catalogue
IVD	Dispositif medical de diagnostic in vitro
TR	Réactif d'essai
	Utiliser jusque AAAA-MM (MM = fin du mois)
	Limites de température
	Consulter les instructions d'utilisation
	l'agglutination sur lame

Date de la présente version: 28/01/2021

sifin diagnostics gmbh | Berliner Allee 317-321 | 13088 Berlin | Germany | www.sifin.de

sifin diagnostics gmbh | Berliner Allee 317-321 | 13088 Berlin | Germany | T: +49 30 700 144-0 | F: +49 30 700 144-30 | info@sifin.de | www.sifin.de

Anti-Salmonella O-Group Pools

Test Reagents for slide agglutination

INFORMATION FOR PROFESSIONAL USE

CE IVD

en

	O-Group-Pool	Vol.
Anti-Salmonella OMA	A, B, D, E, L	1 ml, 3 ml
Anti-Salmonella OMB	C, F, G, H	1 ml, 3 ml
Anti-Salmonella OMC	I, J, K, M, N, O, P	1 ml
Anti-Salmonella OMD	Q, R, S, T, U, V, W	1 ml

	O-Group-Pool	Vol.
Anti-Salmonella OME	X, Y, Z, 51, 52, 53	1 ml
Anti-Salmonella OMF	54-59	1 ml
Anti-Salmonella OMG	60-63, 65-67	1 ml

Intended use
The test reagents are intended for use in the serological identification of *Salmonella* strains isolated from test material of varied origin, using slide agglutination.
Additional serological differentiation must be performed using the group-specific test reagents and the Anti-Salmonella O, Vi test reagents in accordance with the White-Kauffmann-Le-Minor scheme (Kauffmann-White scheme).

Principle of the test
If the isolated strain possesses an antigen that is covered by the test reagent, this *Salmonella* antigen becomes bound when mixed with the specific antibody. The antigen-antibody reaction results in clearly visible agglutination of the strain.

Composition
The test reagents are mixtures of monoclonal antibodies. They are produced from cell culture supernatants of hybridoma cell lines which secrete antibodies against the respective group specific *Salmonella* O-antigens.
For the coverage of the O-Group K, the Anti-Salmonella OMC test reagent contains serum of immunized rabbits from which unspecific agglutinins have been removed by absorption.
Preservative: Sodium azide (NaN_3) 0.9 mg/ml)

Form in which product is supplied, shelf life and conditions for storage
The test reagents are available in liquid form ready to use.
If stored at 2...8 °C before and after opening, they may be used up to the date given on the label. After use, the bottle must be properly closed.
The test reagents may sometimes show turbidity which is not microbially caused. Such turbidity does not impair effectivity and the test reagents can be clarified by centrifugation or filtration.
The test reagents must have their temperatures adjusted to room temperature (18...26 °C) before use.

Warnings and precautions
The biotechnological manufacture of the test reagents means that the risk of contamination by infectious agents can be virtually excluded. Because they contain animal materials (fetal calf serum, stabilizer) and because of the addition of rabbit serum, they should be treated as potentially infectious and handled accordingly.
As these materials contain sodium azide, contact with the skin and mucous membranes must be avoided! In case of contact, rinse with plenty of water.
Since the performance of the slide agglutination test involves working with native pathogenic materials, all necessary work protection procedures must be adhered to (risk of infection)!

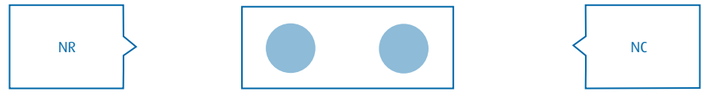
Materials and equipment not supplied
Glass slides, stirring rods, physiological saline, disposal containers for infectious material, White-Kauffmann-Le-Minor scheme.

Test material und methodology
The test material is streaked onto non-selective culture media (Nutrient Agar, Blood Agar) or Kligler Medium. Incubate for 16 – 20 hours at 35...37 °C.
Transfer a small amount of bacterial mass from a suspicious colony under test onto a slide and mix well with a drop of test reagent (ca. 25 µl), so that a homogenous, slightly milky suspension results. The slide should be placed on a dark surface.
The reaction is read with the naked eye by holding the slide in front of a light source against a black background and rocking it (tilting it back and forth).
To control for spontaneous agglutination, a negative control (NC) must be performed at the same time using physiological saline in place of the test reagent.

Test procedure
First carry out the test with the Anti-Salmonella OMA and Anti-Salmonella OMB test reagents. Approx. 98 % of *Salmonella* can be assigned to Anti-Salmonella OMA and Anti-Salmonella OMB.
If the strain does not agglutinate with these both test reagents, it is recommended to test this strain with Anti-Salmonella Vi, REF TR 1316. If this reaction is also negative the strain must then be agglutinated with the Anti-Salmonella OMC, Anti-Salmonella OMD, Anti-Salmonella OME, Anti-Salmonella OMF, and Anti-Salmonella OMG test reagents.

Evaluation
The test can only be evaluated if the negative control (NC) remains milky-opaque.
Positive: visible agglutination after the sample has been tilted back and forth less than 20 times. In a strongly positive reaction (PR), agglutination (coarsely or finely flocculent) appears as soon as the bacterial mass is mixed in. In a weakly positive result, agglutination only appears after the slide has been tilted back and forth 10-20 times.

Negative: if the suspension remains milky-opaque or the reaction begins to occur only after the slide has been tilted back and forth more than 20 times, the result is negative (NR).



Quality assurance during the testing procedure
For the quality control of serological determination of *Salmonella* type-O antigens by slide agglutination test, the good expression of the strain's cell surface antigen is important. The use of strains from interlaboratory tests, or field strains of defined origin that have been fully characterised by an external laboratory, or the use of suitable commercially available *Salmonella* test antigens is therefore recommended for quality control.

Limitations of the method
The test reagents react specifically with individual *Salmonella* strains, i. e. they only agglutinate *Salmonella* strains belonging to the O-Group Pools named in the declaration. In exceptional cases, there is the possibility of cross-reactions with other genera of *Enterobacteriaceae* (e. g. with strains of *Citrobacter* spp., *Hafnia alvei*, *Proteus* spp. or *E. coli*) due to antigen identities or related antigens. For this reason, biochemical examination is required to establish membership of the genus *Salmonella*.

Explanation of the symbols used

LOT	Batch code (Lot)
REF	Catalogue number
IVD	In Vitro Diagnostic Medical Device
TR	Test reagent
	Use by YYYY-MM (MM = end of month)
	Temperature limitation
	Consult instructions for use
	Slide agglutination

Date of revision: 28/01/2021

sifin diagnostics gmbh | Berliner Allee 317-321 | 13088 Berlin | Germany | www.sifin.de

sifin diagnostics gmbh | Berliner Allee 317-321 | 13088 Berlin | Germany | T: +49 30 700 144-0 | F: +49 30 700 144-30 | info@sifin.de | www.sifin.de

Anti-Salmonella pool dei gruppi O

Reagente di prova per agglutinazione su vetrino

INFORMAZIONI PER L'USO PROFESSIONALE

CE IVD

it

	Pool gruppi O	Vol.
Anti-Salmonella OMA	A, B, D, E, L	1 ml, 3 ml
Anti-Salmonella OMB	C, F, G, H	1 ml, 3 ml
Anti-Salmonella OMC	I, J, K, M, N, O, P	1 ml
Anti-Salmonella OMD	Q, R, S, T, U, V, W	1 ml

	Pool gruppi O	Vol.
Anti-Salmonella OME	X, Y, Z, 51, 52, 53	1 ml
Anti-Salmonella OMF	54-59	1 ml
Anti-Salmonella OMG	60-63, 65-67	1 ml

Uso previsto
I reagenti di prova sono stati concepiti per essere utilizzati nell'identificazione sierologica dei ceppi di *Salmonella* isolati da materiale di prova di varia origine mediante agglutinazione su vetrino.
Ulteriori differenziazioni di tipo sierologico devono essere eseguite utilizzando i reagenti di prova gruppo-specifici e i reagenti di prova Anti-Salmonella **O**, Vi conformemente allo schema White-Kauffmann-Le-Minor (schema di Kauffmann-White).

Principio del test
Se il ceppo isolato possiede un antigene incluso nel campo d'azione del reagente di prova, questo antigene di *Salmonella* si lega con l'anticorpo specifico all'atto della miscelazione. La reazione antigene-anticorpo dà luogo all'agglutinazione chiaramente visibile del ceppo.

Composizione
I reagenti di prova sono composti da miscele di anticorpi monoclonali. Essi sono prodotti da surnatanti di colture cellulari di linee cellulari di ibridomi che secernono anticorpi contro i rispettivi antigeni O gruppo-specifici della *Salmonella*.
Per la rilevazione del gruppo **O** K, il reagente di prova Anti-Salmonella **O**MC contiene siero di conigli immunizzati dal quale sono state asportate agglutinine aspecifiche mediante assorbimento.
Conservante: azoturo di sodio (NaN_3) 0,9 mg/ml

Forma di somministrazione, periodo di validità e condizioni di conservazione
I reagenti di prova sono disponibili in forma liquida e pronti all'uso.
Se conservati a una temperatura compresa tra 2 e 8 °C sia prima che dopo l'apertura, essi possono essere utilizzati fino alla data indicata sull'etichetta. Dopo l'uso, il flacone deve essere opportunamente richiuso.
Talvolta i reagenti di prova possono presentare una torbidità non di origine microbica. Tale torbidità non ne compromette l'efficacia e i reagenti di prova possono essere chiarificati mediante centrifugazione o filtrazione.
Prima dell'uso, i reagenti di prova devono essere portati a temperatura ambiente (18-26 °C).

Avvertenze e precauzioni
Data la produzione biotecnologica dei reagenti di prova, si può virtualmente escludere il rischio di contaminazione da agenti infetti. Poiché contengono materiale di origine animale (siero fetale di vitello, stabilizzatore) e siero di coniglio aggiunto, vanno trattati come potenzialmente infetti e dunque devono essere maneggiati di conseguenza.
Dato che questi materiali contengono azoturo di sodio, occorre evitare il contatto con la cute e con le membrane mucose. In caso di contatto, sciacquare con abbondante acqua. Poiché l'esecuzione del test di agglutinazione su vetrino implica l'uso di materiali patogeni naturali, occorre adottare tutte le necessarie procedure di protezione durante il test (rischio d'infezione)!

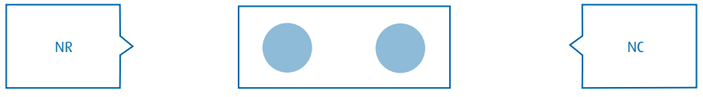
Materiali e attrezzatura non in dotazione
Vetrini, bacchette per agitazione, soluzione salina fisiologica, contenitori per lo smaltimento di materiale infettivo, schema di White-Kauffmann-Le-Minor.

Materiale di prova e metodologia
Il materiale di prova viene steso su mezzi di coltura non selettivi (agar nutriente, agar sangue) o su un mezzo di coltura in Kligler. Incubare per 16-20 ore a 35-37 °C. Trasferire su un vetrino un piccolo quantitativo di massa batterica da una colonia sospetta in esame e miscelare accuratamente con una goccia di reagente di prova (circa 25 µl), in modo tale da ottenere una sospensione omogenea e lievemente latticinosa. Il vetrino deve essere collocato su una superficie scura.
La reazione viene letta a occhio nudo tenendo il vetrino davanti a una fonte luminosa su uno sfondo nero e facendolo oscillare (inclinandolo in avanti e all'indietro). Per verificare ed escludere eventuali agglutinazioni spontanee, è necessario eseguire in contemporanea un controllo negativo (NC) utilizzando la soluzione salina fisiologica al posto del reagente di prova.

Procedura
Per prima cosa eseguire il test con i reagenti di prova Anti-Salmonella OMA e Anti-Salmonella OMB. Circa il 98% delle *Salmonelle* può essere attribuito all'Anti-Salmonella OMA e all'Anti-Salmonella OMB.
Se il ceppo non si agglutina con nessuno di questi due reagenti di pùrova, si raccomanda di testarlo con Anti-Salmonella Vi, REF TR 1316. Se anche questa reazione è negativa, allora il ceppo deve essere agglutinato con i reagenti di prova Anti-Salmonella OMC, Anti-Salmonella OMD, Anti-Salmonella OME, Anti-Salmonella OMF e Anti-Salmonella OMG.

Valutazione
Il test può essere valutato solo se il controllo negativo (NC) resta lattiginoso opaco.
Risultato positivo: agglutinazione visibile dopo che il campione è stato inclinato in avanti e all'indietro meno di 20 volte. In caso di reazione fortemente positiva (PR), l'agglutinazione (di aspetto fioccoso grossolano o fine) compare appena viene inserita e miscelata la massa batterica. In caso di risultato debolmente positivo, l'agglutinazione appare solo dopo che il vetrino è stato inclinato in avanti e all'indietro da 10 a 20 volte.

Risultato negativo: se la sospensione resta lattiginosa opaca o la reazione inizia solo dopo che il vetrino è stato inclinato in avanti e all'indietro per più di 20 volte, il risultato è negativo (NR).



Garanzia di qualità nell'esecuzione delle prove
Ai fini del controllo della qualità della prova sierologica degli antigeni dei gruppi O per i ceppi della *Salmonella* mediante agglutinazione su vetrino, è importante che i ceppi utilizzati esprimano bene i propri antigeni sulla superficie cellulare. Pertanto si consiglia di utilizzare per il controllo di qualità ceppi provenienti da Round Robin Test (prove interlaboratorio) oppure ceppi di campo completamente caratterizzati esternamente di origine definita oppure adeguati antigeni di test della *Salmonella* disponibili in commercio.

Limitazioni del metodo
I reagenti di prova reagiscono in modo specifico con singoli ceppi di *Salmonella*, ossia agglutinano esclusivamente i ceppi di *Salmonella* appartenenti ai pool dei gruppi O dichiarati. In casi eccezionali, è possibile che si verifichino reazioni crociate con altri generi delle *Enterobacteriaceae* (ad es con ceppi di *Citrobacter* spp., *Hafnia alvei*, *Proteus* spp. o *E. coli*) dovute a identità di antigeni o ad antigeni correlati. Per questo motivo è necessario un esame biochimico per determinare l'appartenenza al genere *Salmonella*.

Spiegazione dei simboli utilizzati

LOT	Codice del lotto
REF	Numero catalogo
IVD	Dispositivo medico-diagnostico in vitro
TR	Reagente di prova
	Utilizzare entro il AAAA-MM (MM = fine del mese)
	Limite di temperatura
	Rispettare le istruzioni per l'uso
	Agglutinazione su vetrino

Data di revisione: 28/01/2021

sifin diagnostics gmbh | Berliner Allee 317-321 | 13088 Berlin | Germany | T: +49 30 700 144-0 | F: +49 30 700 144-30 | info@sifin.de | www.sifin.de

Anti-Salmonella O-csoport pool tesztreagensek

Tesztreagensek tárgylemez-agglutinációhoz

PROFESSZIONÁLIS ALKALMAZÁSI TÁJÉKOZTATÓ

CE IVD

hu

	O-csoport pool	Térf.a
Anti-Salmonella OMA	A, B, D, E, L	1 ml, 3 ml
Anti-Salmonella OMB	C, F, G, H	1 ml, 3 ml
Anti-Salmonella OMC	I, J, K, M, N, O, P	1 ml
Anti-Salmonella OMD	Q, R, S, T, U, V, W	1 ml

	O-csoport pool	Térf.a
Anti-Salmonella OME	X, Y, Z, 51, 52, 53	1 ml
Anti-Salmonella OMF	54-59	1 ml
Anti-Salmonella OMG	60-63, 65-67	1 ml

Felhasználás
A tesztreagensek különböző eredetű vizsgálati mintákból izolált *Salmonella* törzsek tárgylemez-agglutinációs módszerrel történő szerológiai azonosítására szolgálnak.
További szerológiai differenciálást kell végezni a csoportspecifikus tesztreagensek és Anti-Salmonella O, Vi tesztreagensek (Enteroclonok) használatával a White-Kauffmann-Le-Minor séma (Kauffmann-White séma) szerint.

A próba elve
Ha az izolált törzs rendelkezik a tesztreagensnek megfelelő *Salmonella* antigénnel, ez a *Salmonella* antigén a specifikus antitesttel keverve megkötődik. Az antigén-antitest reakció eredménye a törzs jól látható agglutinációja.

Összetétel
A tesztreagensek monoklonális antitestek keverékei. Előállításuk a megfelelő csoportspecifikus *Salmonella* O antigénekkel szemben antitesteket kiválasztó hibridóma sejtvonaltenyészetek szupernatánsából történik.
A **K O-csoport** azonosításához az Anti-Salmonella OMC tesztreagens immunizált nyúlszérumot is tartalmaz, amelyből a nem specifikus agglutinineket abszorpcióval eltávolították.
Tartósítószer: nátrium-azid (NaN_3), 0,9 mg/ml.

Kiszerelési forma, eltarthatóság és tárolási feltételek
A tesztreagensek folyékony, használatra kész formában kaphatók.
Felbontatlanul és felbontás után 2-8 °C-on tárolva a címkén megadott dátumig használhatók fel. Használat után a palackot megfelelően le kell zárni.
A tesztreagensekben esetenként nem mikrobiális eredetű zavarosság mutatkozhat. Ez a zavarosság nem rontja a tesztreagensek hatékonyságát, és centrifugálással vagy szűréssel megszüntethető.
Használat előtt meg kell várni, amíg a tesztreagensek szobahőmérsékletre (18-26 °C) melegszenek fel.

Figyelmeztetések és óvintézkedések
A tesztreagensek biotechnológiai gyártási eljárásának köszönhetően a fertőző ágensekkel történő szennyeződés kockázata gyakorlatilag kizárható. Mivel a készítmények állati eredetű anyagot (magzati borjúszérumot, stabilizátort) és hozzáadott nyúlszérumot tartalmaznak, potenciálisan fertőző anyagnak tekintendők, és ennek megfelelően kezelendők. Tekintve, hogy a készítmények nátrium-azidot tartalmaznak, a bőrrel és nyálkahártyával való érintkezést kerülni kell. Ha mégis a bőrre vagy nyálkahártyára kerülnek, bő vízzel kell lemosni.
Mivel a tárgylemez-agglutinációs próba élő kórokozóval végzett tevékenységet is tartalmaz, minden szükséges munkavédelmi eljárást be kell tartani (fertőzésveszély)!

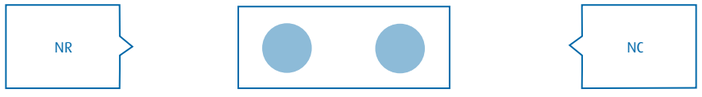
Nem szállított anyagok és eszközök
Üveg tárgylemez, keverőpálca, fiziológiás sóoldat, fertőző anyag kezelésére szolgáló tárolóedény, White-Kauffmann-Le-Minor séma.

Vizsgálati anyagok és módszerek
A vizsgálati anyagot nem szelektív táptalajra (nutrient agar, véres agar) vagy Kligler táptalajra szélesztjük. 16-20 óráig inkubáljuk 35-37 °C-on.
Vigyünk át kis mennyiségű baktériumtömeget egy gyanús vizsgálati telepből egy tárgylemezre, és jól keverjük össze egy csepp tesztreagenssel (kb. 25 µl) úgy, hogy homogén, kissé tejszerű szuszpenziót kapjunk. A lemezt sötét felületre kell helyezni.
Az eredmény leolvasása szabad szemmel történik úgy, hogy a tárgylemezt fekete háttérben fény felé tartjuk, és előre-hátra döntve mozgatjuk.
A spontán agglutináció kizárására egyidejűleg negatív kontroll (NC) vizsgálatot is kell végezni a tesztreagens helyett fiziológiás sóoldattal.

Vizsgálati eljárás
A próbát először az Anti-Salmonella OMA és az Anti-Salmonella OMB tesztreagensekkel végezzük el. A *Salmonella* fajok kb. 98 %-a hozzárendelhető az Anti-Salmonella OMA vagy az Anti-Salmonella OMB poolhoz.
Ha a törzs nem agglutinál a két tesztreagenssel, javasolt a törzset Anti-Salmonella Vi tesztreagenssel vizsgálni, REF TR 1316. Ha ez a reakció is negatív, akkor a törzset agglutinálni kell az Anti-Salmonella OMC, Anti-Salmonella OMD, Anti-Salmonella OME, Anti-Salmonella OMF, Anti-Salmonella OMG poolokkal.

Kiértékelés
A próba csak akkor értékelhető, ha a negatív kontroll (NC) tejszerűen áttetsző marad.
Pozitív: szemmel látható agglutináció, ha a mintát kevesebb, mint 20 alkalommal előre-hátra billentjük. Kifejezett pozitív reakció (PR) esetén az agglutináció (durva vagy finom pelyhesedés) azonnal megjelenik, amikor a baktériumtömeget bekeverjük. Gyenge pozitív reakció esetén az agglutináció csak akkor jelentkezik, ha a tárgylemezt 10-20-szor előre-hátra billentjük.

Negatív: ha a szuszpenzió tejszerűen áttetsző marad, vagy a reakció csak azután kezd fellépni, ha már több mint 20 alkalommal előre-hátra billentettük a tárgylemezt, az eredmény negatív (NR).



Minőségbiztositas a teszt elvegzesekor
A *Salmonella* törszek O-csoport antigénjei szerologiai kimutatasanak targylemezes agglutinacio utjan tortenő minőségellenőrzesehez fontos, hogy az ehhez alkalmazott torzsek antigenjei a sejtfeluleten jol expresszalodjanak. Ezert ajanlatos korvizsgalatokbol szarmazo torzseket vagy extern teljesnek jellemzett, meghatarozott eredetű kornyezeti torzseke, vagy megfelelő, kereskedelmileg kapható *Salmonella*-tesztantigéneket alkalmazni a minőségellenőrzeshez.

A módszer korlátai
A tesztreagensek csak egyes specifikus *Salmonella* törzsekkel lépnek reakcióba, azaz csak a leírásban megnevezett O-csoport poolhoz tartozó *Salmonella* törzsekkel agglutinálnak. Kivételes esetekben keresztreakció alakulhat ki az *Enterobacteriaceae* más nemzetségeinek törzseivel (pl. *Citrobacter* spp., *Hafnia alvei*, *Proteus* spp. vagy *E. coli*) az antigénazonosság vagy antigénrokonság miatt. Emiatt biokémiai vizsgálat szükséges a *Salmonella* genushoz tartozás megállapításához.

Az alkalmazott jelek magyarázata

LOT	Sarzsszám
REF	Rendelési szám
IVD	In vitro diagnosztikum
TR	Tesztreagensek
	Felhasználható -ig éééé-hh (hh = hónap vége)
	Hőmérséklettartomány
	Tartsa be a Használati utasítást
	Tárgylemez-agglutinációhoz

A verzió dátuma: 28/01/2021

sifin diagnostics gmbh | Berliner Allee 317-321 | 13088 Berlin | Germany | T: +49 30 700 144-0 | F: +49 30 700 144-30 | info@sifin.de | www.sifin.de

Пулы Anti-Salmonella к антигенам O-группы

Тест-реагент для реакции агглютинации на предметном стекле

ИНФОРМАЦИЯ ДЛЯ ПРОФЕССИОНАЛЬНОГО ПРИМЕНЕНИЯ

CE IVD

ru

	Пул антигенов O-группы	Объем
Anti-Salmonella OMA	A, B, D, E, L	1 мл, 3 мл
Anti-Salmonella OMB	C, F, G, H	1 мл, 3 мл
Anti-Salmonella OMC	I, J, K, M, N, O, P	1 мл
Anti-Salmonella OMD	Q, R, S, T, U, V, W	1 мл

	Пул антигенов O-группы	Объем
Anti-Salmonella OME	X, Y, Z, 51, 52, 53	1 мл
Anti-Salmonella OMF	54-59	1 мл
Anti-Salmonella OMG	60-63, 65-67	1 мл

Назначение: Тест-реагенты предназначены для серологической идентификации штаммов *Salmonella*, изолированных из исследуемого материала человеческого и иного происхождения, методом агглютинации на предметном стекле. Дополнительно требуется выполнять серологическую дифференциацию с использованием группоспецифических тест-реагентов и тест-реагентов Anti-Salmonella O, Vi согласно схеме Кауфмана-Уайта-ЛеМинора (схеме Кауфмана-Уайта).

Принципы исследования: При наличии у изолированного штамма антигена, способного вступать в реакцию с тест-реагентом, происходит связывание этого антигена *Salmonella* со специфическим антителом при их смешивании. В результате реакции антиген-антитело наблюдается четко видимая агглютинация микроорганизмов.

Состав: Тест-реагенты представляют собой смеси моноклональных антител. Они получены из супернатантов культур гибридных клеточных линий, секретирующих антитела против соответствующих группоспецифических O-антигенов *Salmonella*.
Для включения антител к группе K антигена O тест-реагент Anti-Salmonella OMC дополнительно содержит сыворотку иммунизированных кроликов, очищенных от неспецифических агглютининов путем абсорбции.
Консервант: натрия азид (NaN_3) 0,9 мг/мл

Форма выпуска, срок годности и условия хранения: Тест-реагенты выпускаются в жидкой форме и готовы к применению. При хранении при температуре 2...8 °C до и после вскрытия их можно использовать до даты, указанной на этикетке. После применения следует надлежащим образом закрывать флакон. Иногда в тест-реагентах можно обнаружить помутнение, не связанное с микробным загрязнением. Помутнение не влияет на эффективность, и для отделения от примесей реагенты можно центрифугировать или отфильтровать.
Перед использованием необходимо дождаться, чтобы тест-реагенты приняли комнатную температуру (18...26 °C).

Предупреждения и меры предосторожности: Производство тест-реагентов при помощи биотехнологий фактически исключает риск контаминации возбудителями инфекционных заболеваний. В связи с содержанием материалов животного происхождения (сыворотка эмбрионов коров, стабилизатор) и добавлением сыворотки кроликов, с ними следует обращаться надлежащим образом как с потенциально инфекционным материалом.
В связи с содержанием азида натрия следует избегать контакта этих реагентов с кожей и слизистыми оболочками! В случае контакта промойте пораженные области большим количеством воды.
Так как проведение реакции агглютинации на предметном стекле сопряжено с использованием нативного патогенного материала, следует соблюдать все необходимые правила техники безопасности на рабочем месте (риск инфицирования)!

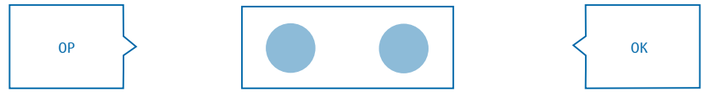
Материалы и оборудование, не входящие в комплект поставки: Предметные стекла, палочки для перемешивания, физиологический раствор, контейнеры для сбора отходов для инфекционного материала, схема Кауфмана-Уайта-ЛеМинора.

Исследуемый материал и методика: Исследуемый материал высевается на неселективные питательные среды (питательный агар, кровяной агар) или среду Клиглера. Инкубация выполняется в течение 16—20 часов при температуре 35...37 °C. Перенесите небольшое количество бактериальной массы из подозрительной исследуемой колонии на предметное стекло и тщательно перемешайте ее с каплей тест-реагента (около 25 мкл) до образования гомогенной, слегка «молочной» суспензии. Предметное стекло должно находиться на темной поверхности. Результат оценивается невооруженным глазом, путем удерживания предметного стекла перед источником света на черном фоне и покачивания предметного стекла (вперед-назад). Для исключения спонтанной агглютинации необходимо одновременно поставить отрицательный контроль (ОК), используя физиологический раствор вместо тест-реагента.

Процедура исследования: Сначала поставьте реакцию с тест-реагентами Anti-Salmonella OMA и Anti-Salmonella OMB. Около 98 % штаммов *Salmonella* можно выявить при помощи тест-реагентов Anti-Salmonella OMA и Anti-Salmonella OMB. При отсутствии агглютинации штамма с данными тест-реагентами рекомендуется исследовать его с тест-реагентом Anti-Salmonella Vi, REF TR 1316. Если результат будет отрицательным, необходимо поставить реакцию агглютинации штамма с тест-реагентами Anti-Salmonella OMC, Anti-Salmonella OMD, Anti-Salmonella OME, Anti-Salmonella OMF, Anti-Salmonella OMG.

Оценка результатов: Оценка результатов исследования допускается, только если отрицательный контроль (ОК) остается молочно-мутным. **Положительная реакция**: видимая агглютинация после покачивания пробы вперед-назад менее 20 раз. В случае выраженной положительной реакции (ПР) агглютинация (крупные или мелкие хлопья) происходит сразу же после перемешивания с бактериальной массой. Если результат слабоположительный, агглютинация происходит только после покачивания предметного стекла вперед-назад 10—20 раз.

Отрицательная реакция: если суспензия остается молочно-мутной, или если реакция начинается только после покачивания предметного стекла вперед-назад более 20 раз, результат является отрицательным (ОР).



Обеспечение качества при выполнении теста: Для контроля качества серологической идентификации антигенов группы O штаммов сальмонеллы путем агглютинации на предметном стекле важно, чтобы используемые для этого штаммы хорошо экспрессировали свои антигены на поверхности клетки. Поэтому для контроля качества рекомендуется использовать штаммы из межлабораторных испытаний или полностью охарактеризованные в независимой лаборатории полевые штаммы уточненного генеза или имеющиеся в продаже подходящие тест-антигены сальмонеллы.

Ограничения метода: Данные тест-реагенты специфическим образом реагируют с отдельными штаммами *Salmonella*, т.е. вызывают агглютинацию только тех штаммов *Salmonella*, которые относятся к заявленным пулам антигенов O-группы. В исключительных случаях возможна перекрестная реактивность с бактериями других родов семейства *Enterobacteriaceae* (например, штаммами *Citrobacter* spp., *Hafnia alvei*, *Proteus* spp. или *E. coli*) в связи с наличием идентичных или родственных антигенов. По этой причине определение принадлежности к роду *Salmonella* требует проведения биохимических исследований.

Разъяснение используемых символов

LOT	Код партии (серии)
REF	Номер по каталогу
IVD	Медицинское устройство для диагностики in vitro
TR	Тест-реагент
	Испо+льзовать до ГГГГ-ММ (ММ = конец месяца)
	Верхний температурный предел
	См. инструкцию по применению
	агглютинация на предметном стекле

Дата редакции: 28/01/2021

sifin diagnostics gmbh | Berliner Allee 317-321 | 13088 Berlin | Germany | T: +49 30 700 144-0 | F: +49 30 700 144-30 | info@sifin.de | www.sifin.de

Anti-Salmonella pools de grupos O

Reactivo de ensayo para la prueba de aglutinación en portaobjetos

INFORMACIÓN DE USO PARA PERSONAL ESPECIALIZADO

CE IVD

es

	Pools de grupos O	Vol.
Anti-Salmonella OMA	A, B, D, E, L	1 ml, 3 ml
Anti-Salmonella OMB	C, F, G, H	1 ml, 3 ml
Anti-Salmonella OMC	I, J, K, M, N, O, P	1 ml
Anti-Salmonella OMD	Q, R, S, T, U, V, W	1 ml

	Pools de grupos O	Vol.
Anti-Salmonella OME	X, Y, Z, 51, 52, 53	1 ml
Anti-Salmonella OMF	54-59	1 ml
Anti-Salmonella OMG	60-63, 65-67	1 ml

Uso previsto
Los reactivos de ensayo se utilizan para la detección serológica de cepas de *Salmonella* aisladas a partir de material de ensayo de origen humano y de otros orígenes mediante la prueba de aglutinación en portaobjetos.
Se debe realizar otra diferenciación serológica utilizando reactivos de ensayo específicos de grupo, así como reactivos de ensayo Anti-Salmonella O, Vi correspondientes al esquema de White-Kauffmann-Le-Minor (esquema Kaufmann-White).

Principio del ensayo
Si la cepa aislada posee un antígeno de *Salmonella* correspondiente al rango de detección del reactivo de ensayo, este se ligará a un anticuerpo específico al mezclarse con él. Como resultado de la reacción antígeno-anticuerpo la cepa se ve claramente aglutinada.

Composición
Los reactivos de ensayo son mezclas de anticuerpos monoclonales. Se forman a partir de sobrenadantes de cultivos de células de líneas celulares de hibridomas que secretan anticuerpos contra los antígenos de *Salmonella* O específicos.
Para la detección del grupo O, el reactivo de ensayo Anti-Salmonella OMC contiene suero K de conejos inmunizados liberado mediante absorción de aglutininas no específicas.
Conservante: azida sódica (NaN_3) 0,9 mg/ml

Forma galénica, vida útil y condiciones de almacenamiento
Los reactivos de ensayo son líquidos y están listos para su uso.
Si se almacenan sin abrir su embalaje original a temperaturas de entre 2 °C y 8 °C, se podrán utilizar hasta la fecha indicada en la etiqueta. Tras utilizar la botella, esta debe volver a cerrarse bien.
En ocasiones puede que los reactivos de ensayo presenten turbidez de origen no microbiano. Sin embargo, la turbidez no disminuye el efecto de los reactivos de ensayo y puede eliminarse mediante centrifugación o filtración.
Antes de utilizar los reactivos de ensayo, los mismos deben aclimatarse a temperatura ambiente (entre 18 °C y 26 °C).

Advertencias y precauciones de uso
Debido a la preparación biotecnológica de los reactivos de ensayo, el riesgo de contaminación por agentes patógenos infecciosos es prácticamente nulo. Puesto que contienen material animal (suero fetal de ternera, estabilizadores), así como un suplemento de suero de conejo, deberían manipularse como productos potencialmente infecciosos.
Además, puesto que contienen azida de sodio, debe evitarse que entren en contacto con la piel y las mucosas.
Si lo hicieran, lavar con agua abundante.
Para realizar la prueba de aglutinación en portaobjetos se trabaja con materiales patógenos nativos, por lo que deberán respetarse las medidas de seguridad laboral (riesgo de infección).

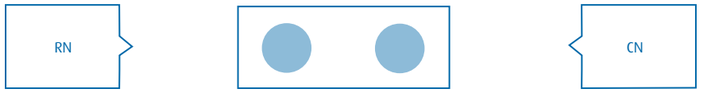
Materiales de trabajo y equipos no suministrados
Portaobjetos de vidrio, agitador, suero fisiológico, contenedores para desechar materiales infecciosos, esquema de White-Kauffmann-Le-Minor.

Material de ensayo y metodología
El material de ensayo se cubre con medios de cultivo no selectivos, por ejemplo agar nutritivo, agar sangre o medio de cultivo de Kliger, y se incuba entre 16 y 20 horas a entre 35 °C y 37 °C.
Transferir pequeñas cantidades de masa bacteriana de una colonia sospechosa a un portaobjetos y mezclarlas con una gota de reactivo de ensayo (25 µl aprox.) para obtener una suspensión homogénea, ligeramente lactosa. El portaobjetos debería colocarse sobre una base oscura.
Para leer el resultado a simple vista, deberá colocarse el portaobjetos delante de una fuente de luz, contra un fondo negro, y girarse.
Para excluir las aglutinaciones espontáneas, deberá realizarse un control negativo con suero fisiológico en lugar de utilizar reactivo de ensayo.

Desarrollo del procedimiento
Realizar la prueba en primer lugar usando los reactivos de ensayo Anti-Salmonella OMA y Anti-Salmonella OMB, con los cuales se detecta aproximadamente el 98 % de las salmonelas. Si la cepa no se aglutina usando estos dos reactivos de control, deberá probarse con Anti-Salmonella Vi (REF TR 1316). Si después de esta prueba el resultado de la reacción es negativo, la cepa deberá aglutinarse con los reactivos de ensayo Anti-Salmonella OMC, Anti-Salmonella OMD, Anti-Salmonella OME, Anti-Salmonella OMF y Anti-Salmonella OMG.

Valoración de los resultados
Solo podrá realizarse una evaluación si el control negativo (CN) permanece turbio y lechoso.
Positivo: Aglutinación visible tras girar la muestra entre 1 y 20 veces. En reacciones positivas (RP) fuertes, la aglutinación (copos gruesos o finos) se detecta directamente en el momento en el que se mezcla la masa bacteriana; mientras que en resultados positivos más débiles se detecta tras girar la muestra entre 10 y 20 veces.

Negativo: Si la suspensión permanece turbia y lechosa o se produce una reacción tras haber girado la muestra más de 20 veces, la reacción es negativa (RN).



Control de calidad durante el procedimiento de ensayo
Para el control de calidad de la detección serológica de los antígenos del grupo O de las cepas de *Salmonella* mediante la prueba de aglutinación en portaobjetos, es importante que las cepas utilizadas expresen bien sus antígenos en la superficie de la célula. Por eso, para realizar los controles de calidad se recomienda utilizar cepas procedentes de ensayos interlaboratorios, cepas silvestres de origen definido caracterizadas en laboratorios totalmente externos o antígenos de prueba adecuados para *Salmonella* disponibles en el mercado.

Limitaciones del método
Los reactivos de ensayo reaccionan de un modo específico con cepas de *Salmonella*, es decir, únicamente aglutinan salmonelas del grupo O indicado. Debido a la identidad de los antígenos o a las semejanzas entre antígenos, puede que, en casos concretos, aparezcan reacciones cruzadas con otros géneros de *Enterobacteriaceae* (p. ej., con cepas de *Citrobacter* spp., *Hafnia alvei*, *Proteus* spp. o *E. coli*). Por eso, desde el punto de vista bioquímico, es necesario clasificarlos al género *Salmonella*.

Explicación de los símbolos utilizados

LOT	Código de lote
REF	Número de referencia del catálogo
IVD	Dispositivo médico de diagnóstico in vitro
TR	Reactivo de ensayo
	Utilizar antes de AAAA-MM (MM = final del mes)
	Límite de temperatura
	Consultar las instrucciones de uso
	Prueba de aglutinación en portaobjetos

Fecha de esta versión: 28/01/2021

sifin diagnostics gmbh | Berliner Allee 317-321 | 13088 Berlin | Germany | T: +49 30 700 144-0 | F: +49 30 700 144-30 | info@sifin.de | www.sifin.de

Pools de grupos O Anti-Salmonella

Reagente de teste para a aglutinação em lâmina de microscópio

INFORMAÇÕES PARA UTILIZAÇÃO POR PROFISSIONAIS

CE IVD

pt

	Pools de grupos O	Vol.
Anti-Salmonella OMA	A, B, D, E, L	1 ml, 3 ml
Anti-Salmonella OMB	C, F, G, H	1 ml, 3 ml
Anti-Salmonella OMC	I, J, K, M, N, O, P	1 ml
Anti-Salmonella OMD	Q, R, S, T, U, V, W	1 ml

	Pools de grupos O	Vol.
Anti-Salmonella OME	X, Y, Z, 51, 52, 53	1 ml
Anti-Salmonella OMF	54-59	1 ml
Anti-Salmonella OMG	60-63, 65-67	1 ml

Utilização indicada
Os reagentes de teste servem como comprovante serológico de estirpes isoladas de *Salmonella* a partir de material de teste humano e de outras origens através da aglutinação em lâmina de microscópio.
A restante diferenciação serológica é realizada com o reagente de teste específico do grupo bem como com o reagente de teste Anti-Salmonella O, Vi de acordo com o esquema White-Kauffmann-Le-Minor (esquema de Kauffmann-White).

Princípio do teste
Se a estirpe isolada possuir um antigene de Salmonella correspondente do reagente de teste da área de cobertura, o mesmo fica ligado ao anticorpo específico aquando da mistura. No seguimento da reação entre antigene e anticorpo, a estirpe fica visivelmente aglutinada.

Composição
Os reagentes de teste são misturas de anticorpos monoclonais. São produzidos a partir de sobrenadantes de linhas de células híbridas que secretaram anticorpos contra os correspondentes grupos específicos do antigen O da Salmonella.
O reagente de teste Anti-Salmonella OMC contém, para recolha do grupo O K soro de coelho imunizado, libertado através da absorção de aglutinina não específica.
Agentes de estabilização: Azida sódica (NaN_3) 0,9 mg/ml

Formato de dosagem, conservação e condições de armazenagem
Os reagentes de teste são fluidos e estão prontos a serem utilizados.
Vêm selados de origem e após abertos pela primeira vez, se armazenados a uma temperatura entre 2...8 °C, podem ser utilizados até à data indicada na etiqueta. Após utilização, os frascos devem ser novamente bem fechados.
Ocasionalmente, podem ocorrer nos reagentes de teste turvações não provocadas por micróbios. Estas não comprometem a eficácia dos reagentes de teste e podem ser eliminadas por centrifugação ou filtragem.
Antes de serem utilizados, os reagentes de teste devem estar à temperatura ambiente (18...26 °C).

Advertências e medidas de segurança
Devido à proveniência biotecnológica do anticorpo monoclonal, o risco de contaminação por agentes patogénicos está praticamente excluído. Devido ao conteúdo do material de origem animal (soro de vitelo fetal, agente estabilizante), bem como devido à adição de soro de coelho, o mesmo deve ser manuseado como sendo potencialmente infeccioso.
Devido ao teor de azida sódica, o contacto com a pele e as mucosas deve ser evitado e, caso necessário, os mesmos devem ser limpos com bastante água.
Uma vez que a aplicação da aglutinação em lâmina de microscópio é trabalhada com agentes patogénicos originais, devem ser respeitadas as medidas de proteção no trabalho (perigo de infeção) !

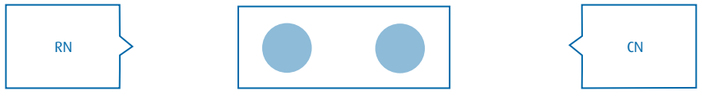
Materiais de trabalho e equipamentos não fornecidos
Lâminas de vidro, agitador, soro fisiológico, contentor para eliminar materiais infecciosos, esquema de White-Kauffmann-Le-Minor.

Material de teste e metodologia
O material de teste foi coberto com meios de cultura não seletivos como agar nutritivo ou agar sangue ou ainda com meio de cultura de Kligler e foi incubado durante 16-20 horas a uma temperatura entre 35...37 °C.
De uma colónia suspeita, é esfregado um pouco de volume de bactérias sobre a lâmina de microscópio numa gota de reagente de teste (cerca de 25 µl) para uma suspensão homogénea e ligeiramente leitosa. A lâmina de microscópio deverá estar pousada numa base escura.
A lâmina de microscópio é levantada contra uma fonte de luz com fundo preto, rodada e o resultado é lido a olho nu.
Para excluir as aglutinações espontâneas, deverá utilizar-se um controlo negativo (CN) com soro fisiológico em vez do reagente de teste.

Procedimento do teste
Em seguida, procede-se ao teste com os reagentes de teste Anti-Salmonella OMA e Anti-Salmonella OMB com os quais podem ser recolhidos cerca de 98 % das Salmonellas. Se uma estirpe não ficar aglutinada com este dois reagentes de teste, deverá ser testada com Anti-Salmonella Vi (REF TR 1316). Se a reação nestes testes for negativa, a estirpe deve ser aglutinada com os reagentes de teste Anti-Salmonella OMC, Anti-Salmonella OMD, Anti-Salmonella OME, Anti-Salmonella OMF, Anti-Salmonella OMG.

Avaliação dos resultados
Só é possível uma avaliação se o controlo negativo (CN) permanecer turvo com aspeto leitoso.
Positivo: Aglutinação visível após 1 a 20 oscilações. No caso de uma forte reação positiva (RP), ocorre uma aglutinação (flocos grossos ou finos) assim que o volume de bactérias é friccionado, com resultado positivo fraco apenas após 10 a 20 oscilações.

Negativo: Uma suspensão que permaneça turva e leitosa ou uma reação que ocorra após mais de 20 oscilações é um resultado negativo (RN).



Garantia de qualidade no procedimento de teste
Para o controlo de qualidade da comprovação serológica do antigénio dos Grupos O de estirpes de *Salmonella* através da aglutinação em lâmina de microscópio, é importante que as estirpes utilizadas para esse fim consigam exprimir bem os seus antigénios na superfície da célula. Recomenda-se, assim, que sejam usadas estirpes de proveniência interlaboratorial ou de proveniência definida de estirpes de campo totalmente caracterizadas como externas ou antigénios de teste de *Salmonella* adequados e disponíveis no mercado, para aplicação do controlo de qualidade.

Limitações do método
Os reagentes de teste reagem com as estirpes específicas de *Salmonella*, ou seja elas aglutinam apenas salmonellas dos pools dos grupos O declarados.
Devido à identidade de antigenes ou relações entre antigenes, podem ocorrer, em casos excecionais, reações cruzadas com outros géneros das enterobactérias (por exemplo, com estirpes de *Citrobacter* spp., *Hafnia alvei*, *Proteus* spp. ou *E. coli*). Por isso, é necessário que o género *Salmonella* seja classificado como bioquímico.

Explication des symboles utilisés

LOT	Número do lote (Denominação do lote)
REF	Número do pedido
IVD	Diagnóstico in vitro
TR	Reagente de teste
	Utilizável até AAAA-MM (MM = Fim do mês)
	Limitação de temperatura
	Esteja atento às instruções de utilização
	Aglutinação em lâmina de microscópio

Data da edição: 28/01/2021

sifin diagnostics gmbh | Berliner Allee 317-321 | 13088 Berlin | Germany | www.sifin.de

Alkaline Saline Peptone Water ISO

Enrichment medium for detection of *Vibrio* spp, according to ISO 21872.

Instructions For Use
ENGLISH

DESCRIPTION

Alkaline Saline Peptone Water ISO (ASPW) is a liquid medium used for the selective enrichment of *Vibrio parahaemolyticus*, *Vibrio cholerae* and *Vibrio vulnificus* from food, animal feed and environmental samples.

This medium complies to ISO 21872-1.

TYPICAL FORMULA*	(g/litre)
Peptone	20.0
Sodium Chloride	20.0
Final pH 8.6 ± 0.2 at 25°C	

*Adjusted and/or supplemented as required to meet performance criteria.

METHOD PRINCIPLE

Peptone provide carbon, nitrogen and amino acids for bacterial growth. The high concentration of sodium chloride along with the alkaline pH provide a favourable environment for growth of *Vibrio* spp while inhibiting other organisms.

PREPARATION

Dehydrated medium — Suspend 40.0 g of the powder in 1 liter of distilled or deionized water. Mix well. Heat to boil and shake until completely dissolved. Dispense into final containers, in quantities required for the examination. Sterilize in autoclave at 121°C for 15 minutes.

TEST PROCEDURE

Following the procedure described by ISO 21872-1

1. Take test portions (25 g or 25 ml) and homogenize in a bottle containing 225 ml of ASPW.
2. Incubate at 41.5 ± 1°C and/or 37 ± 1°C for 6 ± 1 h [a].
3. Transfer 1 ml of the primary enrichment culture into a tube containing 10 ml of ASPW [b].
4. Incubate at 41.5 ± 1°C (*V. parahaemolyticus* and *V. cholerae)* and/or 37 ± 1°C (*V. vulnificus)* for 18 ± 1 h [c].
5. From the cultures obtained in the ASPW, inoculate with 1 µl sampling loop the surface of a TCBS agar plate (ref. 11195) to obtain well-isolated colonies (for more details see the IFU of TCBS agar).

Footnotes

a For primary enrichment, incubation temperature depends on the target species and state of product:
- For detection of *V. parahaemolyticus* and *V. cholerae* in fresh product, incubate at 41.5 ± 1°C.
- For detection of *V. vulnificus* in fresh product, incubate at 37 ± 1°C.
- For detection of all *Vibrio* species in deep frozen, dried or salted product, incubate at 37 ± 1°C.

b Do NOT agitate the sample while taking the aliquot from the surface of the liquid medium.

c When choosing the incubation temperature for secondary enrichment consider the target species only.

INTERPRETING RESULTS

Growth in ASPW is indicated by turbidity compared to an uninoculated control.

For interpreting the test results on TCBS agar see the relevant IFU.

APPEARANCE OF THE MEDIUM

Dehydrated medium: free-flowing, homogeneous, beige.
Prepared medium: clear, light amber.

STORAGE

The powder is very hygroscopic, store the powder at 10-30°C, in a dry environment, in its original container tightly closed. Store tubes and bottles at 10-25°C away from light. Do not use the product beyond its expiry date on the label or if product shows any evidence of contamination or any sign of deterioration.

SHELF LIFE

Dehydrated medium: 4 years.
Tubes/Bottles: 2 years.

QUALITY CONTROL

To check the performance of the medium the following reference strains can be used.

Strain		Inoculum	Incubation	Growth
Vibrio parahaemolyticus	WDCM 00185	≤100 CFU	37 ± 1°C / 18 ± 1 h	Good
Vibrio furnissii	WDCM 00186			Good
Escherichia coli	WDCM 00013	>10^3 CFU		Inhibited

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 intended for professional use only and must be used by properly trained operators.

DISPOSAL OF WASTE

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

BIBLIOGRAPHY

See the references at the end of this document.

TABLE OF SYMBOLS

See the table of symbols at the end of this document.

The product is available in the various configurations listed below. There may be additional product ref. numbers as well. For an updated listing of available products, visit **liofilchem.com**

Product	Format	Packaging	Ref.
Alkaline Saline Peptone Water ISO (ASPW)	Tube	100 x 10 ml	26486
Alkaline Saline Peptone Water ISO (ASPW)	Bottle	6 x 225 ml	414130
Alkaline Saline Peptone Water ISO (ASPW)	Dehydrated medium	500 g of powder	610377

This document is available from the online Support Center:

liofilchem.com/ifu-sds

LIOFILCHEM® s.r.l.
Via Scozia, 64026 Roseto degli Abruzzi (TE) Italy
Tel. +39 0858930745 Fax +39 0858930330 www.liofilchem.com liofilchem@liofilchem.com

Alkaline Saline Peptone Water ISO

Terreno di arricchimento per la ricerca di *Vibrio* spp, secondo ISO 21872

Istruzioni per l'uso
ITALIANO

DESCRIZIONE

Alkaline Saline Peptone Water ISO (ASPW) è un terreno liquido utilizzato per l'arricchimento selettivo di *Vibrio parahaemolyticus*, *Vibrio cholerae* e *Vibrio vulnificus* da alimenti, mangimi e campioni ambientali.

Questo terreno è conforme alla ISO 21872-1.

FORMULA TIPICA*	(g/litro)
Peptone	20.0
Sodio Cloruro	20.0

pH Finale 8.6 ± 0.2 a 25°C

*Adattata e/o integrata per soddisfare le specifiche di performance richieste.

PRINCIPIO DEL METODO

Il peptone fornisce aminoacidi, azoto, carbonio, vitamine e minerali per la crescita batterica. L'alta concentrazione di sodio cloruro insieme al pH alcalino sodio forniscono un ambiente ottimale per la crescita di *Vibrio* spp mentre altri microrganismi vengono inibiti.

PREPARAZIONE

Terreno disidratato: Sospendere 40.0 g di polvere in 1 litro di acqua distillata o deionizzata sterile. Mescolare bene. Riscaldare agitando di frequente e bollire fino a completa dissoluzione. Distribuire nei contenitori finali, nelle quantità richieste per l'esame. Sterilizzare in autoclave a 121°C per 15 minuti.

PROCEDURA DEL TEST

Seguendo la procedura descritta dalla ISO 21872-1

1. Prelevare una porzione del campione (25 g o 25 ml) ed omogenizzare in un flacone contenente 225 ml di ASPW.
2. Incubare a 41.5 ± 1°C e/o 37 ± 1°C per 6 ± 1 ore [a].
3. Trasferire 1 ml della coltura di arricchimento primario in una provetta contenente 10 ml di ASPW [b].
4. Incubare a 41.5 ± 1°C (*V. parahaemolyticus* e *V. cholerae)* e/o 37 ± 1°C (*V. vulnificus)* per 18 ± 1 ore [c].
5. Servendosi di un'ansa, prelevare 1 µl delle colture ottenute in ASPW ed inoculare la superficie di una piastra di TCBS agar (ref. 11195) in modo da ottenere colonie ben isolate [per maggiori dettagli vedere le istruzioni per l'uso (IFU) del TCBS agar].

Note

a Per l'arricchimento primario, la temperatura di incubazione dipende dalle specie oggetto dell'esame (target) e dallo stato del prodotto:
- Per la ricerca di *V. parahaemolyticus* e *V. cholerae* nel prodotto fresco, incubare a 41.5 ± 1°C.
- Per la ricerca di *V. vulnificus* nel prodotto fresco, incubare a 37 ± 1°C.
- Per tutte le specie di *Vibrio* nel prodotto surgelato, essiccato o salato, incubare a 37 ± 1°C.

b NON agitare il campione mentre si preleva l'aliquota dalla superficie del terreno liquido.

c Quando si sceglie la temperatura di incubazione per l'arricchimento secondario bisogna considerare solo le specie target e non lo stato del prodotto.

INTERPRETAZIONE DEI RISULTATI

La crescita in ASPW è indicata dalla torbidità in confronto ad un controllo non inoculato.

For l'interpretazione dei risultati ottenuti su TCBS agar vedere il relativo documento IFU.

ASPETTO

Terreno disidratato: omogeneo, fine granulometria, beige.
Terreno preparato: ambra chiaro, limpido.

CONSERVAZIONE

La polvere è fortemente igroscopica, conservare a 10-30°C, in ambiente asciutto, nel suo contenitore originale chiuso ermeticamente. Conservare i flaconi e le provette a 10-25°C al riparo dalla luce. Non usare il prodotto dopo la sua data di scadenza indicata sull'etichetta o se il prodotto mostra segni di contaminazione o deterioramento.

VALIDITÀ

Terreno disidratato: 4 anni.
Terreno in provette/flaconi: 2 anni.

CONTROLLO DI QUALITÀ

Per il controllo delle performance del terreno, possono essere utilizzati i seguenti ceppi di riferimento.

Ceppo di controllo		Inoculo	Incubazione	Crescita
Vibrio parahaemolyticus	WDCM 00185	≤100 CFU	37 ± 1°C / 18 ± 1 h	Buona
Vibrio furnissii	WDCM 00186			Buona
Escherichia coli	WDCM 00013	>10^3 CFU		Inibita

AVVERTENZE E PRECAUZIONI

Il prodotto non contiene sostanza nocive in concentrazioni superiori ai limiti fissati dall'attuale legislazione e perciò non è classificato come pericoloso. Ciononostante si raccomanda di consultare la scheda di sicurezza per il suo corretto uso. Il prodotto è da intendersi per in ambito professionale e deve essere utilizzato esclusivamente da operatori adeguatamente addestrati.

SMALTIMENTO DEI RIFIUTI

Lo smaltimento dei rifiuti deve essere effettuato in conformità alle normative nazionali e locali in vigore.

BIBLIOGRAFIA

Vedere i riferimenti alla fine di questo documento.

TABELLA DEI SIMBOLI

Vedere la tabella dei simboli alla fine di questo documento.

Il prodotto è disponibile in diverse configurazioni. Vedere l'elenco nella lingua inglese.

Questo documento è disponibile dal Support Center online:
liofilchem.com/ifu-sds

BIBLIOGRAPHY / BIBLIOGRAFIA

1. EN ISO 11133:2014+Amd1:2018. Microbiology of food, animal feed and water – Preparation, production, storage and performance testing of culture media.
2. ISO 21872-1:2017. Microbiology of the food chain – Horizontal method for the determination of *Vibrio* spp. – Part 1: Detection of potentially entecrophatogenic *Vibrio parahaemolyticus*, *Vibrio cholerae* and *Vibrio vulnificus*.
3. Shread P., Donovan T. J., and Lee J. V. (1991) Soc. Gen. Microbiol. Q. 8:184.
4. Cruickshank R. (1968) Medical Microbiology. 11th ed. Livingstone Ltd, London, UK.

TABLE OF SYMBOLS / TABELLA DEI SIMBOLI

LOT	Batch code / Codice del lotto
REF	Catalogue number / Numero di catalogo
	Manufacturer / Fabbricante
	Use by / Utilizzare entro
	Fragile, handle with care / Fragile, maneggiare con cura
	Temperature limitation / Limiti di temperatura
	Contains sufficient for <n> tests / Contenuto sufficiente per <n> saggi
	Consult Instruction For Use / Consultare le istruzioni per l'uso
	Do not reuse / Non riutilizzare
	Keep away from light / Tenere al riparo dalla luce

LIOFILCHEM® s.r.l.

Via Scozia, 64026 Roseto degli Abruzzi (TE) Italy
Tel. +39 0858930745 Fax +39 0858930330 www.liofilchem.com liofilchem@liofilchem.com

Antibiotic Disc

Antibiotic discs for susceptibility tests

Antibiotic Disc

ENGLISH

Antibiotic discs for susceptibility tests

DESCRIPTION

Antibiotic Disc are paper discs with special features, that are impregnated with antibiotic and used for the susceptibility test according to the Kirby-Bauer antibiotic testing (KB testing or disk diffusion antibiotic sensitivity testing).
Antibiotic Disc are available in a large variety of configurations. Each configuration is available in packages of 50 and 250 tests.

CONTENTS OF THE PACKAGES

Discs in cartridge
The 50-test box contains 1 cartridge with 50 discs packed in a desiccant envelope.
The 250-test box contains 5 cartridges of 50 discs, each cartridge individually packed in a desiccant envelope.
Each package also contains a transparent resealable bag.

Discs in canister
The canister contains 250 discs and a desiccant tablet.

METHOD PRINCIPLE

The discs are applied to the surface of a culture medium inoculated with a pure colony suspension of the microorganism under examination. After incubation, the plates are examined, the inhibition halos around each disc are examined and compared with the standard inhibition haloes: in this way the microorganisms are defined as being susceptible, intermediate or resistant to the tested antimicrobial agents.

COMPOSITION

Liofilchem antimicrobial susceptibility test discs are made of high-quality paper in compliance with WHO and FDA specifications.
The discs are manufactured under the quality systems UNI EN ISO 9001 and EN ISO 13485, and to DIN specification for potency, i.e. the concentration of each antibiotic is within 90-125% of the concentration stated on the disc.

GATHERING AND KEEPING SAMPLES

The colonies that are to be subjected to the susceptibility test are taken up by culture media that have been previously swabbed with the sample under examination. In the case of mixed colonies the bacterial strains must be purified before they are swabbed on the plates for the susceptibility test.

TEST PROCEDURE

1. Allow discs to equilibrate to room temperature before opening the container (cartridge or canister) in order to minimize the condensation on the discs, which could affect long-term stability.
2. Make a suspension of the test organism to the density of a 0.5 McFarland turbidity standard.
3. Using a swab, inoculate a suitable agar plate medium by uniformly spreading the suspension over the entire agar surface.
4. Apply discs firmly to the surface of the inoculated agar plate.
5. Incubate plates in an inverted position at the appropriate temperature, atmosphere and time, following the methodology chosen (e.g. CLSI, EUCAST).
6. Return unused discs to the refrigerator/freezer as soon as the application of the discs has been completed (see STORAGE).

NOTE 1: The medium to be used depends on the organism under investigation and the methodology followed, and must be validated by the media manufacturer for antimicrobial susceptibility testing. A list of recommended agar media can be found at the end of this IFU.

NOTE 2: It is recommended to use the inoculum suspension within 15 minutes of preparation, apply discs within 15 minutes of inoculation and incubate plates within 15 minutes of disc application.

For more details, please refer to the current published standards.

EVALUATING THE RESULTS / QUALITY CONTROL

At the end of the incubation period, measure the inhibition halos and interpret according to the current reference standards:
Antibiotic Disc Interpretative Criteria and Quality Control (pdf file)

CLINICAL INTERPRETATION

The susceptibility test carried out *in vitro* cannot exactly reproduce *in vivo* conditions. Nevertheless, it shows the effect of the concentration of the antibiotic, which varies in the culture medium in relation to the growth of the microbial population.

The final choice of antibiotic to administer to the patient is the responsibility of the clinician who possesses all the information on the patient.

LIMITS

Diffusion susceptibility tests use an *in vitro* technique and cannot therefore reproduce the extremely complex *in vivo* conditions. Nevertheless, it is a useful and important tool that helps the clinician choose the correct therapy. Many variable factors influence the final result of the diffusion susceptibility test. The main ones are: the culture medium used, impregnation of the discs, inoculation of the medium, temperature, time and incubation atmosphere of the plates, pre-incubation and pre-diffusion conditions, depth of the medium, etc.

PRECAUTIONS

The Antibiotic Disc cannot be classified as being hazardous according to current legislation. Antibiotic Disc are disposable products. Antibiotic Disc are only for diagnostic *in vitro* use and are intended for professional use. They must be used in the laboratory by properly trained operators using approved aseptic and safety methods for pathogenic agents.

STORAGE

The unopened package of Antibiotic Disc can be stored in most cases at –20°C to +8°C till the expiry date. Some products have to be stored at –20°C as maximum storage temperature. The recommended temperature limits can be found both on the product envelop and on the box label. Leftover discs from an opened CARTRIDGE need to be stored at 2-8°C for no more than 7 days. The cartridge containing unused discs should be returned into its desiccant envelope and then inserted into the resealable bag. Discs in a CANISTER can be used for up to 2 months from first opening and must be stored at the label storage temperature. Dispose of expired discs.

ELIMINATING USED MATERIAL

After use, Antibiotic Disc and the material that comes into contact with the sample must be decontaminated and disposed of in accordance with current laboratory techniques for the decontamination and disposal of potentially infected material.

Antibiotic Disc

ITALIANO

Dischi antibiotici per antibiogramma

DESCRIZIONE

Antibiotic Disc sono dischi di carta, con caratteristiche peculiari, impregnati con antibiotico, utilizzati per l'antibiogramma secondo il metodo Kirby-Bauer (test KB o antibiogramma a disco diffusione).
Antibiotic Disc sono previsti in una larga varietà di configurazioni. Ciascuna configurazione è disponibile nella variante da 50 e 250 test.

CONTENUTO DELLE CONFEZIONI

Dischi in cartuccia
La confezione da 50 test contiene 1 cartuccia con 50 dischi inserita in una bustina con film essiccante.
La confezione da 250 test contiene 5 cartucce da 50 dischi, ognuna in una bustina con film essiccante.
Ciascuna confezione contiene inoltre una bustina trasparente con chiusura a pressione.

Dischi in barattolo
Il barattolo contiene 250 dischi e una compressa essiccante.

PRINCIPIO DEL METODO

I dischi vengono applicati sulla superficie di un terreno di coltura inoculato con una sospensione di una coltura pura del microrganismo in esame. Dopo l'incubazione, vengono esaminate le piastre, misurati gli aloni di inibizione intorno a ciascun disco e confrontati con i diametri degli aloni di inibizione standard: in tal modo i microrganismi vengono definiti sensibili, intermedi o resistenti agli agenti antimicrobici testati.

COMPOSIZIONE

I dischi Liofilchem per i test di sensibilità agli antimicrobici sono preparati con carta di alta qualità in conformità alla specifiche fornite dall'OMS e dalla FDA. I dischi sono prodotti secondo i sistemi di qualità UNI EN ISO 9001 ed EN ISO 13485, ed in conformità con le specifiche DIN per la potenza, ovvero la concentrazione di ciascun antibiotico rientra nell'intervallo del 90-125% della concentrazione indicata sul disco.

RACCOLTA E CONSERVAZIONE DEI CAMPIONI

Le colonie da sottoporre all'antibiogramma vengono riprese dai terreni colturali seminati preventivamente con il campione in esame. In caso di colonie miste è necessario procedere alla purificazione dei ceppi batterici prima della semina sulle piastre per l'antibiogramma.

PROCEDURA DEL TEST

1. Attendere che i dischi raggiungano la temperatura ambiente prima di aprire il contenitore (cartuccia o barattolo) per minimizzare la formazione di condensa sui dischi, che potrebbe influire sulla stabilità a lungo termine.
2. Preparare una sospensione del microrganismo da testare con torbidità pari allo 0.5 McFarland.
3. Utilizzando un tampone, inoculare un terreno idoneo in piastra distribuendo la sospensione in maniera uniforme sull'intera superficie dell'agar.
4. Applicare i dischi sulla superficie della piastra inoculata.
5. Incubare le piastre capovolte alla temperatura, atmosfera e tempo appropriati, seguendo la metodologia scelta (es. CLSI, EUCAST).
6. Non appena terminata l'applicazione dei dischi, riporre i dischi non utilizzati nel frigorifero/congelatore (vedere CONSERVAZIONE).

NOTA 1: Il terreno da utilizzare dipende dall'organismo in esame e dalla metodologia seguita e deve essere convalidato dal produttore del terreno per i test di sensibilità agli antimicrobici. Alla fine di questo documento è disponibile un elenco dei terreni solidi raccomandati.

NOTA 2: Si raccomanda di utilizzare la sospensione di inoculo entro 15 minuti dalla preparazione, applicare i dischi entro 15 minuti dall'inoculo e incubare le piastre entro 15 minuti dall'applicazione del disco.

Per maggiori dettagli si rimanda agli standard attualmente pubblicati.

INTERPRETAZIONE DEI RISULTATI / CONTROLLO QUALITÀ

Al termine del periodo di incubazione, procedere alla misurazione degli aloni di inibizione ed interpretarli secondo gli standard di riferimento in vigore:

Antibiotic Disc Interpretative Criteria and Quality Control (pdf file)

INTERPRETAZIONE CLINICA

L'antibiogramma eseguito *in vitro* non può riprodurre esattamente le condizioni che si trovano *in vivo*, tuttavia è in grado di evidenziare l'effetto della concentrazione dell'antibiotico, che varia nel terreno colturale, nei confronti della popolazione microbica in fase di sviluppo. La scelta finale dell'antibiotico da somministrare al paziente spetta al clinico che è in possesso di tutti i dati riguardanti il paziente stesso.

LIMITI

L'antibiogramma per diffusione, utilizzando una tecnica *in vitro*, non è in grado di riprodurre le condizioni estremamente complesse che si trovano *in vivo*; costituisce tuttavia un utile ed importante supporto alla scelta terapeutica del clinico. Molte sono le variabili che influenzano il risultato finale dell'antibiogramma per diffusione; le principali sono rappresentate da: terreno di coltura utilizzato, impregnazione dei dischi, inoculo del terreno, temperatura, tempo ed atmosfera di incubazione delle piastre, condizioni di pre-incubazione e pre-diffusione, spessore del terreno etc.

PRECAUZIONI

Il prodotto Antibiotic Disc non è classificabile come pericoloso ai sensi della legislazione vigente. Antibiotic Disc è un dispositivo monouso. Antibiotic Disc è solo per uso diagnostico *in vitro*, è destinato ad un ambito professionale e deve essere usato in laboratorio da operatori adeguatamente addestrati, con metodi approvati di asepsi e di sicurezza nei confronti degli agenti patogeni.

CONSERVAZIONE

La confezione integra di Antibiotic Disc può essere conservata nella maggior parte dei casi tra –20°C e +8°C fino alla data di scadenza. Per alcuni prodotti la temperatura massima di conservazione è –20°C. I limiti di temperatura raccomandati sono riportati sulla bustina del prodotto e sull'etichetta esterna. I dischi rimanenti in una CARTUCCIA aperta devono essere conservati a 2-8°C per non più di 7 giorni. Riporre la cartuccia nel suo involucro con film essiccante e quindi nella bustina richiudibile. I dischi in BARATTOLO possono essere utilizzati fino a 2 mesi dopo la prima apertura e vanno conservati alla temperatura indicata in etichetta. Eliminare i dischi scaduti.

ELIMINAZIONE DEL MATERIALE USATO

Dopo l'utilizzazione Antibiotic Disc ed il materiale venuto a contatto con il campione devono essere decontaminati e smaltiti in accordo con le tecniche in uso in laboratorio per la decontaminazione e lo smaltimento di materiale potenzialmente infetto.

Antibiotic Disc

Discos antibióticos para antibiograma

ESPAÑOL

DESCRIPCIÓN

Antibiotic Disc son discos de papel de características especiales, impregnados con un antibiótico, utilizados para el test de susceptibilidad según el test de antibióticos de Kirby.Bauer (Test KB o test de sensibilidad antibiótica por difusión).
Antibiotic Disc están previstos en una ancha variedad de configuraciones. Cada configuración está disponible en la variante de 50 y 250 tests.

CONTENIDO DE LOS ESTUCHES

Discos en cartucho

La caja de 50 ensayos incluye: 1 cartucho con 50 discos en envase desecante.
La caja de 250 ensayos incluye: 5 cartuchos de 50 discos, cada uno en envase desecante.
Cada paquete también incluye una bolsa transparente resellable.

Discos en caja

La caja contiene 250 discos y una tableta desecante.

PRINCIPIO DEL MÉTODO

Los discos se aplican en la superficie de un medio de cultivo inoculado con una suspensión de colonias puras del microorganismo en examen. Después de la incubación, se examinan las placas, se miden los halos de inhibición alrededor de cada disco y se comparan con los diámetros de los halos de inhibición estándar: de esta manera los microorganismos se definen sensibles, intermedios o resistentes a los agentes antimicrobiano testados.

COMPOSICIÓN

Los discos Liofilchem para las pruebas de sensibilidad a antimicrobianos están fabricados con papel de alta calidad de conformidad con las especificaciones de la OMS y la FDA. Los discos son fabricados bajo los sistemas de calidad UNI EN ISO 9001 y EN ISO 13485, y también de acuerdo a las especificaciones DIN sobre potencia, es decir, la concentración de antibiótico en el disco está entre el 90 y el 125% de la indicada.

RECOLECCIÓN Y CONSERVACIÓN DE LAS MUESTRAS

Las colonias que se van a someter al antibiograma se toman del medio de cultivo inoculado previamente con la muestra a examinar. En caso de colonias mixtas es necesario proceder a la purificación de las cepas bacterianas antes de la aplicación en las placas para el antibiograma.

PROCEDIMIENTO DEL TEST

1. Dejar equilibrar a temperatura ambiente el envoltorio antes de abrirlo para minimizar la posible condensación en lo discos, lo cual podría afectar la estabilidad a largo plazo;
2. Hacer una suspensión del organismo de prueba a la densidad de un estándar de turbidez de 0.5 McFarland.
3. Con un hisopo, inocular un medio adecuado en placa de agar esparciendo uniformemente la suspensión sobre toda la superficie del agar.
4. Aplicar los discos firmemente a la superficie de la placa de agar inoculada.
5. Incubar las placas en posición invertida, a la temperatura, atmósfera y tiempo adecuados, siguiendo la metodología elegida (por ejemplo, CLSI, EUCAST).
6. Guardar los discos sin usar en refrigerador / congelador tan pronto como se haya completado la aplicación de los discos (consultar ALMACENAMIENTO).

NOTA 1: El medio que se utilizará depende del organismo que se esté investigando y de la metodología seguida, debe ser validado por el fabricante del medio para las pruebas de sensibilidad a los antimicrobianos. Puede encontrar una lista de los medios de agar recomendados al final de este inserto.

NOTA 2: Se recomienda usar la suspensión de inóculo dentro de los 15 minutos posteriores a la preparación, aplicar los discos dentro de los 15 minutos posteriores a la inoculación e incubar las placas dentro de los 15 minutos posteriores a la aplicación del disco.

Para obtener más detalles, consultar los estándares publicados actualmente.

INTERPRETACIÓN DE LOS RESULTADOS / CONTROL CALIDAD

Al finalizar el período de incubación, mida los halos de inhibición y haga la interpretación según los estándares de referencia:

Antibiotic Disc Interpretative Criteria and Quality Control (pdf file)

INTERPRETACIÓN CLÍNICA

El antibiograma realizado *in vitro* no puede reproducir exactamente las condiciones que se hallan *in vivo*, sin embargo puede evidenciar el efecto de la concentración del antibiótico, que varía en el medio de cultivo, con respecto a la población microbiana en fase de desarrollo.
La elección final del antibiótico que se administrará al paciente corresponde al clínico que posee todos los datos relativos al paciente mismo.

LÍMITES

El antibiograma por difusión, utilizando una técnica *in vitro*, no puede reproducir las condiciones extremamente complejas que se hallan in vivo; sin embrago representa un útil e importante soporte para el clínico a la hora de escoger la terapia. Muchas son las variables que influyen en el resultado final del antibiograma por difusión; las principales están representadas por: medio de cultivo utilizado, impregnación de los discos, inoculación del cultivo, temperatura, tiempo y atmósfera de incubación de las placas, condiciones de pre-incubación y pre-difusión, espesor del cultivo, etc.

PRECAUCIONES

El producto Antibiotic Disc no se puede clasificar como peligroso según la legislación vigente. Antibiotic Disc es un dispositivo desechable. Antibiotic Disc es sólo para uso diagnóstico *in vitro,* está destinado a un ámbito profesional y tiene que ser utilizado en laboratorio por operadores adecuadamente formados, con métodos aprobados de asepsia y seguridad con respecto a los agentes patógenos.

CONSERVACIÓN

El paquete de discos de antibiótico sin abrir se puede almacenar en la mayoría de los casos de –20ºC a + 8°C hasta la fecha de caducidad. Algunos productos tienen que ser almacenados a –20ºC como temperatura máxima de almacenamiento. Los límites de temperatura recomendados se pueden encontrar tanto en el envase del producto como en la etiqueta de la caja. Los discos sobrantes de un CARTUCHO abierto deben almacenarse a 2-8°C durante no más de 7 días. El cartucho que contiene los discos sin usar debe devolverse a su sobre desecante y guardarse en la bolsa resellable. Los discos en CAJA se pueden usar hasta 2 meses desde la primera apertura y deben almacenarse a la temperatura de almacenamiento indicada en la etiqueta. Desechar los discos caducados.

ELIMINACIÓN DEL MATERIAL USADO

Después de la utilización Antibiotic Disc y el material que ha entrado en contacto con la muestra tienen que ser descontaminados y eliminados de acuerdo con las técnicas en uso en laboratorio para la descontaminación y la eliminación de material potencialmente infecto.

DEUTSCH

Antibiotic Disc

Antibiotika Discs zur Empfindlichkeitstestung

BESCHREIBUNG

Antibiotic Disc sind Papierdiscs mit speziellen Eigenschaften. Sie sind mit Antibiotika imprägniert und werden für die Empfindlichkeitstestung entsprechend der Kirby-Bauer Anibiotika Testung (KB Testung oder Disk Diffusion Antibiotika Sensitivitätstestung) verwendet.
Antibiotic Disc sind in vielen verschiedenen Konfigurationen erhältlich, jede Konfiguration ist in Packungen zu 50 und 250 Tests erhältlich.

INHALT DER PACKUNG

Discs in Kartuschen
Die 50-Test Box enthält 1 Kartusche mit 50 Discs verpackt in beschichteten Folienbeuteln.
Die 250-Test Box enthält 5 Kartuschen von 50 Discs, jede Kartusche einzeln verpackt in beschichteten Folienbeuteln.
Jedes Packung enthält auch eine transparenten wiederverschließbaren Beutel.

Discs in Röhrchen
Das Röhrchen enthält 250 discs und eine Trockenmitteltablette.

TESTPRINZIP

Die Discs werden auf die Oberfläche eines Nährbodens aufgebracht, der mit einer reinen Kolonie-Suspension des zu untersuchenden Mikroorganismus beimpft ist. Nach der Inkubation werden die Platten ausgewertet, indem der Hemmhofdurchmesser bestimmt und mit den entsprechenden Standardvorgaben verglichen wird. Auf diese Weise kann eine

Einteilung der Mikroorganismen in die Kategorien „empfindlich“, „intermediär“ oder „resistent“ gegenüber der getesteten antimikrobiellen Substanz vorgenommen werden.

ZUSAMMENSETZUNG

Die Liofilchem Discs für die antimikrobielle Empfindlichkeitstestung sind aus einem hochqualitativen Papier und gemäß den WHO und FDA Spezifikationen. Die Discs werden entsprechend den Qualitätssicherungssystemen UNI EN ISO 9001, EN ISO 13485 und nach DIN Spezifikation für die Wirksamkeit hergestellt, d.h. die Konzentration des Antibiotikums beträgt 90-125% der auf der Disc angegeben Konzentration.

PROBENENTNAHME UND -LAGERUNG

Die für die Empfindlichkeitstestung vorgesehenen Kolonien werden von einem vorher mit der zu prüfenden Probe inokulierten Medium abgenommen. Bei Mischkulturen müssen vorher Reinkulturen hergestellt werden.

TESTDURCHFÜHRUNG

1. Lassen Sie die Discs auf Raumtemperatur kommen, bevor Sie die Kartusche öffnen, um die Kondensation auf den Discs zu minimieren, da diese die Langzeitstabilität beeinträchtigen könnte.
2. Stellen Sie eine Suspension mit der Dichte eines 0,5 McFarland-Trübungsstandards her.
3. Beimpfen Sie mit einem Tupfer ein geeignetes Agarplattenmedium, indem Sie die Suspension gleichmäßig auf der gesamten Agaroberfläche verteilen.
4. Bringen Sie Discs fest auf die Oberfläche der beimpften Agarplatte auf.
5. Inkubieren Sie die Platten im umgedrehten Zustand (Agar nach oben) bei geeigneter Temperatur, Atmosphäre und Zeit, entsprechend der gewählten Methodik (z. B. CLSI, EUCAST).
6. Legen Sie unbenutzte Discs wieder in den Kühlschrank/Gefrierschrank, sobald die Anwendung der Discs abgeschlossen ist (siehe LAGERUNG).

HINWEIS 1: Das zu verwendende Medium hängt von dem zu untersuchenden Organismus und der angewandten Methodik ab und muss vom Medienhersteller für antimikrobielle Empfindlichkeitstests validiert werden. Eine Liste der empfohlenen Agarmedien finden Sie am Ende dieser Gebrauchsanweisung.

HINWEIS 2: Es wird empfohlen, die Inokulumsuspension innerhalb von 15 Minuten nach der Zubereitung zu verwenden, die Discs innerhalb von 15 Minuten nach der Inokulation aufzutragen und die Platten innerhalb von 15 Minuten nach dem Auftragen der Discs zu inkubieren.

Weitere Details entnehmen Sie bitte den aktuellen veröffentlichten Standards.

AUSWERTUNG DER ERGEBNISSE / QUALITÄTSKONTROLLE

Am Ende der Inkubationszeit die Hemmhöfe messen und basierend auf gegenwärtige Referenzstandards interpretieren:

Antibiotic Disc Interpretative Criteria and Quality Control (pdf file)

KLINISCHE INTERPRETATION

Ein *in-vitro* durchgeführter Empfindlichkeitstest kann *in-vivo* Bedingungen nicht exakt reproduzieren. Dennoch kann die Wirkung der im Nährboden vorhandenen unterschiedlichen Antibiotikakonzentrationen auf das Keimwachstum dargestellt werden.
Die Entscheidung, welches Antibiotikum zur Therapie des Patienten eingesetzt wird, muss der behandelnde Arzt unter Berücksichtigung aller Patienteninformationen treffen.

GRENZEN DES VERFAHRENS

Agardiffusionsmethoden zur Empfindlichkeitstestung werden unter *in-vitro* Bedingungen durchgeführt und können daher die extrem komplexen *in-vivo* Vorgänge nicht genau wiedergeben. Sie sind trotzdem ein nützliches und wichtiges Hilfsmittel zur Identifizierung der richtigen Therapie. Viele verschiedene Faktoren können das Endergebnis der Agardiffusionsmethode zur Empfindlichkeitstestung beeinflussen. Hierzu gehören hauptsächlich das verwendete Kulturmedium, die Beschichtung der Discs, die Inokulation des Nährmediums, Temperatur, Dauer der Inkubation und Inkubationsathmosphäre, Zustand vor Inkubation und Diffusion, Tiefe des Nährmediums etc.

VORSICHTSMAßNAHMEN

Nach gegenwärtiger Gesetzgebung können Antibiotic Disc nicht als gefährlich eingestuft werden. Antibiotic Disc sind zum einmaligen Gebrauch bestimmt. Antibiotic Disc sind nur für die diagnostische *in-vitro* Testung und den professionellen Einsatz. Sie müssen im Labor von gut geschultem Personal unter Berücksichtigung der Regeln für das Arbeiten mit pathogenen Keimen angewendet werden.

LAGERUNG

Die ungeöffnete Verpackung der Antibiotika Discs kann in den meisten Fällen bei –20°C bis + 8°C gelagert werden. Einige Produkte müssen bei –20°C als maximale Lagertemperatur aufbewahrt werden. Die empfohlene Lagertemperatur befindet

sich auf dem Produktumschlag und auf dem Label der Verpackung. Übrig gebliebene Discs aus einer geöffneten Kartusche müssen bei 2-8°C für nicht mehr als 7 Tage gelagert werden. Die Kartusche mit den unbenutzten Discs sollte in ihre Trockenmittelhülle zurückgelegt und dann in den wiederverschließbaren Beutel eingesetzt werden. Discs in einem BEHÄLTER können bis zu 2 Monate nach dem ersten Öffnen verwendet werden und müssen bei der auf dem Etikett angegebenen Lagertemperatur gelagert werden. Verwerfen Sie Discs mit Ablauf des Verfallsdatums.

ENTSORGUNG VON GEBRAUCHTEM MATERIAL

Nach Gebrauch werden Antibiotic Disc zusammen mit dem Material, das in Kontakt mit den Proben gekommen ist, dekontaminiert und entsprechend den Laborrichtlinien für infektiöses Material entsorgt.

Antibiotic Disc

FRANÇAIS

Disques antibiotiques pour antibiogramme

DESCRIPTION

Antibiotic Disc sont des disques papier élaborés avec une matière spécifique. Ils sont imprégnés avec un antibiotique et utilisés pour les tests de sensibilité selon la méthode de tests antibiotiques de Kirby-Bauer (Tests KB ou test de sensibilité sur disque antibiotique).
Les Antibiotic Disc existent dans une large variété de configuration. Chaque configuration est disponible dans la variante à 50 et 250 tests.

CONTENU DES EMBALLAGES

Disques en cartouche
La version de 50 tests contient 1 cartouche avec 50 disques dans un emballage dessicant.
La version de 250 tests contient 5 cartouches de 50 disques, chaque cartouche emballé individuellement dans un emballage dessicant.
Chaque paquet contient également un sachet refermable transparent.

Disques en flacon
Le flacon contient 250 disques avec un dessicant.

PRINCIPE DE LA MÉTHODE

Les disques sont appliqués sur la surface d'un milieu de culture, inoculé avec une suspension monobacterienne du micro-organisme à étudier. Après l'incubation, les plaques sont examinées, les zones d'inhibition circulaires autour de chaque disque sont mesurées et comparées avec les diamètres des zones d'inhibition circulaires standard: les micro-organismes sont ainsi définis sensibles, intermédiaires ou résistants aux agents antimicrobiens testés.

COMPOSITION

Les disques Liofilchem pour les tests de sensibilité antimicrobienne sont fabriqués avec un papier haute qualité selon les spécifications WHO et FDA.
Les disques sont fabriqués sous les systèmes de qualité UNI EN ISO 9001 et EN ISO 13485 et la spécification DIN pour la puissance, c'est-à-dire que la concentration de chaque antibiotique est comprise entre 90 et 125%.

PRÉLÈVEMENT ET CONSERVATION DES ÉCHANTILLONS

Les colonies à soumettre à l'antibiogramme sont reprises des milieux de culture préalablement ensemencés avec l'échantillon à étudier. En cas de colonies mixtes, procéder à la purification des souches bactériennes avant l'ensemencement sur les plaques pour l'antibiogramme.

PROCÉDURE DU TEST

1. Laisser les disques revenir à température ambiante avant d'ouvrir le conditionnement (cartouche ou flacon) pour éviter la formation de condensation qui pourrait affecter la stabilité à long terme.
2. Réaliser une suspension bactérienne à 0.5 McF de la bactérie à tester.
3. A l'aide d'un écouvillon, ensemencer la suspension bactérienne sur toute la surface de la gélose appopriée de façon uniforme.
4. Appliquer les disques sur la surface de la gélose.
5. Incuber les boites à l'envers à température et conditions appropriées selon les référentiels (CLSI, EUCAST…)
6. Après utilisation remettre les disques non utilisés suivant les recommandations de stockage fournisseur.

NOTE 1: Le milieu à utiliser dépend de la bactérie à tester et doit faire l'objet d'une validation dans le cas d'un test d'étude de la sensibilité antibactérienne. Une liste des milieux gélosés recommandés se trouve à la fin de cette notice.

NOTE 2: Il est recommandé d'appliquer la règle des 3x15 mn pour la réalisation de l'antibiogramme:
- Inoculum utilisé avant 15 mn ;
- Appliquer les disques 15 mn après ensemencement ;
- Incuber les géloses 15 mn après dépôt des disques.

Pour plus d'information, veuillez vous référer aux référentiels en vigueur.

INTERPRÉTATION DES RÉSULTATS / CONTRÔLE QUALITÉ

A la fin de période d'incubation, mesurer le halo d'inhibition et l'interpréter selon le standard de référence actuel:

Antibiotic Disc Interpretative Criteria and Quality Control (pdf file)

INTERPRÉTATION CLINIQUE

L'antibiogramme effectué *in vitro* ne peut pas reproduire exactement les conditions qui se trouvent *in vivo*, il est toutefois en mesure de mettre en évidence l'effet de la concentration de l'antibiotique, qui varie dans le milieu de culture, vis-à-vis de la population microbienne en phase de développement. Le choix final de l'antibiotique à administrer au patient revient au clinicien qui est en possession de toutes les données concernant le patient.

LIMITES

L'antibiogramme par diffusion, utilisant une technique *in vitro*, n'est pas en mesure de reproduire les conditions très complexes qui se trouvent in vivo ; il représente toutefois un support utile et important pour le choix thérapeutique du clinicien. Nombreuses sont les variables qui influencent le résultat final de l'antibiogramme par diffusion ; les principales sont représentées par : le milieu de culture utilisé, l'imprégnation des disques, l'inoculation du milieu, la température, le temps et l'atmosphère d'incubation des plaques, les conditions de pré-incubation et de pré-diffusion, l'épaisseur du milieu, etc.

PRÉCAUTIONS

Le produit Antibiotic Disc ne peut être classé comme dangereux aux termes de la législation en vigueur. Antibiotic Disc est un dispositif à usage unique. Antibiotic Disc est uniquement destiné à un usage diagnostique in vitro, et à un usage professionnel ; il doit être utilisé en laboratoire par des opérateurs correctement formés, avec des méthodes approuvées d'asepsie et de sécurité à l'égard des agents pathogènes.

CONSERVATION

L'emballage non ouvert des disques antibiotiques peut être stocké dans la plupart des cas entre –20°C et +8°C jusqu'à la date de péremption. Certains produits doivent être stockés à –20°C comme température de stockage maximale. Les limites de températures recommandées peuvent être trouvées sur l'enveloppe du produit et sur la boite d'étiquettes. Les disques non-utilisés contenus dans la cartouche doit être conservés à 2-8°C pendant 7 jours maximum. La cartouche doit être remis dans son emballage initial avec dessicant. Les disques conditionnés dans le flacon peuvent être utiliser jusqu'à 2 mois après leur première ouverture et doivent être conservés à la température de stockage indiquée sur l'étiquette poduit. Jeter les disques périmés.

ÉLIMINATION DU MATÉRIEL UTILISÉ

Après utilisation, les Antibiotic Disc et le matériel ayant été au contact avec l'échantillon doivent être décontaminés et éliminés conformément aux techniques utilisées en laboratoire pour la décontamination et l'élimination de matériel potentiellement infecté.

Antibiotic Disc

ΕΛΛΗΝΙΚΑ

Αντιβιοτικά δισκία για αντιβιογράμματα

ΠΕΡΙΓΡΑΦΗ

Οι δίσκοι αντιβοτικών είναι χάρτινα δισκία με ιδιαίτερα χαρακτηριστικά,εμποτισμένα με αντιβιοτικά,που χρησιμοποιούνται για τη δοκιμασία ευαισθησίας σύμφωνα με τη μέθοδο Bauer-Kirby ή με τη μέθοδο διάχυσης. Τα Antibiotic Disc παράγονται σε μια μεγάλη γκάμα σχηματισμών. Κάθε σχηματισμός είναι διαθέσιμος σε παραλλαγές των 50 και 250 τεστ.

ΠΕΡΙΕΧΟΜΕΝΟ ΣΥΣΚΕΥΑΣΙΩΝ

Δίσκοι σε φυσίγγιο

Το κιτ των 50 τεστ περιέχει 1 φυσίγγιο 50 δίσκων συσκευασμένων σε φάκελο με αφυγραντικό.
Το κουτί των 250 τεστ περιέχει 5 φυσίγγια των 50 δίσκων, κάθε ένα ξεχωριστά συσκευασμένο σε φάκελο με αφυγραντικό.
Κάθε συσκευασία επίσης περιέχει ένα διάφανο, επανασφραγιζόμενο σακουλάκι.

Δίσκοι σε δοχείο
Το δοχείο περιέχει 250 δίσκους και αφυγραντικό πώμα.

ΑΡΧΗ ΤΗΣ ΜΕΘΟΔΟΥ

Οι δίσκοι τοποθετούνται σε υπόστρωμα καλλιέργειας στην επιφάνεια του οποίου έχει εμβολιαστεί ζωμός που περιέχει καθαρές αποικίες του μικροοργανισμού υπό εξέταση. Μετά την επώαση εξετάζονται τα τρυβλία, μετρώνται οι ζώνες αναστολής γύρω από κάθε δίσκο και συγκρίνονται με τις στάνταρ διαμέτρους των ζωνών αναστολής. Με τον τρόπο αυτό οι μικροοργανισμοί ορίζονται ως ευαίσθητοι, ενδιάμεσοι ή ανθεκτικοί στους εξεταζόμενους αντιμικροβιακούς παράγοντες.

ΣΥΝΘΕΣΗ

Τα δισκία ευαισθησίας αντιβιοτικών της Liofilchem είναι κατασκευασμένα από χαρτί υψηλής ποιότητας , σύμφωνα με τις προδιαγραφές και τα πρότυπα που έχει ορίσει ο ΠΟΥ και το FDA.
Οι δίσκοι κατασκευάζονται σύμφωνα με τα συστήματα ποιότητας UNI EN ISO 9001 και EN ISO 13485, και σύμφωνα με τις προδιαγραφές DIN, δηλαδή η συγκέντρωση του κάθε αντιβιοτικού αγγίζει το 90-125% της αναγραφόμενης στο δισκίο συγκέντρωσης.

ΣΥΛΛΟΓΗ ΚΑΙ ΔΙΑΤΗΡΗΣΗ ΔΕΙΓΜΑΤΩΝ

Οι αποικίες που θα υποβληθούν σε αντιβιόγραμμα παραλαμβάνονται από υποστρώματα καλλιεργιών στις οποίες σπέρνεται προηγουμένως το υπό εξέταση δείγμα. Σε περίπτωση μικτών καλλιεργιών πρέπει να γίνει καθαρισμός των βακτηριδιακών στελεχών πριν από τη σπορά στα τριβλία για το αντιβιόγραμμα.

ΔΙΑΔΙΚΑΣΙΑ ΤΕΣΤ

1. Αφήστε τους δίσκους να έρθουν σε θερμοκρασία δωματίου πριν το άνοιγμα (φυσίγγιο ή δοχείο), για την ελαχιστοποίηση της συμπύκνωσης των δίσκων, κάτι που θα μπορούσε να επηρεάσει τη μακροπρόθεσμη σταθερότητα.
2. Κάντε ένα εναιώρημα του δοκιμαστικού οργανισμού στην πυκνότητα ενός προτύπου θολερότητας 0,5 McFarland.
3. Χρησιμοποιώντας ένα στυλεό, εμβολιάστε ένα κατάλληλο μέσο πλάκας άγαρ απλώνοντας ομοιόμορφα το εναιώρημα σε ολόκληρη την επιφάνεια άγαρ.
4. Εφαρμόστε τους δίσκους σταθερά στην επιφάνεια της πλάκας άγαρ.
5. Επωάστε τις πλάκες σε ανεστραμμένη θέση στην κατάλληλη θερμοκρασία, ατμόσφαιρα και χρόνο, ακολουθώντας τη μεθοδολογία που επιλέξατε (π.χ. CLSI, EUCAST).
6. Επιστρέψτε τους αχρησιμοποίητους δίσκους στο ψυγείο / καταψύκτη μόλις ολοκληρωθεί η εφαρμογή των δίσκων (βλέπε ΑΠΟΘΗΚΕΥΣΗ).

ΣΗΜΕΙΩΣΗ 1: Το μέσο που χρησιμοποιείται εξαρτάται από τον οργανισμό που βρίσκεται υπό διερεύνηση και τη μεθοδολογία που ακολουθείται και πρέπει να επικυρωθεί από τον κατασκευαστή μέσων για τη δοκιμή ευαισθησίας κατά των μικροβίων. Μια λίστα με τα προτεινόμενα μέσα άγαρ μπορεί να βρεθεί στο τέλος αυτής της IFU.

ΣΗΜΕΙΩΣΗ 2: Συνιστάται η χρήση του εναιωρήματος εμβολίου εντός 15 λεπτών από την προετοιμασία, η εφαρμογή δίσκων εντός 15 λεπτών από τον εμβολιασμό και των πλακών επώασης εντός 15 λεπτών από την εφαρμογή του δίσκου.

Για περισσότερες λεπτομέρειες, ανατρέξτε στα τρέχοντα δημοσιευμένα πρότυπα.

ΕΡΜΗΝΕΙΑ ΤΩΝ ΑΠΟΤΕΛΕΣΜΑΤΩΝ / ΕΛΕΓΧΟΣ ΠΟΙΟΤΗΤΑΣ

Στο τέλος του χρόνου επώασης μετρείστε τη ζώνη αναστολής που δημιουργήθηκε και ερμηνεύστε σύμφωνα με τα τρέχοντα πρότυπα αναφοράς:

Antibiotic Disc Interpretative Criteria and Quality Control (pdf file)

ΚΛΙΝΙΚΗ ΕΡΜΗΝΕΙΑ

Το αντιβιόγραμμα που εκτελείται *in vitro* δεν μπορεί να αναπαράγει επακριβώς τις συνθήκες *in vivo*, ωστόσο είναι σε θέση να προβάλει την επίδραση της συγκέντρωσης αντιβιοτικού, που μεταβάλλεται στο υπόστρωμα καλλιέργειας, σε σε σχέση με τον μικροβιακό πληθυσμό που βρίσκεται υπο ανάπτυξη. Η τελική επιλογή του αντιβιοτικού που θα χορηγηθεί στον ασθενή εναπόκειται στον κλινικό γιατρό ο οποίος διαθέτει όλα τα στοιχεία που αφορούν τον ίδιο τον ασθενή.

ΠΕΡΙΟΡΙΣΜΟΙ

Το αντιβιόγραμμα με τη μέθοδο διάχυσης, χρησιμοποιώντας μια τεχνική *in vitro*, δεν είναι σε θέση να αναπαράγει τις εξαιρετικά σύνθετες συνθήκες της κατάστασης in vivo, ωστόσο αποτελεί μια χρήσιμη και σημαντική υποστήριξη για τη θεραπευτική επιλογή του κλινικού γιατρού.

Πολλές είναι οι μεταβλητές που επηρεάζουν το τελικό αποτέλεσμα του αντιβιογράμματος με διάχυση. Οι κυριότερες από αυτές είναι: χρησιμοποιούμενο υπόστρωμα καλλιέργειας, εμποτισμός των δίσκων,, εμβολιασμός (στρώσιμο) υποστρώματος, θερμοκρασία, χρόνος και ατμόσφαιρα επώασης των τριβίων, συνθήκες προ-επώασης και προ-διάχυσης, πάχος του υποστρώματος, κλπ.

ΠΡΟΦΥΛΑΞΕΙΣ

Το προϊον Antibiotic Disc, με βάση την ισχύουσα νομοθεσία, δεν ταξινομείται ως επικίνδυνο. Τα Antibiotic Disc είναι μίας μόνο χρήσης. Τα Antibiotic Disc προορίζονται μόνο για διαγνωστική *in vitro* χρήση, μόνο για επαγγελματικούς σκοπούς και πρέπει να χρησιμοποιούνται σε εργαστήριο από κατάλληλα εκπαιδευμένο προσωπικό, με μεθόδους που έχουν εγκριθεί για θέματα ασηψίας και ασφάλειας σε σχέση με τους παθογόνους παράγοντες.

ΑΠΟΘΗΚΕΥΣΗ

Αποθήκευση της αρχικής σφραγισμένης συσκευασίας των δίσκων αντιβιοτικών, στην πλειονότητα των περιπτώσεων, στους -20°C έως +8°C μέχρι την αναγραφόμενη ημερομηνία λήξης. Κάποια είδη θα πρέπει να αποθηκευτούν στους -20°C, ως μέγιστη θερμοκρασία αποθήκευσης. Οι προτεινόμενες συνθήκες και θερμοκρασίες φύλαξης, αναγράφονται τόσο στη εσώκλειστο του είδους όσο και στην ετικέτα της συσκευασίας του. Οι υπολειπόμενοι δίσκοι από ανοικτό φυσίγγιο θα πρέπει να φυλάσσονται όχι για περισσότερο από 7 ημέρες. Το φυσίγγιο που περιέχει αχρησιμοποίητους δίσκους θα πρέπει να επιστραφεί στον ξηραντικό φάκελο και στη συνέχεια να εισαχθεί στο επανασφραγιζόμενο σακουλάκι. Οι δίσκοι σε δοχείο μπορούν να χρησιμοποιηθούν έως και 2 μήνες από το πρώτο άνοιγμα και πρέπει να αποθηκευτούν στη θερμοκρασία αποθήκευσης που αναγράφεται στην ετικέτα της συσκευασίας. Απορρίψτε τους δίσκους που έχουν λήξει.

ΑΠΟΡΡΙΨΗ ΤΟΥ ΧΡΗΣΙΜΟΠΟΙΗΜΕΝΟΥ ΥΛΙΚΟΥ

Μετά τη χρήση, τα Antibiotic Disc και τα υλικά που ήρθαν σε επαφή με το δείγμα πρέπει να απολυμαίνονται και να απορρίπτονται σύμφωνα με τις συνήθεις τεχνικές εργαστηρίου για την απολύμανση και την απόρριψη πιθανώς μολυσμένου υλικού.

Antibiotic Disc

PORTUGUÊS

Discos antibiótico para antibiograma

DESCRIÇÃO

Antibiotic Disc, são discos em papel, com características especiais, impregnados com antibiótico, e destinados a ensaios de susceptibilidade anti-microbiano, pelo teste de Kirby-Bauer (Teste de KB ou teste de sensibilidade antibiótica por difusão em disco).
Antibiotic Disc estão disponíveis numa ampla variedade de apresentações.. Cada configuração é disponível na variante de 50 e 250 testes.

CONTEÚDO DAS CONFECÇÕES

Discos em cartucho
A caixa de 50 testes contém 1 cartucho de 50 discos num envelope dissecante.
A caixa de 250 testes contém 5 cartuchos de 50 discos, cada embalado individualmente em envelope dissecante.
Cada embalagem também é fornecida em saco transparente selado.

Discos em embalagem.
A embalagem contém 250 discos e um tablete dessecante.

PRINCÍPIO DO MÉTODO

Os discos são aplicados na superfície do meio de cultura inoculado com uma suspensão colónia pura do microrganismo em estudo. Depois da incubação, são examinadas as chapas, misturadas as auréolas de inibição ao redor de cada disco e comparados com os diâmetros das auréolas de inibição padrão: deste modo os microrganismos são definidos sensíveis, intermédios ou resistentes aos agentes anti-micróbios testados.

COMPOSIÇÃO

Os discos de suscetibilidade antimicrobiana da Liofilchem são produzidos em papel de alta Qualidade em conformidade com as especificações da WHO e FDA.
Os discos são produzidos segundo os sistemas de Qualidade da UNI EN ISO 9001 e EN ISO 13485, e ainda de acordo com a DIN nos termos de, concentração de cada antibiótico encontra-se na gama entre os 90-125% da concentração legislada para cada disco.

RECOLHA E CONSERVAÇÃO DAS AMOSTRAS

As colónias que devem ser submetidas ao antibiograma são recolhidas nos terrenos de cultura semeados preventivamente com a amostra em exame. Em caso de colónias mistas é necessário proceder à purificação das estirpes bactéricas antes da semeadura nas chapas para o antibiograma.

PROCEDIMENTO DO TESTE

1. Permite que os discos equilibrem à temperatura ambiente antes usar e abrir a embalagem de forma a minimizar condensações no disco, que poderia afetar a estabilidade a longo prazo.
2. Fazer uma suspensão do organismo teste com uma densidade padrão de turvação de McFarland de 0.5.
3. Usando uma zaragatoa, inocular no meio adequado a suspensão por toda a placa de forma a obter um espalhamento uniforme.
4. Aplicar os discos firmemente à superfície do meio inoculado.
5. Incubar as places, em posição invertida, à temperatura, atmosfera e tempo adequado, de acordo com a metodologia escolhida (ex.: CLSI, EUCAST).
6. Colocar novamente os discos não utilizados no frigorífico assim que a aplicação dos discos estiver terminada (ver ARMAZENAMENTO).

NOTE 1: O meio a usar depende do organismos em estudo e da metodologia escolhida, e deverá estar validada pelo fabricante para testes antimicrobianos de susceptibilidade. Uma lista de meios recomendados está disponível no fim desta IFU.

NOTE 2: É recomendado a utilização de uma suspensão de inóculo até 15 minutos pós preparação, aplicar os discos até 15 minutos após inoculação e incubar as placas até 15 minutos após a aplicação do disco.

Para obter mais detalhes, consulte os padrões publicados atuais.

ANALISE DOS RESULTADOS / CONTROLO DA QUALIDADE

Após incubação, meça os halos de inibição e interprete-os, de acordo com as normas de referência:

Antibiotic Disc Interpretative Criteria and Quality Control (pdf file)

INTERPRETAÇÃO CLÍNICA

O antibiograma realizado *in vitro* não pode reproduzir exactamente as condições que se encontram *in vivo*, contudo é em grau de evidenciar o efeito da concentração de antibiótico, que varia no terreno cultural, nas comparações da população de micróbios em fase de desenvolvimento.
A escolha final do antibiótico a subministrar ao paciente concerne ao clínico que é em posse de todos os dados que dizem respeito ao próprio paciente.

LIMITES

O antibiograma para difusão, utilizando uma técnica *in vitro*, não é em grau de reproduzir as condições extremamente complexas que se encontram “in vivo”; contudo, constitui um útil e importante suporte à escolha terapêutica do clínico. Muitas são as variáveis que influenciam o resultado final do antibiograma por difusão; as principais são representadas pelo: terreno de cultura utilizado, impregnação dos discos, inóculo do terreno, temperatura, tempo e atmosfera de incubação das chapas, condições de pré-incubação e pré-difusão, espessura do terreno, etc.

PRECAUÇÕES

O produto Antibiotic Disc não é classificável como perigoso em conformidade com a legislação em vigor. Antibiotic Disc é um dispositivo de uso único. Antibiotic Disc é somente para o uso diagnóstico *in vitro,* é destinado a um âmbito profissional e deve ser utilizado em laboratório por operadores adequadamente treinados, com métodos aprovados de assepsia e de segurança nos confrontos dos agentes patogénicos.

ARMAZENAMENTO

Depois de aberta a embalagem de Antibioc Disc, esta pode ser armazenada, na maioria dos casos, de –20ºC a +8ºC, até ao fim da data de validade. Alguns dos produtos deverão ser armazenados a –20ºC, como temperatura máxima de armazenamento. As recomendações dos limites de temperatura, vêm descritos na embalagem do produto, assim como no seu rótulo. Os discos não utilizados do cartucho aberto deverão ser armazenados novamente a 2-8ºC até um máximo de 7 dias. Os cartuchos não utilizados deverão ser colocados novamente na embalagem com o dessecante, dentro da embalagem resselável. Os discos em cartucho podem ser utilizados pelo período de 2 meses após a primeira data de abertura e deverão ser armazenados de acordo com a temperatura indicada no rótulo da embalagem. Descartar os discos fora de validade.

ELIMINAÇÃO DO MATERIAL UTILIZADO

Depois da utilização do Antibiotic Disc e do material que entrou a contacto com a amostra, devem ser descontaminados e eliminados em acordo com as técnicas em uso no laboratório para a descontaminação e a eliminação de material potencialmente infecto.

Antibiotic Disc

POLSKI

Krążki antybiotykowe do oznaczania lekowrażliwości

OPIS

Antibiotic Disc są krążkami bibułowymi o specjalnych cechach, które nasączone są antybiotykiem i stosowane są do badań lekowrażliwości zgodnie z metodą Kirby-Bauera (badanie KB lub badanie lekowrażliwości metodą dyfuzyjno-krążkową). Krążki antybiotykowe dostępne są w wielu konfiguracjach. Każda konfiguracja dostępna jest w opakowaniach po 50 i 250 testów.

ZAWARTOŚĆ OPAKOWANIA

Krążki w kartridżu

Opakowanie 50 testów zawiera 1 kartridż z 50 krążkami zapakowanymi w saszetkę ze środkiem pochłaniającym wilgoć.
Opakowanie 250 testów zawiera 5 kartridży z 50 krążkami zapakowanymi indywidualnie w saszetkę ze środkiem pochłaniającym wilgoć.
Każde opakowanie zawiera także przezroczysty zapinany woreczek.

Krążki w pojemniku

Pojemnik zawiera 250 krążków i tabletkę środka pochłaniającego wilgoć.

ZASADA METODY

Krążki nanoszone są na powierzchnię pożywki hodowlanej zaszczepionej zawiesiną bakteryjną, zawierającą czyste kolonie badanego mikroorganizmu. Po inkubacji płytki są badane, strefy zahamowania wokół każdego krążka są mierzone i porównywane ze standardowymi strefami zahamowania wzrostu: w ten sposób mikroorganizmy są określane jako wrażliwe, średniowrażliwe lub oporne na testowane czynniki przeciwbakteryjne.

SKŁAD

Krążki do badania wrażliwości na środki przeciwdrobnoustrojowe marki Liofilchem są wykonane z wysokiej jakości bibuły zgodnie ze specyfikacjami WHO i FDA.
Krążki produkowane są zgodnie z systemem jakości UNI EN ISO 9001 i EN ISO 13485 oraz specyfikacją DIN na wysycenie t.j. stężenie każdego antybiotyku jest w zakresie 90–125% stężenia opisanego na krążku.

POBIERANIE I PRZYGOTOWANIE PRÓBEK

Kolonie, które będą poddawane badaniu lekowrażliwości pobierane są z pożywek hodowlanych, które uprzednio zostały zaszczepione badaną próbką. W przypadku kolonii mieszanych, przed zaszczepieniem na płytki do badania lekowrażliwości, szczepy bakteryjne należy oczyścić.

PROCEDURA TESTU

1. Pozostawić krążki do osiągnięcia temperatury pokojowej przed otwarciem opakowania (kartridża lub pojemnika) w celuzminimalizowania kondensacji na krążku, ponieważ taka kondensacja może wpływać na długoterminową stabilność;
2. Sporządzić zawiesinę badanego organizmu do gęstości wzorca zmętnienia McFarlanda 0,5.
3. Za pomocą wymazówki zaszczepić odpowiednią pożywkę na płytce agarowej, równomiernie rozprowadzając zawiesinę na całej powierzchni agaru.
4. Mocno przycisnąć krążki do powierzchni zaszczepionej płytki agarowej.
5. Inkubować płytki w pozycji odwróconej w odpowiedniej temperaturze, warunkach i czasie, zgodnie z wybraną metodologią (np. CLSI, EUCAST).
6. Odłożyć nieużyte krążki do lodówki/zamrażarki natychmiast po zakończeniu aplikowania krążków (patrz PRZECHOWYWANIE).

UWAGA 1: Wybór pożywki zależy od badanego organizmu i stosowanej metodologii i musi być zwalidowane przez producenta pożywki w celu badania wrażliwości na środki przeciwdrobnoustrojowe. Lista rekomendowanych pożywek agarowych znajduje się na końcu niniejszej instrukcji użytkowania.

UWAGA 2: Zaleca się użycie zawiesiny inokulum w ciągu 15 minut od przygotowania, nałożenie krążków w ciągu 15 minut od posiewu i inkubację płytek w ciągu 15 minut od nałożenia krążka.

Więcej informacji można znaleźć w aktualnie opublikowanych normach.

OCENA WYNIKÓW / KONTROLA JAKOŚCI

Pod koniec okresu inkubacji, zmierzyć strefy inhibicji i interpretować zgodnie z obowiązującymi standardami referencyjnymi:

Antibiotic Disc Interpretative Criteria and Quality Control (pdf file)

INTERPRETACJA KLINICZNA

Test lekowrażliwości przeprowadzony *in vitro* nie może dokładnie odtwarzać warunków *in vivo*. Jednakże pokazuje on efekt stężenia antybiotyku, który różni się na pożywce hodowlanej w stosunku do wzrostu populacji bakterii.
Ostateczny wybór antybiotyku podanego pacjentowi należy do lekarza, który posiada wszystkie informacje o pacjencie.

OGRANICZENIA

Dyfuzyjne testy oznaczania lekowrażliwości wykorzystują technikę *in vitro* i dlatego nie mogą odtworzyć niezwykle złożonych warunków *in vivo*. Niemniej jednak jest to użyteczne i ważne narzędzie, które pomaga lekarzowi wybrać właściwą terapię. Wiele zmiennych czynników wpływa na ostateczny wynik dyfuzyjnego testu lekowrażliwości. Główne z nich to: użyta pożywka hodowlana, wysycenie krążka, zaszczepienie pożywki, temperatura, czas i warunki inkubacji płytki, warunki przed inkubacją i przed nałożeniem krążków, grubość agaru, etc.

ŚRODKI OSTROŻNOŚCI

Zgodnie z obowiązującymi przepisami krążki antybiotykowe nie mogą być sklasyfikowane jako niebezpieczne. Krążki są produktami jednorazowego użytku. Służą wyłącznie do diagnostyki *in vitro* i przeznaczone są do użytku profesjonalnego. Należy je stosować w laboratorium przez odpowiednio wyszkolonych operatorów przy użyciu zatwierdzonych metod aseptycznych i dotyczących bezpieczeństwa czynników chorobotwórczych.

PRZECHOWYWANIE

Nieotwarte opakowanie Antibiotic Disc można przechowywać w większości przypadków w –20°C do +8°C do upływu terminu ważności. Niektóre produkty muszą być przechowywane w –20°C jako maksymalnej temperaturze przechowywania. Zalecane zakresy temperatur można znaleźć zarówno na saszetce produktu jak i na etykiecie opakowania. Pozostałe krążki z otwartych kartridży należy przechowywać w temperaturze 2–8°C nie więcej niż 7 dni. Kartridż zawierający nieużyte krążki powinien być umieszczony ponownie w saszetce ze środkiem pochłaniającym wilgoć, a następnie włożony do zapinanego woreczka. Krążki w POJEMNIKU mogą być używane w czasie nieprzekraczającym 2 miesięcy po otwarciu i muszą być przechowywane w temperaturze podanej na etykiecie. Przeterminowane krążki należy wyrzucić.

SUWANIE ZUŻYTYCH MATERIAŁÓW

Po użyciu krążki i materiały, które weszły w kontakt z próbką należy odkazić i wyrzucić zgodnie z wytycznymi stosowanymi w laboratorium odnośnie do odkażania i likwidacji potencjalnie zakaźnych materiałów.

TABELLA DEI SIMBOLI / TABLE OF SYMBOLS / TABLA DE LOS SÍMBOLOS / TABELLE DER SYMBOLE / TABLEAU DES SYMBOLES / ΠΊΝΑΚΑΣ ΣΥΜΒΌΛΩ / MESA DE SÍMBOLOS / TABELA SYMBOLI

IVD	**It**: Dispositivo medico-diagnostico *in vitro* / **En**:*In Vitro* Diagnostic Medical Device / **Es**: Producto sanitario para diagnóstico *in vitro* / **De**: für *In Vitro* Diagnostik / **Fr**: Dispositif médical de diagnostic *in vitro* / **El:** *In Vitro* Διαγνωστικό Ιατροτεχνολογικό προϊόν / **Pt**: Dispositivo médico para diagnóstico *in vitro* / **Pl:** Wyrób medyczny do diagnostyki in vitro
	**It**: Non riutilizzare / **En**: Do not reuse / **Es**: No reutilizar / **De**: Nicht wiederverwenden / **Fr**: Ne pas réutiliser / **El**: Μην κάνετε επαναληπτική χρήση / **Pt**: Não reutilizar / **Pl:** Nie używać ponownie
	**It**: Fabbricante / **En**:Manufacturer / **Es**: Fabricante / **De**: Hersteller / **Fr**:Fabricant / **El**: Κατασκευαστής / **Pt**: Fabbricante / **Pl:** Producent
	**It**: Contenuto sufficiente per "n" saggi / **En**: Contains sufficient for <n> tests / **Es**: Contenido suficiente para <n> ensayos / **De**: Enthält Material für <n>Tests / **Fr**: Contenu suffisant pour "n" tests / **El**: Περιεχόμενο επαρκές για «ν» εξετάσεις / **Pt**: Conteúdo suficiente para "n" ensaios / **Pl:** Zawiera ilość wystarczającą na <n> testów
REF	**It**: Numero di catalogo / **En**: Catalogue number / **Es**: Número de catálogo / **De**: Bestellnummer / **Fr**: Référence du catalogue / **El**: Αριθμός καταλόγου / **Pt**: Referência de catálogo / **Pl:** Numer katalogowy
	**It**: Fragile, maneggiare con cura / **En**: Fragile, handle with care / **Es**: Frágil, manipular con precaución / **De**: Zerbrechlich, mit Vorsicht behandeln / **Fr**: Fragile, manipuler avec précaution / **El**: Εύθραυστο, να χρησιμοποιείται με προσοχή / **Pt**: Frágil, manusear com cuidado / **Pl:** Delikatne, obchodzić się ostrożnie
	**It**: Utilizzare entro / **En**: Use By / **Es**: Fecha de caducidad / **De**: Verwendbar bis / **Fr**: Date limite d'utilisation / **El**: Ημερομηνία λήξης / **Pt**: Prazo de validade / **Pl:** Używany przez
	**It**: Attenzione, vedere le istruzioni per l'uso / **En**: Caution,consult accompanying documents / **Es**: Atención,ver instrucciones de uso / **De**: Vorsicht, Begleitdokumente beachten / **Fr**: Attention voir notice d'instructions / **El**: Προειδοποίηση, συμβουλευτείτε τα συνοδά έντυπα / **Pt**: Atenção, consulte a documentação incluída / **Pl:** Zapoznaj się z instrukcją użytkowania
	**It**: Limiti di temperatura / **En**: Temperature limitation / **Es**: Limite de temperatura / **De**: Temperaturbereich / **Fr**: Limites de température / **El**: Περιορισμοί θερμοκρασίας / **Pt**: Limites de temperatura / **Pl:** Ograniczenie temperatury
	**It**: Limite superiore di temperatura / **En**: Upper limit of temperature / **Es**: Limite superior de temperatura / **De**: Temperaturobergrenze / **Fr**: Limite supérieure de température / **El**: ανώτερο όριο θερμοκρασίας / **Sv**: högsta temperaturen / **Pt**: Limite superior de temperatura / **Pl:** Górna granica temperatury
LOT	**It**: Codice del lotto / **En**: Batch code / **Es**: Codigo de lote numéro de lot / **De**: Charge / **Fr**: Numéro de lot / **El**: Αριθμός Παρτίδας / **Pt**: Código do lote / **Pl:** Kod partii

The following list of products might be out-of-date

View the complete range of Antimicrobial Discs in cartridge and canister on Liofilchem's website

Antibiotic discs in cartridge and canister							
Description		**µg**		**CLSI** [1]	**EUCAST** [3,4]		**Ref.***
Amikacin	AK	30		✓	✓		9004
Amoxicillin	AML	2				●	9151
Amoxicillin	AML	10			✓	●	9133
Amoxicillin	AML	25				●	9179
Amoxicillin	AML	30				●	9005
Amoxicillin-clavulanic acid	AUG	3	(2/1)		✓	●	9191
Amoxicillin-clavulanic acid	AUG	7.5				●	9255
Amoxicillin-clavulanic acid	AUG	30	(20/10)	✓	✓	●	9048
Amoxicillin 2 + Clavulanic acid 0.1	AC	2.1	(2/0.1)			●	9273 ◆
Amoxicillin 2 + Clavulanic acid 0.5	AC	2.5	(2/0.5)			●	9274 ◆
Amoxicillin 5 + Clavulanic acid 0.1	AC	5.1	(5/0.1)			●	9275 ◆
Amoxicillin 5 + Clavulanic acid 0.5	AC	5.5	(5/0.5)			●	9276 ◆
Amoxicillin 5 + Clavulanic acid 1	AC	6	(5/1)			●	9277 ◆
Amoxicillin 10 + Clavulanic acid 0.1	AC	10.1	(10/0.1)			●	9278 ◆
Amoxicillin10 + Clavulanic acid 0.5	AC	10.5	(10/0.5)			●	9279 ◆
Amoxicillin 10 + Clavulanic acid 1	AC	11	(10/1)			●	9280 ◆
Ampicillin	AMP	2			✓	●	9115
Ampicillin	AMP	10		✓	✓	●	9006
Ampicillin-sulbactam	AMS	20	(10/10)	✓	✓	●	9031
Ampliclox (Ampicillin + Cloxacillin)	ACL	30	(25/5)			●	9122
Azithromycin	AZM	15		✓	✓		9105
Azlocillin	AZL	75		✓			9007
Aztreonam	ATM	30		✓	✓		9008
Bacitracin	BA	10	units				9051
Carbenicillin	CAR	100		✓			9009
Cefaclor	CEC	30		✓	✓		9010
Cefadroxil	CDX	30			✓		9052
Cefamandole	MA	30		✓			9014
Cefazolin	KZ	30		✓	✓		9015
Cefepime	FEP	10					9220
Cefepime	FEP	30		✓	✓		9104
Cefepime + Clavulanic acid	FEL	40	(30/10)		✓ [10]	●	9143
Cefiderocol	FDC	30		✓	✓	●	9266
Cefixime	CFM	5		✓	✓		9089
Cefoperazone	CFP	30					9016
Cefoperazone	CFP	75		✓			9108
Cefotaxime	CTX	5			✓		9152
Cefotaxime	CTX	30		✓			9017
Cefotaxime	CTX	75					9134
Cefotaxime + Clavulanic acid	CTL	40	(30/10)		✓ [10]	●	9182
Cefotaxime + Clavulanic acid + Cloxacillin	CTLC				✓ [10]	●	9203
Cefotaxime + Cloxacillin	CTC	230	(30/200)		✓ [10]	●	9224
Cefotetan	CTT	30		✓			9081
Cefoxitin	FOX	30		✓	✓		9018
Cefoxitin + Cloxacillin	FOC	230	(30/200)				9144
Cefpirome	CR	30					9185
Cefpodoxime	PX	10		✓	✓		9064
Cefpodoxime + Clavulanic acid	PXL	11	(10/1)			●	9190
Cefprozil	CPR	30		✓			9112
Cefsulodin	CSD	30					9053
Ceftaroline	CPT	5			✓		9195
Ceftaroline	CPT	30		✓			9198
Ceftazidime	CAZ	10			✓		9153
Ceftazidime	CAZ	30		✓			9019
Ceftazidime-avibactam	CZA	14	(10/4)		✓		9206

Antibiotic discs in cartridge and canister							
Description		**µg**		**CLSI** [1]	**EUCAST** [3,4]		**Ref.***
Ceftazidime-avibactam	CZA	50	(30/20)	✓			9205
Ceftazidime + Clavulanic acid	CAL	20	(20/10)		✓[9]	●	9258 ◆
Ceftazidime + Clavulanic acid	CAL	40	(30/10)		✓[10]	●	9145
Ceftazidime + Clavulanic acid + Cloxacillin	CALC				✓[10]	●	9204
Ceftazidime + Cloxacillin	CAC				✓[10]	●	9225
Ceftibuten	CTB	30		✓	✓		9101
Ceftizoxime	CZX	30		✓			9054
Ceftobiprole	BPR	5		✓	✓		9242
Ceftolozane-tazobactam	C/T	40	(30/10)	✓	✓	●	9246
Ceftriaxone	CRO	30		✓	✓		9020
Cefuroxime	CXM	1					9232
Cefuroxime	CXM	5					9236 ◆
Cefuroxime	CXM	30		✓	✓		9021
Cephalexin	CL	30			✓		9011
Cephalothin	KF	30		✓			9013
Cephradine	CE	30					9055
Chloramphenicol	C	10					9128
Chloramphenicol	C	30		✓	✓		9022
Cinoxacin	CIN	100		✓			9057
Ciprofloxacin	CIP	5		✓	✓		9056
Clarithromycin	CLR	15		✓			9098
Clavulanic acid	CLA	1				●	9229 ◆
Clavulanic acid	CLA	2				●	9228 ◆
Clavulanic acid	CLA	5				●	9230 ◆
Clavulanic acid	CLA	10				●	9231 ◆
Clindamycin	CD	2		✓	✓		9047
Clindamycin	CD	10					9146
Cloxacillin	CX	5					9058
Colistin sulfate	CS	10		✓			9023 ◆
Colistin sulfate	CS	25					9184 ◆
Colistin sulfate	CS	30	units				9141 ◆
Daptomycin (includes Ca^{2+})	DAP	30					9090
Dicloxacillin	DCX	1					9093
Dipicolinic acid	DP					●	9194
Doripenem	DOR	10		✓	✓		9154
Doxycycline	DXT	30		✓			9059
EDTA	ED					●	9087
Eravacycline	ERV	20		✓	✓		9238
Eravacycline	ERV	50					9237 ◆
Ertapenem	ETP	10		✓	✓	●	9061
Ertapenem + Cloxacillin	ET+CL					●	9199
Ertapenem + Phenylboronic acid	ET+BO					●	9202
Erythromycin	E	2					9180
Erythromycin	E	15		✓	✓		9024
Fosfomycin	FOS	50					9025
Fosfomycin (includes G6P 50)	FOS	100	(100/50)				9121
Fosfomycin (includes G6P 50)	FOS	200	(200/50)	✓	✓		9109
Fosfomycin 200 + G6P 200	FGP	200	(200/200)				9214 ◆
Furazolidone	FR	50					9099
Fusidic acid	FC	10		✓	✓		9049
Fusidic acid	FC	30					9111
Gatifloxacin	GAT	5		✓			9169
Gentamicin	CN	10		✓	✓		9026
Gentamicin	CN	30			✓		9125
Gentamicin	CN	120		✓			9124
Gentamicin	CN	500					9288 ◆
Imipenem	IMI	10		✓	✓	●	9079
Imipenem-relebactam	I/R	35	(10/25)	✓	✓	●	9253
Imipenem + Cloxacillin	IMI+CL					●	9086
Imipenem + EDTA	IM+ED					●	9183

Antibiotic discs in cartridge and canister							
Description		**µg**		**CLSI [1]**	**EUCAST [3,4]**		**Ref.***
Imipenem + Phenylboronic acid	IMI+BO					●	9085
Kanamycin	K	30		✓			9027
Lefamulin	LMU	5			✓		9249 ◆
Lefamulin	LMU	20		✓			9250 ◆
Levofloxacin	LEV	5		✓	✓		9102
Levonadifloxacin	LND	10		✓			9267
Lincomycin	MY	2					9028
Lincomycin	MY	15					9116
Linezolid	LNZ	10			✓		9155
Linezolid	LNZ	30		✓			9136
Lomefloxacin	LOM	10		✓			9113
Mecillinam	MEC	10		✓	✓		9156
Meropenem	MRP	10		✓	✓	●	9068
Meropenem + Cloxacillin	MR+CL				✓ [10]	●	9175
Meropenem + EDTA	MR+ED				✓ [10]	●	9178
Meropenem + Phenylboronic acid	MR+BO				✓ [10]	●	9176
Methicillin	MET	5					9029
Metronidazole	MTZ	5			✓		9076
Metronidazole	MTZ	50					9119
Mezlocillin	MEZ	75					9062
Minocycline	MN	30		✓	✓		9030
Moxifloxacin	MXF	5		✓	✓		9103
Mupirocin	MUP	5					9189
Mupirocin	MUP	200		✓	✓		9157
Nafcillin	NAF	1		✓			9174
Nalidixic acid	NA	30		✓	✓		9001
Neomycin	N	10			✓		9287 ◆
Netilmicin	NET	10			✓		9170
Netilmicin	NET	30		✓			9033
Nitrofurantoin	F	50					9181
Nitrofurantoin	F	100			✓		9158
Nitrofurantoin	F	300		✓			9034
Nitroxoline	NI	30			✓		9209
Norfloxacin	NOR	10		✓	✓		9035
Novobiocin	NO	5					9117
Novobiocin	NO	30					9063
Ofloxacin	OFX	5		✓	✓		9080
Omadacycline	OMC	30		✓		●	9252 ◆
Oritavancin	ORI	5				●	9201
Oxacillin	OX	1		✓	✓		9036
Oxacillin	OX	5					9135
Oxolinic acid	OA	2					9002
Oxytetracycline	OT	30					9065
Pefloxacin	PEF	5		✓	✓		9091
Penicillin G	P	1	unit		✓		9130
Penicillin G	P	2	units				9127
Penicillin G	P	10	units	✓			9037
Penicillin G 1 + Clavulanic acid 2	PC	2	(1 unit/2)			●	9227 ◆
Penicillin G 1 + Clavulanic acid 5	PC	5	(1 unit/5)			●	9240 ◆
Penicillin G 1 + Clavulanic acid 10	PC	10	(1 unit/10)			●	9241 ◆
Penicillin G 1 + Clavulanic acid 20	PC	20	(1 unit/20)			●	9254 ◆
Phenoxymethylpenicillin	PV	10					9171
Phenylboronic acid	BO					●	9193
Pipemidic acid	PI	20					9003
Piperacillin	PRL	30			✓		9159
Piperacillin	PRL	100		✓			9038
Piperacillin-tazobactam	TZP	36	(30/6)		✓		9160
Piperacillin-tazobactam	TZP	110	(100/10)	✓			9100
Polymyxin B	PB	100	units				9066
Polymyxin B	PB	300	units	✓			9120

Antibiotic discs in cartridge and canister						
Description		**µg**		**CLSI [1]**	**EUCAST [3,4]**	**Ref.***
Quinupristin-dalfopristin	QDA	15		✓	✓	9161
Rifampicin	RD	5		✓	✓	9118
Rifampicin	RD	30				9039
Roxithromycin	RXT	15				9060
Sisomycin	SIS	30				9046
Sodium Fusidate	FC	30				9131
Spectinomycin	SPC	100		✓		9067
Streptomycin	S	10		✓		9040
Streptomycin	S	300		✓	✓	9162
Sulbactam	SU	20				9129
Sulfadiazine	SUZ	300				9150
Sulfafurazole	SF	300				9041
Sulfamethoxazole	SMX	50				9084
Sulfamethoxazole	SMX	100				9187
Sulfaprim	SXT	50				9132
Sulfonamide	S3	300		✓		9126
Tedizolid	TZD	2		✓	✓	9243
Tedizolid	TZD	20				9245
Teicoplanin	TEC	30		✓	✓	9050
Telithromycin	TEL	15		✓	✓	9172
Temocillin	TMO	30			✓	9186
Tetracycline	TE	30		✓	✓	9043
Ticarcillin	TC	75		✓	✓	9070
Ticarcillin-clavulanic acid	TTC	85	(75/10)	✓	✓	9096
Tigecycline	TGC	15		✓	✓	9147
Tobramycin	TOB	10		✓	✓	9044
Tobramycin	TOB	30				9163
Trimethoprim	TM	2.5				9083
Trimethoprim	TM	5		✓	✓	9110
Trimethoprim-sulfamethoxazole	SXT	2.5	(0.125/2.375)			9299 ◆
Trimethoprim-sulfamethoxazole	SXT	25	(1.25/23.75)	✓	✓	9042
Vancomycin	VA	5			✓	9164
Vancomycin	VA	30		✓		9045

●, to be stored at -20°C ◆, not CE marked

Storage Unless otherwise indicated, discs may be stored at any temperature between –20°C and +8°C.

CE Marking Unless otherwise indicated, all products intended for clinical applications are CE marked according to the European Directive 98/79/EC for In Vitro Diagnostic Medical Devices.

* **Packaging** 5 cartridges of 50 discs (5x50 discs = 250 discs).
Single cartridges of 50 discs are available: add /1 to the catalogue ref. no. to indicate the relevant item.
Example: ref. 9045/1 indicates Vancomycin 30 µg in one single cartridge of 50 discs.

250 discs in a CANISTER: add /2 to the catalogue ref. no. to indicate the relevant item.
Example: ref. 9045/2 indicates Vancomycin 30 µg in a canister of 250 discs.

Note that testing and reporting antimicrobial agents for which there are no interpretive criteria are the responsibility of the chief microbiologist and such decisions should be made with input from the infectious disease clinicians.

For full details on specific organism/agent combinations refer to current CLSI and EUCAST recommendations.
Also see the EUCAST guidance at http://www.eucast.org/clinical_breakpoints/when_there_are_no_breakpoints/

Antibiotic discs in cartridge and canister Veterinary				
Description		**µg**	**CLSI [7]**	**Ref.***
Aminosidine	AM	60		9301
Apramycin	AP	15	✓	9300
Ceftiofur	FUR	30	✓	9251
Enrofloxacin	ENR	5	✓	9233
Florfenicol	FFC	30	✓	9234
Flumequine	UB	30		9208
Lincomycin-spectinomycin	MSP	100		9302
Marbofloxacin	MAR	5	✓	9297
Neomycin	N	30	✓	9032
Spectinomycin	SPC	100	✓	9067
Spiramycin	SP	100		9088
Tiamulin	T	30	✓	9094
Tilmicosin	TIL	15	✓	9298
Tylosin	TY	30	✓	9082

* **Packaging** 5 cartridges of 50 discs (5x50 discs = 250 discs).

Single cartridges of 50 discs are available: add /1 to the catalogue ref. no. to indicate the relevant item.
Example: ref. 9082/1 indicates Tylosin 30 µg in one single cartridge of 50 discs.

250 discs in a CANISTER: add /2 to the catalogue ref. no. to indicate the relevant item.
Example: ref. 9082/2 indicates Tylosin 30 µg in a canister of 250 discs.

REFERENCES

1. CLSI. Performance Standards for Antimicrobial Susceptibility Testing; 33rd ed. CLSI Supplement M100S. Wayne, PA: Clinical and Laboratory Standards Institute; 2023.
2. CLSI. Performance Standards for Antimicrobial Disk Susceptibility Tests; 13th ed. CLSI standard M02. Wayne, PA: Clinical and Laboratory Standards Institute; 2018.
3. The European Committee on Antimicrobial Susceptibility Testing. Breakpoint tables for interpretation of MICs and zone diameters. Version 13.2, 2023. http://www.eucast.org
4. The European Committee on Antimicrobial Susceptibility Testing. Routine and extended internal quality control for MIC determination and disk diffusion as recommended by EUCAST, Version 13.2, 2023. http://www.eucast.org
5. EUCAST Disk Diffusion Method for Antimicrobial Susceptibility Testing - Version 11.0, January 2023.
6. EUCAST Disk Diffusion Anaerobic Bacteria - Version 2.0, January 2023.
7. CLSI. Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated From Animals, 4th ed. CLSI supplement VET08. Wayne, PA: Clinical and Laboratory Standards Institute; 2018.
8. CLSI. Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated From Animals; 5th ed. CLSI standard VET01. Wayne, PA: Clinical and Laboratory Standards Institute; 2018.
9. EUCAST technical guidance on the use of the combination disk test (CDT) for confirmation of ESBL in Enterobacterales. New disk potencies for combination disks containing cefotaxime and ceftazidime without and with clavulanic acid (February 2019).
10. EUCAST guidelines for detection of resistance mechanisms and specific resistances of clinical and/or epidemiological importance. Version 2.0, July 2017.
11. DIN 58940-2 Medical microbiology - Susceptibility testing of microbial pathogens to antimicrobial agents - Part 2: Active substance carriers for the agar diffusion test; 2007-10.
12. FDA (1978) Codes of Fed.Rebs. 21.Part 460.
13. WHO (1977) Tech rep.Ser.n°610.

Agar media for AST of bacteria (*)

Description		CLSI	EUCAST	Packaging	REF
Mueller Hinton II Agar (cation-adjusted), for non-fastidious bacteria	**MHA**	✓	✓	20 plates 90 mm 10 plates 140 mm	10031 10231
Mueller Hinton II Agar with 5% Sheep Blood, for fastidious organisms, including *Pasteurella multocida* and *Mannheimia haemolytica*	**MHA + 5% blood**	✓		20 plates 90 mm 10 plates 140 mm	10131 11231
Mueller Hinton Fastidious Agar: MHA with 20 mg/l β-NAD and 5% Horse Blood, for *Streptococcus* spp., *Haemophilus* spp., and other fastidious organisms	**MH-F**		✓	20 plates 90 mm 10 plates 140 mm	10132 11132
Haemophilus Test Agar, for *Haemophilus* spp.	**HTM**	✓		20 plates 90 mm	10080
GC Agar Base with 1% defined growth supplement, for *Neisseria gonorrhoeae*		✓			
Mueller Hinton Chocolate Agar, for *Actinobacillus pleuropneumoniae* and *Histophilus somni*	**Chocolate MHA**	✓		20 plates 90 mm	10335
Fastidious Anaerobe Agar with defibrinated Horse Blood, for selected rapidly growing anaerobic bacteria (i.e. *Bacteroides* spp., *Prevotella* spp., *F. necrophorum*, *C. perfringens* and *C. acnes*)	**FAA-HB**		✓	20 plates 90 mm	10062

*Available as ready to use plated media in the Liofilchem Catalog.

NOTE: MHA, MH-F and FAA-HB into Petri dishes should have a level depth of 4 ± 0.5 mm.

Find out more on Disc Diffusion Antimicrobial Susceptibility Tests

F01511

LIOFILCHEM® s.r.l.

Via Scozia, 64026 Roseto degli Abruzzi (TE) Italy
Tel. +39 0858930745 Fax +39 0858930330 www.liofilchem.com

CE IVD

Bile Aesculin Agar

Selective medium for the preliminary identification of group D streptococci.

TYPICAL FORMULA	(g/l)
Beef Exract	3.0
Meat Peptone	5.0
Esculin	1.0
Oxgall	40.0
Ferric Citrate	0.5
Agar	14.0
Final pH 6.4 ± 0.2 at 25°C	

DESCRIPTION

Bile Aesculin Agar is used for the selective isolation and differentiation of enterococci and streptococci (serology group D) from food and pharmaceutical products. This medium is not intended for use in the diagnosis of disease or other conditions in humans.

PRINCIPLE

Beef extract and meat peptone provide amino acids, nitrogen, carbon, vitamins and minerals. Oxgall is ox bile purified and dehydrated. It contains a mix of biliary salts and is used in media for enterobacteria, as selective agent, because inhibit Gram-positive bacteria other than Group D streptococci. Esculin is a glucoside which is hydrolysed by Group D streptococci to form aesculetin and dextrose. Aesculetin combines with ferric citrate in the medium to form a dark brown or black complex which is indicative of a positive result. Agar is the solidifying agent.

PREPARATION

Suspend 63.5 g of powder in 1 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. Dispense in Petri dishes.

TECHNIQUE

Inoculate the medium by streaking directly the sample onto the agar surface.

Incubate at 36 ± 1°C for 18-24 hours in aerobic atmosphere.

INTERPRETATION OF RESULTS

Group D streptococci grow on this medium producing a dark brown color around the colonies.

STORAGE

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

For professional use only. Operators must be trained and have certain experience in the laboratory methods. Please read the instructions carefully before using this product. Reliability of assay results cannot be guaranteed if there are any deviations from the instructions in this document.

Consult the Safety Data Sheet (SDS) for information regarding hazards and safe handling practices.

DISPOSAL OF WASTE

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

REFERENCES

1. European Pharmacopoeia 9.0 (2017) - 2.6.12. Microbiological examination of non-sterile products: Microbial enumeration tests. 2.6.13. Microbiological examination of non-sterile products: Test for specified microorganisms.
2. Swan, A. 1954.The use of bile-esculin medium and of Maxted's technique of Lancefield grouping in the identification of enterococci (group D streptococci). J. Clin. Pathol. 7:160.
3. Facklam, R. R., and M. D. Moody. 1970. Presumptive identification of group D streptococci: the bileesculin test. Appl. Microbiol. 20:245.
4. Rochaix, A. 1924. Milieux a leculine pour le diagnostid differentieldes bacteries du grojps strepto-enteropneumocoque. Comt. Rend. Soc. Biol. 90:771-772.
5. Meyer, K., and H. Schönfeld. 1926. Über die Unterscheidung des Enterococcus vom Streptococus viridans und die Beziehunger beider zum Strptoccus lactis. Zentralb. Bakteriol Parasitenkd. Infektionskr. Hyg. Abt. I orig. 99:402-416.
6. Schleifer, K. H., and R. Kilpper-Balz. 1987. Molecular and chemotaxonomic approaches to the classification of streptococci, enterococci and lactococci: a review. Syst. Appl. Microbiol. 10:1-19.
7. Vanderzant, C., and D. F. Splittstoesser (eds.). Compendium of methods for the microbiological examination of foods, 4th ed. American Public Health Association, Washington, D.C.
8. FDA-BAM. www.fda.gov/Food/ScienceResearch/LaboratoryMethods/BacteriologicalAnalytical manualBAM/ default.htm.
9. Marshall, R. T. (ed.). 2004. Standard methods for the examination of dairy products, 17th ed. American Public Health Association, Washington, D.C.

LIOFILCHEM® S.r.l.
Via Scozia, 64026 Roseto degli Abruzzi (TE) ITALY
Tel +39 0858930745 Fax +39 0858930330 www.liofilchem.com liofilchem@liofilchem.com

PRODUCT SPECIFICATIONS

NAME
Bile Aesculin Agar

PRESENTATION
Dehydrated medium

STORAGE
10-30°C

PACKAGING

Ref.	Content	Packaging
620210	100 g	100 g of powder in plastic bottle
610210	500 g	500 g of powder in plastic bottle
6102105	5 kg	5 kg of powder in plastic bottle

pH OF THE MEDIUM
6.4 ± 0.2

USE
Bile Aesculin Agar is used for the selective isolation and differentiation of enterococci and streptococci (serology group D) from food and pharmaceutical products

TECHNIQUE
Refer to technical sheet of the product

APPEARANCE OF THE MEDIUM
Powder medium
Appearance: free-flowing, homogeneous
Colour: greenish, light to medium beige
Ready-to-use medium
Appearance: slightly opalescent
Colour: greenish to medium amber

SHELFLIFE
4 years

QUALITY CONTROL
1. Control of general characteristics, label and print
2. Microbiological control
   Inoculum for productivity: 50-100 CFU
   Inoculum for selectivity: 10^4-10^6 CFU
   Incubation Conditions: 24 h/ 36 ± 1°C

Microorganism		Growth	Characteristics
Enterococcus faecalis	ATCC 19433	Good	Blackening of the medium
Streptococcus pyogenes	ATCC 19615	Inhibited	---

TABLE OF SYMBOLS

LOT Batch code	Consult instructions for use	Manufacturer	Use by
REF Catalogue number	Temperature limitation	Contains sufficient for <n> tests	Keep away from sunlight

LIOFILCHEM® S.r.l.
Via Scozia, 64026 Roseto degli Abruzzi (TE) ITALY
Tel +39 0858930745 Fax +39 0858930330 www.liofilchem.com liofilchem@liofilchem.com

Instructions For Use
ENGLISH

MacConkey Broth

Differential medium for detection of coliform bacteria.

DESCRIPTION

MacConkey Broth is a liquid medium used for the detection and enumeration of coliforms from water, and milk.

TYPICAL FORMULA*	(g/l)
Peptone	20.0
Lactose	10.0
Sodium Chloride	5.0
Bile Salts No.3	5.0
Bromocresol Purple	0.01
Final pH 7.4 ± 0.2 at 25°C	

*Adjusted and/or supplemented as required to meet performance specifications.

METHOD PRINCIPLE

Peptone provides amino acids, nitrogen, carbon, vitamins and minerals for organisms growth. Lactose is the fermentable carbohydrate. Sodium Chloride maintain the osmotic balance of the medium. Bile salts inhibit the growth of Gram-positive bacteria. Bromocresol purple is the pH indicator.

PREPARATION

Dehydrated medium

Suspend 40.0 g of the powder in 1 liter of distilled or deionized water. Mix well. Heat to boil shaking frequently until completely dissolved. Dispense into tubes containing Durham tubes. Autoclave at 121°C for 15 minutes.

Ensure there are no air bubbles in the Durham tube prior to use as it may lead to a misinterpretation (false-positive reaction in gas production) of results. If needed, invert the tube to allow the air being released from the Durham tube.

TEST PROCEDURE

For the presumptive coliform examination, inoculate a series of tubes with the test sample and its dilutions and incubate at 35-37°C for 18-24 hours.

The number of total coliforms is estimated by counting the number of tubes giving a positive reaction for acid (yellow colouration of the broth) and gas production and comparing the pattern of positive results with standard charts to find MPN (Most Probabile Number) of total 100 ml sample.

For the differential test, subculture from each tube showing positive reaction into a fresh tube of MacConkey Broth. Incubate at 42-44°C for up to 48 h to detect *Escherichia coli*.

INTERPRETING RESULTS

Turbidity of MacConkey Broth indicates microbial growth.

Acid production due to lactose fermentation causes a color change of the medium to yellow. Gas is also produced, collecting in Durham tubes.

The ability to form gas in MacConkey Broth at 44°C is specific for *E. coli,* which is indicative of faecal pollution of the original sample.

Non-fermenting Gram-negative organisms produce good growth but without significant changes in color nor gas formation.

STORAGE

The powder is very hygroscopic, store the powder at 10-30°C, in a dry environment, in its original container tightly closed. Do not use the product beyond its expiry date on the label or if product shows any evidence of contamination or any sign of deterioration.

SHELF LIFE

Dehydrated medium: 4 years.

QUALITY CONTROL

Appearance of Dehydrated Medium: Free-flowing, homogeneous, light beige.

Appearance of Prepared Medium: Clear, purple.

Expected Cultural Response:

Control strain		Inoculum	Incubation	Growth	Acid	Gas
Salmonella Typhimurium	WDCM 00031 (ATCC® 14028; NCTC 12023)	≤100 CFU	18-24 h / 35-37°C	Good	Neg. (purple)	Neg.
Escherichia coli	WDCM 00012 (ATCC® 8739; NCTC 12923)	≤100 CFU	18-24 h / 42-44°C	Good	Pos. (yellow)	Pos.
Staphylococcus aureus	WDCM 00032 (ATCC® 6538; NCTC 10788)	1000 CFU	48 h / 35-37°C	Inhibition	—	—

Please refer to the actual batch related Certificate of Analysis (CoA).

WARNING AND PRECAUTIONS

For professional use only. Operators must be trained and have certain experience in the laboratory methods. Please read the instructions carefully before using this product. Reliability of assay results cannot be guaranteed if there are any deviations from the instructions in this document.

Consult the Safety Data Sheet (SDS) for information regarding hazards and safe handling practices.

DISPOSAL OF WASTE

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

BIBLIOGRAPHY

See the references at the end of this document.

TABLE OF SYMBOLS

See the table of symbols at the end of this document.

The product is available in the configurations listed below. There may be additional product ref. numbers as well. For an updated listing of available products, visit **liofilchem.com**

Product	Format	Packaging	Ref.
MacConkey Broth	Dehydrated medium	100 g	620171
		500 g	610171

Significant changes from previous version:

Document	Release Date	Change Summary
610171_IFU-0	2022-11-04	Document creation

This IFU document and the SDS are available from the online Support Center:

liofilchem.com/ifu-sds

Istruzioni per l'uso
ITALIANO

MacConkey Broth

Terreno differenziale per la ricerca di batteri coliformi.

DESCRIZIONE

MacConkey Broth è un terreno liquido utilizzato per la ricerca ed il conteggio dei coliformi da acqua e latte.

FORMULA TIPICA*	(g/l)
Peptone	20.0
Lattosio	10.0
Sodio Cloruro	5.0
Sali di Bile No.3	5.0
Porpora di Bromocresolo	0.01
pH Finale 7.4 ± 0.2 a 25°C	

*Adattata e/o integrata per soddisfare le specifiche di performance richieste.

PRINCIPIO DEL METODO

Il peptone fornisce aminoacidi, azoto, carbonio, vitamine e minerali per la crescita dei microrganismi. Il lattosio è il carboidrato fermentabile. Il sodio cloruro mantiene il bilancio osmotico del terreno. I sali di bile inibiscono i batteri Gram positivi. Il porpora di bromocresolo è l'indicatore di pH.

PREPARAZIONE

Terreno disidratato

Sospendere 40.0 g di polvere in 1 litro di acqua distillata o deionizzata sterile. Mescolare bene. Riscaldare agitando di frequente e bollire fino a completa dissoluzione. Distribuire in provette con campanella di Durham. Autoclavare a 121°C per 15 minuti.

Assicurarsi che non vi siano bolle d'aria nella campanella di Durham prima dell'uso in quanto ciò potrebbe portare a un'interpretazione errata (reazione falsa positiva nella produzione di gas) dei risultati. Se necessario, capovolgere la provetta per consentire la fuoriuscita dell'aria dalla campanella di Durham.

PROCEDURA DEL TEST

Per l'esame presuntivo dei coliformi, inoculare una serie di provette con il campione in esame e le sue diluizioni ed incubare a 35-37°C per 18-24 ore.

Il numero di coliformi totali viene stimato contando il numero di provette che danno una reazione positiva per la produzione di acido (colorazione gialla del brodo) e di gas e confrontando l'andamento dei risultati positivi con i grafici standard per trovare l'MPN (numero più probabile) riferito al campione totale (100 ml).

Per il test differenziale, da ciascuna delle provette positive trasferire un volume del brodo di coltura in una nuova provetta di MacConkey Broth. Incubare a 44°C fino a 48 h per rilevare *Escherichia coli*.

INTERPRETAZIONE DEI RISULTATI

La torbidità in MacConeky Broth indica la crescita microbica.

La produzione di acidi causata dalla fermentazione del lattosio provoca il cambiamento di colore del terreno a giallo. Ciò comporta anche la produzione di gas, raccolto nelle campanelle di Durham.

La capacità di formare gas in MacConkey Broth a 44°C è specifica per *E. coli*, che è un indicatore di inquinamento fecale del campione originale.

I microrganismi Gram negativi non fermentanti producono una buona crescita che non è però accompagnata da significativi cambiamenti di colore né da formazione di gas.

CONSERVAZIONE

La polvere è fortemente igroscopica, conservare a 10-30°C, in ambiente asciutto, nel suo contenitore originale chiuso ermeticamente. Non usare il prodotto dopo la sua data di scadenza indicata sull'etichetta o se il prodotto mostra segni di contaminazione o deterioramento.

VALIDITÀ

Terreno disidratato: 4 anni.

CONTROLLO DI QUALITÀ

Aspetto del Terreno Disidratato: Omogeneo, granulometria fine, beige chiaro.

Aspetto del Terreno Preparato: Viola, limpido.

Risultati Attesi dei Test Colturali:

Ceppi di controllo		Inoculo	Incubazione	Crescita	Acido	Gas
Salmonella Typhimurium	WDCM 00031 (ATCC® 14028; NCTC 12023)	≤100 CFU	18-24 h / 35-37°C	Buona	Neg. (viola)	Neg.
Escherichia coli	WDCM 00012 (ATCC® 8739; NCTC 12923)	≤100 CFU	18-24 h / 42-44°C	Buona	Pos. (giallo)	Pos.
Staphylococcus aureus	WDCM 00032 (ATCC® 6538; NCTC 10788)	1000 CFU	48 h / 35-37°C	Inibizione	—	—

Fare riferimento al certificato di analisi (CoA) relativo al lotto effettivo.

AVVERTENZE E PRECAUZIONI

Esclusivamente per uso professionale. Gli operatori devono essere formati e avere una certa esperienza nei metodi di laboratorio. Si prega di legger attentamente le istruzioni prima di utilizzare questo prodotto. L'affidabilità dei risultati del test non può essere garantita se ci sono deviazioni dalle istruzioni riportate in questo documento.

Consultare la scheda di sicurezza (SDS) per informazioni sui pericoli e sulle modalità di manipolazione sicure.

SMALTIMENTO DEI RIFIUTI

Lo smaltimento dei rifiuti deve essere effettuato in conformità alle normative nazionali e locali in vigore.

BIBLIOGRAFIA

Vedere i riferimenti alla fine di questo documento.

TABELLA DEI SIMBOLI

Vedere la tabella dei simboli alla fine di questo documento.

Per le configurazioni disponibili di questo prodotto vedere l'elenco nella lingua inglese.

Questo documento IFU e la SDS sono disponibili dal Support Center online:

liofilchem.com/ifu-sds

References / Riferimenti

1. European Pharmacopoeia (EP) 2.6.13. Microbiological examination of non-sterile products: Test for specified microorganisms.
2. United States Pharmacopoeia (USP) <62> Microbiological examination of non-sterile products: Test for specified microorganisms.
3. Japanese Pharmacopoeia (JP) 4.05 Microbiological examination of non-sterile products: Test for specified microorganisms.
4. Departments of the Environment, Health, Social Security and Public Health Laboratory Service (1982) The Bacteriological Examination of Drinking Water Supplies, Report No. 71, HMSO London.
5. World Health Organization (1963) International Standards for Drinking Water, 2nd ed., WHO, Geneva.
6. Murray, Baron, Jorgensen, Landry and Pfaller ed. (2007) Manual of clinical microbiology, 9th ed. American Society for Microbiology, Washington, D.C.
7. MacConkey, A. (1908) Bile salt media and their advantages in some bacteriological examinations. J. Hyg. 8: 322-334.
8. MacConkey, A. (1905) Lactose-fermenting bacteria in faeces. J. Hyg. 8: 333-379.

Table of Symbols / Tabella dei Simboli

LOT	Batch code / Codice del lotto
REF	Catalogue number / Numero di catalogo
	Manufacturer / Fabbricante
	Use by / Utilizzare entro
	Fragile, handle with care / Fragile, maneggiare con cura
	Temperature limitation / Limiti di temperatura
	Contains sufficient for <n> tests / Contenuto sufficiente per <n> saggi
	Consult instructions for use / Consultare le istruzioni per l'uso
	Do not reuse / Non riutilizzare
	Keep away from sunlight / Tenere al riparo dalla luce solare
	Warninng / Avvertimento

Liofilchem® s.r.l.

Via Scozia, 64026 Roseto degli Abruzzi (TE) Italy
Tel. +39 0858930745 Fax +39 0858930330 www.liofilchem.com liofilchem@liofilchem.com

CIC-C1q ELISA (IgG)
Test instruction

ORDER NO.		SUBSTRATE	FORMAT
EA 1818-9601 G	Circulating Immune Complexes (CIC-C1q)	C1q-coated microplate wells	96 x 01 (96)

Indication: The ELISA test kit provides a semiquantitative or quantitative in vitro assay against C1q-binding-circulating immune complexes (CIC) containing IgG in human serum or plasma for the diagnosis of rheumatic, autoimmune and infectious diseases, allergy, haematological and neoplastic disorders.

Application: Increased levels of circulating immune complexes can be detected in patients with different autoimmune, microbial and neoplastic diseases. Immune complexes are produced as a consequence of the body's immune response. Investigation of these complexes can therefore be useful for assessing the disease activity, for therapy control and for establishing a prognosis. Moreover, circulating immune complexes can become pathogenetically active through complement activation.

Principle of the test: The test kit contains microplate strips, each with 8 break-off reagent wells coated with complement fragment C1q. In the first reaction step, diluted patient samples are incubated in the wells. If samples are positive, circulating immune complexes bind to the C1q via the Fc fragment of the immunoglobulins. To detect the bound immune complexes, a second incubation is carried out using an enzyme-labelled anti-human IgG (enzyme conjugate) catalysing a colour reaction. The intensity of the colour formed is proportional to the concentration of circulating immune complexes against C1q.

Contents of the test kit:

Component	Colour	Format	Symbol
1. Microplate wells coated with C1q 12 microplate strips each containing 8 individual break-off wells in a frame, ready for use	---	12 x 8	STRIPS
2. Calibrator 1 200 RU/ml (CIC-C1q, human), lyophilised	colourless	1 x 2.0 ml	CAL 1
3. Calibrator 2 20 RU/ml (CIC-C1q, human), lyophilised	colourless	1 x 2.0 ml	CAL 2
4. Calibrator 3 2 RU/ml (CIC-C1q, human), lyophilised	colourless	1 x 2.0 ml	CAL 3
5. Positive control (CIC-C1q, human), lyophilised	colourless	1 x 2.0 ml	POS CONTROL
6. Negative control (CIC-C1q, human), lyophilised	colourless	1 x 2.0 ml	NEG CONTROL
7. Enzyme conjugate peroxidase-labelled anti-human IgG (rabbit), ready for use	light green	1 x 12 ml	CONJUGATE
8. Sample buffer ready for use	bluish	1 x 100 ml	SAMPLE BUFFER
9. Wash buffer 10x concentrated	colourless	1 x 100 ml	WASH BUFFER 10x
10. Chromogen/substrate solution TMB/H_2O_2, ready for use	colourless	1 x 12 ml	SUBSTRATE
11. Stop solution 0.5 M sulphuric acid, ready for use	colourless	1 x 12 ml	STOP SOLUTION
12. Plastic foil	---	2 pieces	
13. Test instruction	---	1 booklet	
14. Quality control certificate	---	1 protocol	

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

Preparation and stability of the reagents

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

- **Coated wells:** Ready for use. Tear open the resealable protective wrapping of the microplate at the recesses above the grip seam. Do not open until the microplate has reached room temperature to prevent the individual strips from moistening. Immediately replace the remaining wells of a partly used microplate in the protective wrapping and tightly seal with the integrated grip seam (Do not remove the desiccant bag).
  Once the protective wrapping has been opened for the first time, the wells coated with C1q can be stored in a dry place and at a temperature between +2°C and +8°C for 4 months.
- **Calibrators and controls:** Lyophilised. Reconstitute the content of each vial with 2 ml deionised or distilled water. The reconstituted calibrators and controls must be mixed thoroughly before use.
  The reconstituted calibrators and controls are stable at +18°C to +25°C for up to 4 hours. If the reconstituted calibrators and controls are to be stored for a longer period of time, they should be kept aliquotated at -20°C or lower until expiry date. Repeated freeze-thaw cycles of calibrators and controls should be avoided.
- **Enzyme conjugate:** Ready for use. The enzyme conjugate must be mixed thoroughly before use.
- **Sample buffer:** Ready for use.
- **Wash buffer:** The wash buffer is a 10x concentrate. If crystallisation occurs in the concentrated buffer, warm it to +37°C and mix well before diluting. The quantity required should be removed from the bottle using a clean pipette and diluted with deionised or distilled water (1 part reagent plus 9 parts distilled water).
  For example: For 1 microplate strip, 5 ml concentrate plus 45 ml water.
  The working strength wash buffer is stable for 4 weeks when stored at +2°C to +8°C and handled properly.
- **Chromogen/substrate solution:** Ready for use. Close the bottle immediately after use, as the contents are sensitive to light. The chromogen/substrate solution must be clear on use. Do not use the solution if it is blue coloured.
- **Stop solution:** Ready for use.

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

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

Warning: The calibrators and controls of human origin have tested negative for HBsAg, anti-HCV, anti-HIV-1 and anti-HIV-2. Nonetheless, all materials should be treated as being a potential infection hazard and should be handled with care. Some of the reagents contain the agent sodium azide in a non-declarable concentration. Avoid skin contact.

Preparation and stability of the patient samples

Samples: Human serum or EDTA plasma.

Stability: Patient samples to be investigated can generally be stored at +2°C to +8°C for up to 14 days. Diluted samples should be incubated within one working day.

Sample dilution: Patient samples are diluted **1:51** in sample buffer. For example: dilute 10 µl sample in 0.5 ml sample buffer and mix well by vortexing (sample pipettes are not suitable for mixing).

NOTE: The calibrators and controls are prediluted and ready for use after reconstitution, do not dilute them.

Incubation

For **semiquantative analysis** incubate **calibrator 2** along with the positive and negative controls and patient samples. For **quantitative analysis** incubate **calibrators 1, 2 and 3** along with the positive and negative controls and patient samples.

(Partly) manual test performance

Sample incubation: (1st step)
Transfer 100 µl of the calibrators, positive and negative controls or diluted patient samples into the individual microplate wells according to the pipetting protocol. Cover the wells and incubate for **30 minutes** at room temperature (+18°C to +25°C) on a **microplate shaker** (**600 rpm**, horizontal rotary shaker with a shaking amplitude of approx. 3 mm, e.g. microplate shaker TPM 4 from Sarstedt or Compact Digital Microplate Shaker from Thermo Scientific).*

Washing:
Manual: Remove the plastic foil. Empty the wells and subsequently wash 3 times using 350 µl of working strength wash buffer for each wash.
Automatic: Remove the plastic foil and wash the reagent wells 3 times with 450 µl of working strength wash buffer (program setting: e.g. TECAN Columbus Washer "Overflow Mode").

Leave the wash buffer in each well for 30 to 60 seconds per washing cycle, then empty the wells. After washing (manual and automated tests), thoroughly dispose of all liquid from the microplate by tapping it on absorbent paper with the openings facing downwards to remove all residual wash buffer.

Note: Residual liquid (> 10 µl) in the reagent wells after washing can interfere with the substrate and lead to false low extinction values. Insufficient washing (e.g. less than 3 wash cycles, too small wash buffer volumes, or too short residence times) can lead to false high extinction values.
Free positions on the microplate strip should be filled with blank wells of the same plate format as that of the parameter to be investigated.

Conjugate incubation: (2nd step)
Pipette 100 µl of enzyme conjugate (peroxidase-labelled anti-human IgG) into each of the microplate wells. Incubate for **30 minutes** at room temperature (+18°C to +25°C) on a **microplate shaker** (**600 rpm**, horizontal rotary shaker with a shaking amplitude of approx. 3 mm, e.g. microplate shaker TPM 4 from Sarstedt or Compact Digital Microplate Shaker from Thermo Scientific).

Washing:
Empty the wells. Wash as described above.

Substrate incubation: (3rd step)
Pipette 100 µl of chromogen/substrate solution into each of the microplate wells. Incubate for **15 minutes** at room temperature (+18°C to +25°C) (protect from direct sunlight).

Stopping:
Pipette 100 µl of stop solution into each of the microplate wells in the same order and at the same speed as the chromogen/substrate solution was introduced.

Measurement:
Photometric measurement of the colour intensity should be made at a **wavelength of 450 nm** and a reference wavelength between 620 nm and 650 nm **within 30 minutes of adding the stop solution.** Prior to measuring, slightly shake the microplate to ensure a homogeneous distribution of the solution.

* Please note that in case of deviating device specificiations customers are required to carry out their own validation.

Test performance using fully automated analysis devices

Sample dilution and test performance are carried out fully automatically using an analysis device. The incubation conditions programmed in the respective software authorised by EUROIMMUN may deviate slightly from the specifications given in the ELISA test instruction. However, these conditions were validated in respect of the combination of the DSX from Dynex and this EUROIMMUN ELISA. Validation documents are available on enquiry.
Automated test performance using other fully automated, open-system analysis devices is possible. However, the combination should be validated by the user.

Pipetting protocol

	1	2	3	4	5	6	7	8	9	10	11	12
A	C 2	P 6	P 14	P 22			C 1	P 4	P 12	P 20		
B	pos.	P 7	P 15	P 23			C 2	P 5	P 13	P 21		
C	neg.	P 8	P 16	P 24			C 3	P 6	P 14	P 22		
D	P 1	P 9	P 17				pos.	P 7	P 15	P 23		
E	P 2	P 10	P 18				neg.	P 8	P 16	P 24		
F	P 3	P 11	P 19				P 1	P 9	P 17			
G	P 4	P 12	P 20				P 2	P 10	P 18			
H	P 5	P 13	P 21				P 3	P 11	P 19			

The pipetting protocol for microtiter strips 1 to 4 is an example for the **semiquantitative analysis** of 24 patient samples (P 1 to P 24).
The pipetting protocol for microtiter strips 7 to 10 is an example for the **quantitative analysis** of 24 patient samples (P 1 to P 24).

The calibrators (C 1 to C 3), the positive (pos.) and negative (neg.) controls, and the patient samples have each been incubated in one well. The reliability of the ELISA test can be improved by duplicate determinations for each sample.
The wells can be broken off individually from the strips. This makes it possible to adjust the number of test substrates used to the number of samples to be examined and minimises reagent wastage.
Both positive and negative controls serve as internal controls for the reliability of the test procedure. They should be assayed with each test run.

Calculation of results

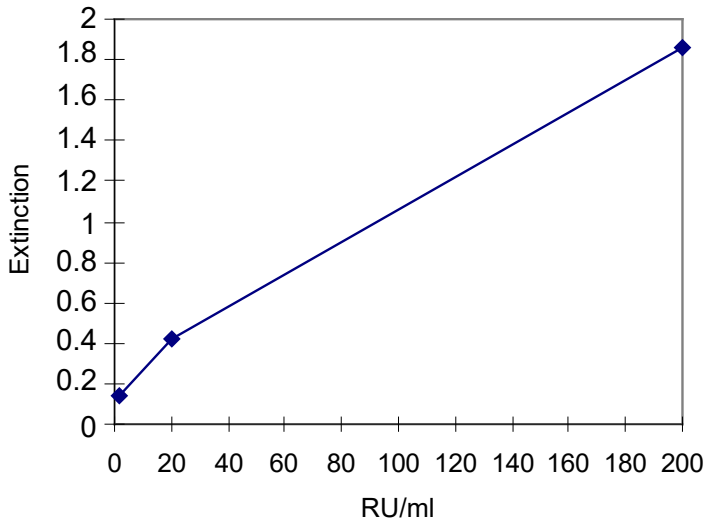
Semiquantitative: Results can be evaluated semiquantitatively by calculating a ratio of the extinction value of the control or patient sample over the extinction value of calibrator 2. Calculate the ratio according to the following formula:

$$\frac{\text{Extinction of the control or patient sample}}{\text{Extinction of calibrator 2}} = \text{Ratio}$$

EUROIMMUN recommends interpreting results as follows:

Ratio <1.0:	**negative**
Ratio ≥1.0:	**positive**

Quantitative: The calibration curve from which the concentration of antibodies in the patient samples can be taken is obtained by point-to-point plotting of the extinction values measured for the 3 calibrators against the corresponding units (linear/linear). Use “point-to-point” plotting for calculation of the calibration curve by computer. The following plot is an example of a typical calibration curve. Please do not use this curve for the determination of circulating immune complex concentrations in patient samples.



If the extinction for a patient sample lies above the value of calibrator 1 (200 RU/ml), the result should be reported as “>200 RU/ml”. It is recommended that the sample be re-tested at a dilution of 1:201. The result in RU/ml read from the calibration curve for this sample must then be multiplied by a factor of 4.

The upper limit of the normal range (cut-off value) recommended by EUROIMMUN is 20 relative units per millilitre (RU/ml). This corresponds to 4 µg Eq/ml (µg equivalents of heat-aggregated human IgG per ml). EUROIMMUN recommends interpreting results as follows:

<20 RU/ml:	**negative**
≥20 RU/ml:	**positive**

For duplicate determinations the mean of the two values should be taken. If the two values deviate substantially from one another, EUROIMMUN recommends retesting the samples.

For diagnosis, the clinical picture of the patient always needs to be taken into account along with the serological findings.

Test characteristics

Calibration: The calibration is performed in relative units (RU). Five RU/ml correspond to one µg Eq/ml (µg equivalents of heat-aggregated human IgG per ml).

For every group of tests performed, the extinction values of the calibrators and the relative units and/or ratio determined for the positive and negative controls must lie within the limits stated for the relevant test kit lot. A quality control certificate containing these reference values is included. If the values specified for the controls are not achieved, the test results may be inaccurate and the test should be repeated.

The binding activity of the antibodies and the activity of the enzyme used are temperature-dependent. It is therefore recommended using a thermostat in all three incubation steps. The higher the room temperature (+18°C to +25°C) during the incubation steps, the greater will be the extinction values. Corresponding variations apply also to the incubation times. However, the calibrators, controls and patient samples are subject to the same influences, with the result that such variations will be largely compensated in the calculation of the result.

Antigen: The microplate wells were coated with the complement factor C1q prepared from human serum. The circulating immune complexes bind to the C1q via the Fc fragment of the immunoglobulins. C1q is a glycoprotein composed of 18 polypeptide chains and consisting of three nonidentical subunits with M.W. of 29,000, 26,000 and 19,000 respectively. C1q is complexed with two C1r and two C1s molecules to form the first component of complement (C1). Activation of complement via the classical pathway is triggered by binding of C1q to immune complexes or to a variety of other activating substances. Subsequent to C1q binding, C1r and C1s are converted to proteolytic enzymes, which are responsible for continuation of activation via the classical pathway.

Linearity: The linearity of the CIC-C1q ELISA (IgG) was determined by assaying at least 4 serial dilutions of different patient samples. The CIC-C1q ELISA (IgG) is linear at least in the tested concentration range (8 RU/ml to 193 RU/ml).

Detection limit: The lower detection limit is defined as the mean value of an analyte-free sample plus three times the standard deviation and is the smallest detectable antibody titer. The lower detection limit of the CIC-C1q ELISA (IgG) is 0.1 RU/ml

Analytical specificity: The ELISA presented here specifically detects circulating immune complexes containing IgG with the ability to bind to human complement fragment C1q. Elevated concentrations of CIC-C1q are observed in up to 10% of blood donors without any clinical manifestations.

Interference: Haemolytic, lipaemic and icteric samples showed no influence on the result up to a concentration of 10 mg/ml for haemoglobin, 20 mg/ml for triglycerides and 0.4 mg/ml for bilirubin in this ELISA.

Reproducibility: The reproducibility of the test was investigated by determining the intra- and inter-assay coefficients of variation (CV) using 3 samples. The intra-assay CVs are based on 20 determinations and the inter-assay CVs on 4 determinations performed in 6 different test runs.

Intra-assay variation, n = 20		
Sample	**Mean value (RU/ml)**	**CV (%)**
1	10	7.3
2	17	8.0
3	115	4.8

Inter-assay variation, n = 4 x 6		
Sample	**Mean value (RU/ml)**	**CV (%)**
1	8	10.6
2	14	14.0
3	100	10.2

Sensitivity and specificity: The CIC-C1q levels were determined in 55 serum samples from patients with systemic lupus erythematosus (SLE) or rheumatoid arthritis (RA) and from apparently healthy blood donors (origin: Europe, China) using the EUROIMMUN ELISA and another commercially available ELISA (Bühlmann Laboratories) as reference. Of the analysed sera, the same 22 were positive and the same 30 were negative in both assays. Two sera were found positive by the EUROIMMUN ELISA only, and one serum by the reference ELISA (Bühlmann Laboratories) only. The sensitivity amounted to 96%, with a specificity of 94%.

Reference range: The CIC-C1q concentrations were determined in serum samples from a total of 250 apparently healthy blood donors (origin: Germany) using this EUROIMMUN ELISA. CIC-C1q was detected at concentrations ranging from 1 to 296 RU/ml. The 90.0 percentile of the reference interval was calculated to be 15 RU/ml and the 95.0 percentile was found to be 31 RU/ml.

Clinical significance

Immune complexes are protein aggregates that form via the binding of antibodies to antigens. They are continually formed as a result of the immune response. Since they are rapidly degraded, circulating immune complexes (synonym: circulating Ab-Ag complexes, CIC) are not normally detected in serum or only in low concentrations. If CIC are present in disease in such high concentrations that they cannot be quickly eliminated from the body, e.g. if phagocyte capacity is overwhelmed, accumulation of the immune complexes occurs. This can influence the coagulation system and lead to increased thromobocyte and erythrocyte aggregation. Through activation of the complement system CIC can induce inflammatory reactions and cause damage to organs, as is the case, for example, in glomerulonephritis, Crohn’s disease, ulcerative colitis, rheumatic diseases and viral or bacterial infections.

In the classical activation pathway the antigen-antibody complexes bind to the complement components C1, C4 and C2 and trigger the enzyme cascade. This is continued and finished with the components C3, C5, C6, C7, C8 and C9. The factors, which include C1q, act as indicators of immune complex diseases with complement use.

Circulating or extravascular immune complexes can become pathogenetically active. When immune complexes accumulate on or in tissues this can lead to inflammatory processes with corresponding clinical symptoms. The involvement of immune complexes can be detected in a range of immune complex-associated autoimmune diseases, such as systemic lupus erythematosus (CLE), rheumatoid arthritis (RA) and immune complex glomerulonephritis. Elevated CIC serum concentrations are also found in bacterial, viral and parasitic infections as post- and parainfectious immune complex diseases, as well as in allergies, chronic skin diseases and neurological diseases.

Although the detection of CIC is not specific for a particular disease these analyses can yield useful information about the immunopathology, course and prognosis of disease. The presence of CIC is an indicator of an overwhelmed immune defence or an autoimmune conflict, which should be clarified by targeted tests. In autoimmune, microbial or neoplastic diseases measurement of CIC is used as a marker for disease activity and also for assessing organ manifestation and for monitoring treatment. The test method of choice is ELISA, which allows quantitative in vitro determination of C1q-binding, IgG-containing CIC. Complement CIC are bound to immobilised purified C1q proteins. Persistently elevated CIC-C1q indicates chronic active disease. Normalisation is considered an indicator of successful treatment. It is often possible to correlate the CIC level with clinical symptoms and relapses. The WHO recommends always performing a second determination several weeks later in order to evaluate the persistence of the immune complexes in the circulation and thereby the pathological significance. The second determination also allows treatment to be monitored. With appropriate therapy a clear decrease in CIC should occur.

According to the WHO the following diseases are CIC-associated: rheumatic diseases (rheumatoid arthritis, systemic lupus erythematosus, Sjögren's syndrome, mixed connective tissue disease, periarteritis nodosa, Felty's syndrome, Bechterew's disease, Reiter's disease), viral infections (hepatitis B, cytomegaly, mononucleosis, subacute and sclerosing panencephalitis), bacterial infections (infectious endocarditis, disseminated gonorrhoea, syphilis, streptococcus and meningococcus infections), parasitosis (malaria, schistosomiasis, trypanosomiasis, toxoplasmosis), glomerulonephritis, neoplastic diseases and other diseases such as Crohn's disease, ulcerative colitis, idiopathic interstitial pneumonia, cystic fibrosis, multiple sclerosis, thrombotic thrombocytopenic purpura, Behcet's syndrome Churg-Strauss syndrome and chronic liver diseases.

Literature references

1. Aziz M, Akhtar S, Malik A. **Evaluation of cell-mediated immunity and circulating immune complexes as prognostic indicators in cancer patients**. Cancer detection and prevention 22.2 (1997): 87-99.
2. Braun A, Sis J, Max R, Mueller K, Fiehn C, Zeier M, Andrassy K. **Anti-chromatin and anti-C1q antibodies in systemic lupus erythematosus compared to other systemic autoimmune diseases.** Scand J Rheumatol 36 (2007) 291-298.
3. EUROIMMUN AG. Stöcker W, Schlumberger W, Krüger C. **Alle Beiträge zum Thema Autoimmundiagnostik.** In: Gressner A, Arndt T (Hrsg.) Lexikon der Medizinischen Laboratoriumsdiagnostik. 2. Auflage. Springer Medizin Verlag, Heidelberg (2012).
4. Flierman R, Daha MR. **Pathogenic role of anti-C1q autoantibodies in the development of lupus nephritis--a hypothesis.** Mol Immunol 44 (2007) 133-138.
5. Oelzner P, Deliyska B, Fünfstück R, Hein G, Herrmann D, Stein G. **Anti-C1q antibodies and antiendothelial cell antibodies in systemic lupus erythematosus - relationship with disease activity and renal involvement.** Clin Rheumatol 22 (2003) 271-278.
6. O'Flynn J, Flierman R, van der Pol P, Rops A, Satchell SC, Mathieson PW, van Kooten C, van der Vlag J, Berden JH, Daha MR. **Nucleosomes and C1q bound to glomerular endothelial cells serve as targets for autoantibodies and determine complement activation.** Mol Immunol 49 (2011) 75-83.

7. Saadoun, David, et al. **Anti-C1q antibodies in hepatitis C virus infection.** Clinical & Experimental Immunology 145.2 (2006): 308-312.

8. Shoenfeld Y, Szyper-Kravitz M, Witte T, Doria A, Tsutsumi A, Tatsuya A, Dayer JM, Roux-Lombard P, Fontao L, Kallenberg CG, Bijl M, Matthias T, Fraser A, Zandman-Goddard G, Blank M, Gilburd B, Meroni PL. **Autoantibodies against protective molecules--C1q, C-reactive protein, serum amyloid P, mannose-binding lectin, and apolipoprotein A1: prevalence in systemic lupus erythematosus.** Ann N Y Acad Sci 1108 (2007) 227-239.

9. Smykał-Jankowiak K, Niemir ZI, Polcyn-Adamczak M. **Do circulating antibodies against C1q reflect the activity of lupus nephritis?** Pol Arch Med Wewn 121 (2011) 287-295.

10. Trouw LA, Groeneveld TW, Seelen MA, Duijs JM, Bajema IM, Prins FA, Kishore U, Salant DJ, Verbeek JS, van Kooten C, Daha MR. **Anti-C1q autoantibodies deposit in glomeruli but are only pathogenic in combination with glomerular C1q-containing immune complexes.** J Clin Invest 114 (2004) 679-688.

11. Walport MJ. **Complement and systemic lupus erythematosus.** Arthritis Res 4 (2002) 279-293.

12. Zabaleta-Lanz ME, Muñoz LE, Tapanes FJ, Vargas-Arenas RE, Daboin I, Barrios Y, Pinto JA, Bianco NE. **Further description of early clinically silent lupus nephritis.** Lupus 15 (2006) 845-851.

Instructions For Use
ENGLISH

EE Broth-Mossel

Liquid medium for the cultivation and selective enrichment of
Enterobacteriaceae from different types of samples, according to USP/EP/JP.

DESCRIPTION

Enterobacteriaceae Enrichment Broth-Mossel is a selective medium used for the detection of bile-tolerant Gram-negative bacteria in food and other materials of sanitary importance.

This medium complies with the recommendations of the harmonized method in the United States Pharmacopoeia (USP), European Pharmacopoeia (EP) and Japanese Pharmacopoeia (JP) for the microbiological examination of nonsterile products.

TYPICAL FORMULA	(g/l)
Pancreatic Digest of Gelatin	10.0
Glucose Monohydrate	5.0
Dehydrated Ox Bile	20.0
Potassium Dihydrogen Phosphate	2.0
Disodium Hydrogen Phosphate, Anhydrous	6.4*
Brilliant Green	0.015

Final pH 7.2 ± 0.2 at 25°C

* Equivalent to 8.0 g of Disodium Hydrogen Phosphate Dihydrate.

METHOD PRINCIPLE

Pancreatic digest of gelatin provides amino acids, nitrogen, carbon, vitamins and minerals for organisms growth. Glucose is the fermentable carbohydrate. Ox bile and brilliant green are selective agents effective against Gram-positive cocci. Potassium phosphate and sodium phosphate act as buffer.

PREPARATION

Dehydrated medium — Suspend 43.4 g of the powder in 1 liter of distilled or deionized water. Mix well. Heat to boil shaking frequently until completely dissolved. DO NOT AUTOCLAVE.

TEST PROCEDURE

As in the Pharmacopoeia, prepare the sample using a 1 in 10 dilution of not less than 1 g of the product to be examined by choosing Tryptic Soy Broth (ref. 24513 or 452080) as diluent and incubate at 20-25°C for 2-5 hour to resuscitate bacteria.

For qualitative test (test for absence), transfer the volume of the pre-enrichment broth corresponding to 1 g of the product to be examined to EE Broth-Mossel.

For quantitative test, enumerate Enterobacteriaceae found per milliliter or per gram of test sample by using the Most Probable Number (MPN) technique. Use the volume of the pre-enrichment broth containing 0.1 g, 0.01 g and 0.001 g (or 0.1 ml, 0.01 ml and 0.001 ml) of the product to be examined to inoculate EE Broth-Mossel.

For both types of test incubate EE Broth-Mossel at 30-35°C for 24-48 h and continue analysis by subculturing on Violet Red Bile Glucose Agar (ref. 11184). Incubate plates aerobically at 30-35°C for 18-24 hours.

INTERPRETING RESULTS

Turbidity of EE Broth-Mossel indicates microbial growth; acid production causes a color change of the medium to yellow.

No growth of colonies on Violet Red Bile Glucose Agar is reported as absence of bile-tolerant Gram-negative bacteria. Growth of colonies constitutes a positive result and the probable number of bacteria is determined from the table below.

MPN Table.

Results for each quantity of product			Probable number of bacteria
0.1 g or 0.1 ml	0.01 g or 0.01 ml	0.001 g or 0.001 ml	per gram or per milliliter of product
+	+	+	$>10^3$
+	+	–	10^3 - 10^2
+	–	–	10^2 - 10
–	–	–	<10

APPEARANCE

Dehydrated medium: free-flowing, homogeneous, light beige to light green.
Prepared medium: clear, green.

STORAGE

The powder is very hygroscopic, store the powder at 10-30°C, in a dry environment, in its original container tightly closed. Store bottles and prepared plates at 10-25°C away from light. Do not use the product beyond its expiry date on the label or if product shows any evidence of contamination or any sign of deterioration.

SHELF LIFE

Dehydrated medium: 4 years.
Medium in tubes/bottles: 1 year.

QUALITY CONTROL

The medium is inoculated with the microbial strains indicated in the QC table.
Inoculum for productivity: ≤100 CFU.
Inoculum for selectivity: >100 CFU.
Incubation conditions: 18-24 h at 30-35°C (Pharmacopoeia growth promotion).

QC Table.

Microorganism		Specification
Escherichia coli	ATCC® 8739	Good growth
Pseudomonas aeruginosa	ATCC® 9027	Good growth
Staphylococcus aureus	ATCC® 6538	Inhibition

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 intended for professional use only and must be used by properly trained operators.

DISPOSAL OF WASTE

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

BIBLIOGRAPHY

1. EN ISO 11133:2014. Microbiology of food, animal feed and water – Preparation, production, storage and performance testing of culture media.
2. European Pharmacopoeia 6.5 (2009) 2.6.13. Microbiological examination of non-sterile products: Test for specified microorganisms.
3. United States Pharmacopoeia 32 NF 27 (2009) <62> Microbiological examination of non-sterile products: Test for specified microorganisms.
4. Japanese Pharmacopoeia 4.05 (2008) Microbiological examination of non-sterile products: Test for specified microorganisms.
5. ISO 21528-1:2004. Microbiology of food and animal feeing stuffs – Horizontal method for the detection and enumeration of Enterobacteriaceae – Detection and enumeration by MPN technique with pre-enrichment.
6. ISO 21528-2:2004. Microbiology of food and animal feeing stuffs – Horizontal method for the detection and enumeration of Enterobacteriaceae – Colony count method.
7. Davidson, Roth, and Gambrel-Lenarz (2004) In Wehr and Frank (ed.) Standard methods for the microbiological examination of dairy products, 17th ed. American Public Health Association, Washington, D.C.
8. Kornacki and Johnson (2001) In Downes and Ito (ed.) Compendium of methods for the microbiological examination of foods, 4th ed. American Public Health Association, Washington D.C.
9. Mossel, Vissar, and Cornellisen (1963) J. Appl. Bacteriol. 26:444.

PRESENTATION		Contents	Ref.
EE Broth-Mossel	Tubes	20 x 10 ml tubes	24096
EE Broth-Mossel	Bottles (screw cap)	6 x 100 ml bottles	402480
EE Broth-Mossel	Bottles (flip-off cap)	25 x 100 ml bottles	453080
EE Broth-Mossel	Bottles (perforable cap)	6 x 100 ml bottles	495000
EE Broth-Mossel	Dehydrated medium	500 g of powder	610017
EE Broth-Mossel	Dehydrated medium	100 g of powder	620017

TABLE OF SYMBOLS

LOT Batch code	Keep away from sunlight	Manufacturer	Use by	Fragile, handle with care
REF Catalogue number	Temperature limitation	Contains sufficient for <n> tests	Caution, consult Instruction For Use	Do not reuse

LIOFILCHEM® s.r.l.

Via Scozia zona ind.le, 64026 Roseto degli Abruzzi (Te) Italy
Tel. +39 0858930745 Fax +39 0858930330 www.liofilchem.net liofilchem@liofilchem.net

Istruzioni per l'uso
ITALIANO

EE Broth-Mossel

Terreno liquido per la coltivazione e l'arricchimento selettivo delle Enterobacteriaceae da differenti tipologie di campioni, secondo USP/EP/JP.

DESCRIZIONE

Enterobacteriaceae Enrichment Broth-Mossel è un terreno selettivo utilizzato per la ricerca dei batteri Gram negativi bile tolleranti negli alimenti, acqua ed altri materiali di importanza.

Questo terreno è conforme alle raccomandazioni del metodo armonizzato delle Farmacopee Statunitense (USP) Europea (EP) e Giapponese (JP) per l'esame microbiologico dei prodotti non sterili.

FORMULA TIPICA	(g/l)
Digerito Pancreatico di Gelatina	10.0
Glucosio Monoidrato	5.0
Bile di Bue Disidratata	20.0
Potassio Diidrogeno Fosfato	2.0
Sodio Idrogeno Fosfato Bibasico, Anidro	6.4*
Verde Brillante	0.015

pH Finale 7.2 ± 0.2 a 25°C

* Equivalente a 8.0 g di Sodio Idrogeno Fosfato Bibasico Biidrato

PRINCIPIO DEL METODO

Il digerito pancreatico di gelatina fornisce aminoacidi, azoto, carbonio, vitamine e minerali per la crescita dei microrganismi. Il glucosio è il carboidrato fermentabile. Il sodio cloruro mantiene il bilancio osmotico del terreno. Bile di bue e verde brillante sono agenti selettivi efficaci contro i cocchi Gram positivi. Potassio fosfato e sodio fosfato agiscono da tampone.

PREPARAZIONE

Terreno disidratato: Sospendere 43.4 g di polvere in 1 litro di acqua distillata o deionizzata sterile. Mescolare bene. Riscaldare agitando di frequente e bollire fino a completa dissoluzione. NON AUTOCLAVARE.

PROCEDURA DEL TEST

Come da Farmacopea, preparare il campione utilizzando una diluizione 1 a 10 con almeno 1 g del prodotto da esaminare scegliendo come diluente Tryptic Soy Broth (ref. 24513 o 452080) ed incubare a 20-25°C per 2-5 ore per recuperare i batteri.

Per test qualitativi (test per assenza), trasferire in EE Broth-Mossel il volume del brodo di pre-arricchimento corrispondente ad 1 g del prodotto da esaminare.

Per test quantitativi, contare le Enterobacteriaceae trovate per millilitro o per grammo di campione utilizzando la tecnica MPN (Most Probable Number).

Per entrambe le tipologie di test, incubare EE Broth-Mossel a 30-35°C per 24-48 ore e procedere con l'analisi seminando parte del brodo su Violet Red Bile Glucose Agar (ref. 11184). Incubare le piastre in atmosfera aerobica a 30-35°C per 18-24 ore.

INTERPRETAZIONE DEI RISULTATI

La torbidità in EE Broth-Mossel indica la crescita microbica; la produzione di acidi provoca il cambiamento di colore del terreno a giallo.

Il mancato sviluppo di colonie su Violet Red Bile Glucose Agar è riportata come assenza di batteri Gram begativi bile tolleranti. La crescita di colonie costituisce un risultato positivo ed il numero probabile di batteri viene determinato consultando la tabella sottostante.

Tabella MPN.

Risultati in base alle quantità di prodotto			Numero probabile di batteri per grammo o per millilitro di prodotto
0.1 g o 0.1 ml	0.01 g o 0.01 ml	0.001 g o 0.001 ml	
+	+	+	$>10^3$
+	+	–	10^3 - 10^2
+	–	–	10^2 - 10
–	–	–	<10

ASPETTO

Terreno disidratato: omogeneo, fine granulometria, da beige a verde chiaro.
Terreno preparato: verde, limpido.

CONSERVAZIONE

La polvere è fortemente igroscopica, conservare a 10-30°C, in ambiente asciutto, nel suo contenitore originale chiuso ermeticamente. Conservare i flaconi e le provette a 10-25°C al riparo dalla luce. Non usare il prodotto dopo la sua data di scadenza indicata sull'etichetta o se il prodotto mostra segni di contaminazione o deterioramento.

VALIDITÀ

Terreno disidratato: 4 anni.
Terreno in provette/flaconi: 1 anno.

CONTROLLO DI QUALITÀ

Il terreno viene inoculato con i ceppi microbici indicati nella tabella CQ.
Inoculo per produttività: ≤100 UFC.
Inoculo per selettività: >100 UFC.
Condizioni di incubazione: 18-24 ore a 30-35°C (Pharmacopoeia growth promotion);

Tabella CQ.

Microrganismo		Specifiche
Escherichia coli	ATCC® 8739	Crescita buona
Pseudomonas aeruginosa	ATCC® 9027	Crescita buona
Staphylococcus aureus	ATCC® 6538	Inibizione

AVVERTENZE E PRECAUZIONI

Il prodotto non contiene sostanza nocive in concentrazioni superiori ai limiti fissati dall'attuale legislazione e perciò non è classificato come pericoloso. Ciononostante si raccomanda di consultare la scheda di sicurezza per il suo corretto uso. Il prodotto è da intendersi per uso in ambito professionale e deve essere utilizzato esclusivamente da operatori adeguatamente addestrati.

SMALTIMENTO DEI RIFIUTI

Lo smaltimento dei rifiuti deve essere effettuato in conformità alle normative nazionali e locali in vigore.

BIBLIOGRAFIA

1. EN ISO 11133:2014. Microbiology of food, animal feed and water – Preparation, production, storage and performance testing of culture media.
2. European Pharmacopoeia 6.5 (2009) 2.6.13. Microbiological examination of non-sterile products: Test for specified microorganisms.
3. United States Pharmacopoeia 32 NF 27 (2009) <62> Microbiological examination of non-sterile products: Test for specified microorganisms.
4. Japanese Pharmacopoeia 4.05 (2008) Microbiological examination of non-sterile products: Test for specified microorganisms.
5. ISO 21528-1:2004. Microbiology of food and animal feeing stuffs – Horizontal method for the detection and enumeration of Enterobacteriaceae – Detection and enumeration by MPN technique with pre-enrichment.
6. ISO 21528-2:2004. Microbiology of food and animal feeing stuffs – Horizontal method for the detection and enumeration of Enterobacteriaceae – Colony count method.
7. Davidson, Roth, and Gambrel-Lenarz (2004) In Wehr and Frank (ed.) Standard methods for the microbiological examination of dairy products, 17th ed. American Public Health Association, Washington, D.C.
8. Kornacki and Johnson (2001) In Downes and Ito (ed.) Compendium of methods for the microbiological examination of foods, 4th ed. American Public Health Association, Washington D.C.
9. Mossel, Vissar, and Cornellisen (1963) J. Appl. Bacteriol. 26:444.

PRESENTAZIONE		Contenuto	Ref.
EE Broth-Mossel	Provette	Provette 20 x 10 ml	24096
EE Broth-Mossel	Flaconi (tappo a vite)	Flaconi 6 x100 ml	402480
EE Broth-Mossel	Flaconi (tappo flip-off)	Flaconi 25 x100 ml	453080
EE Broth-Mossel	Flaconi (tappo perforabile)	Flaconi 6 x 100 ml	495000
EE Broth-Mossel	Terreno disidratato	500 g di polvere	610017
EE Broth-Mossel	Terreno disidratato	100 g di polvere	620017

TABELLA DEI SIMBOLI

LOT Codice del lotto	Tenere al riparo dalla luce	Fabbricante	Utilizzare entro	Fragile, maneggiare con cura
REF Numero di catalogo	Limiti di temperatura	Contenuto sufficiente per <n> saggi	Attenzione, Consultare le istruzioni per l'uso	Non riutilizzare

LIOFILCHEM® s.r.l.

Via Scozia zona ind.le, 64026 Roseto degli Abruzzi (Te) Italy
Tel. +39 0858930745 Fax +39 0858930330 www.liofilchem.net liofilchem@liofilchem.net

INTEGRAL SYSTEM ENTEROBATTERI

REF. 71714

- ***Sistema colorimetrico per l'identificazione biochimica e l'antibiogramma degli enterobatteri***
- ***Colorimetric system for biochemical identification and susceptibility testing of enterobacteria***
- ***Système colorimétrique pour l'identification biochimique et l'antibiogramme des entérobactéries***
- ***Sistema colorimétrico para la identificación bioquímica y el antibiograma de las enterobacterias***
- ***Sistema colorimétrico para a identificação bioquímica e o antibiograma das enterobactérias***
- ***Χρωματικό σύστημα για τον βιοχημικό προσδιορισμό και το αντιβιόγραμμα των εντεροβακτηριδίων***

Code *F00113*
Rev. 2 / 15.05.2006

INTEGRAL SYSTEM ENTEROBATTERI

Sistema colorimetrico per l'identificazione biochimica e l'antibiogramma degli enterobatteri

DESCRIZIONE

INTEGRAL SYSTEM ENTEROBATTERI è un sistema a 24 pozzetti contenenti substrati biochimici ed antibiotici essiccati per l'identificazione biochimica e l'antibiogramma degli enterobatteri.
Il sistema viene inoculato con la sospensione batterica del microrganismo in esame ed incubato a 36 °C ± 1°C per 18-24 ore. I test per l'identificazione e per l'antibiogramma vengono interpretati valutando il viraggio di colore dei vari pozzetti del sistema.

CONTENUTO DELLE CONFEZIONI

La confezione contiene:
- 20 Sistemi INTEGRAL SYSTEM ENTEROBATTERI
- 20 Fiale di Suspension Medium (7.0 ml/fiala)
- 20 Oxidase Disc
- 1 Foglio istruzioni

PRODOTTI NECESSARI NON CONTENUTI

- Olio di vaselina per uso microbiologico (Vaseline oil 2 flaconi da 50 ml)..... ….cod. 80278
- Physiological Solution (20 fiale da 7.0 ml/fiala) ……..…cod. 20095
- Kovac's Reagent (2 flaconi da 25 ml) …… .cod. 80270
- Materiale vario per laboratorio di microbiologia

CONFIGURAZIONE

Il sistema presenta la configurazione indicata in tabella n°1.

Tabella n°1

Pozzetto	***IDENTIFICAZIONE BIOCHIMICA***
1-LDC	Decarbossilazione della lisina
2-ODC	Decarbossilazione dell'ornitina
3-H_2S	Produzione di idrogeno solforato
4-IND	Test dell'indolo
5-LAC	Fermentazione del lattosio
6-DUL	Fermentazione del dulcitolo
7-PA	Deaminazione della fenilalanina
8-UR	Idrolisi dell'urea
9-CIT	Utilizzazione del citrato
10-OX	Test dell'ossidasi
Pozzetto	***ANTIBIOGRAMMA* (*)**
11-AK	Amikacina - 32 µg/ml
12-CN	Gentamicina - 8 µg/ml
13-TOB	Tobramicina - 8 µg/ml
14-TZP	Piperacillina+Tazobactam - 128/4 µg/ml
15-FOS	Fosfomicina - 200 µg/ml
16-CFP	Cefoperazone - 64 µg/ml
17-CTX	Cefotaxime - 64 µg/ml
18-CAZ	Ceftazidime - 32 µg/ml
19-AMS	Ampicillina + Sulbactam – 32/16 µg/ml
20-NA	Acido Nalidixico - 32 µg/ml
21-CIP	Ciprofloxacina - 4 µg/ml
22-LEV	Levofloxacin - 8 µg/ml
23-SXT	Co-Trimossazolo - 8 µg/ml
24-C	Controllo di crescita per l'antibiogramma

(*):La concentrazione di ogni antibiotico è conforme alle norme aggiornate NCCLS-January 2004, Vol 24 N°1.[1]

PRINCIPIO DEL METODO

INTEGRAL SYSTEM ENTEROBATTERI permette di eseguire simultaneamente l'identificazione biochimica e l'antibiogramma degli Enterobatteri isolati da campioni clinici.

- L'identificazione si basa su prove biochimiche eseguite su terreni colturali contenenti substrati specifici nei pozzetti da **1-LDC** a **10-OX**.
- L'antibiogramma viene valutato in base alla crescita o all'inibizione dei microrganismi in terreni contenenti l'antibiotico ed un indicatore di crescita nei pozzetti da **11-AK** a **23-SXT**. Il pozzetto **24-C** non contiene antibiotici, ma solo il terreno colturale e l'indicatore e serve come controllo della crescita microbica per l'antibiogramma.

COMPOSIZIONE

Tabella n°2

Pozzetto	***Contenuto***
1-LDC	Terreno per evidenziare la decarbossilazione della lisina
2-ODC	Terreno per evidenziare la decarbossilazione dell'ornitina
3-H_2S	Terreno per evidenziare la produzione di idrogeno solforato
4-IND	Terreno per evidenziare la produzione dell'indolo
5-LAC	Terreno per evidenziare la fermentazione del lattosio
6-DUL	Terreno per evidenziare la fermentazione del dulcitolo
7-PA	Terreno per evidenziare la deaminazione della fenilalanina
8-UR	Terreno per evidenziare l'idrolisi dell'urea
9-CIT	Terreno per evidenziare l'utilizzazione del citrato
10-OX	Terreno per evidenziare l'enzima citocromo-ossidasi
11-AK	Terreno contenente Amikacina - 32 µg/ml
12-CN	Terreno contenente Gentamicina - 8 µg/ml
13-TOB	Terreno contenente Tobramicina - 8 µg/ml
14-TZP	Terreno contenente Piperacillina+Tazobactam - 128/4 µg/ml
15-FOS	Terreno contenente Fosfomicina - 200 µg/ml
16-CFP	Terreno contenente Cefoperazone - 64 µg/ml
17-CTX	Terreno contenente Cefotaxime - 64 µg/ml
18-CAZ	Terreno contenente Ceftazidime - 32 µg/ml
19-AMS	Terreno contenente Ampicillina + Sulbactam - 32/16 µg/ml
2-NA	Terreno contenente Acido Nalidixico - 32 µg/ml
21-CIP	Terreno contenente Ciprofloxacina - 4 µg/ml
22-LEV	Terreno contenente Levofloxacin - 8 µg/ml
23-SXT	Terreno contenente Co-Trimossazolo - 8 µg/ml
24-C	Terreno senza antibiotici

Suspension Medium(g/l): *Estratto di lievito **5 g**; Peptone di carne **3 g**; Glucosio **2 g**; Acqua distillata **1000.0 ml** pH **6.8±0.2***

RACCOLTA E CONSERVAZIONE DEI CAMPIONI

Le colonie da sottoporre al test di identificazione biochimica ed all'antibiogramma con INTEGRAL SYSTEM ENTEROBATTERI devono essere riprese da un terreno colturale, selettivo o non selettivo, utilizzato per l'isolamento degli enterobatteri.

PROCEDURA DEL TEST

1. Prelevare un sistema dal kit.
2. Dopo essersi accertati con opportune indagini che le colonie sviluppate sui terreni colturali appartengono presuntivamente al gruppo degli Enterobatteri, prelevare una o più colonie morfologicamente simili, ben isolate, da un terreno di coltura solido e sospenderle in 5 ml di fisiologica per uso microbiologico in modo da ottenere una torbidità equivalente a 0,5 McFarland **(Sospensione batterica)**.
3. Trasferire:
   - 0.2 ml di **Sospensione batterica** nei primi 10 pozzetti ed aggiungere ai pozzetti **1-LDC**, **2-ODC**, **3-H_2S** ed **8-UR** 2 gocce di olio di vaselina per uso microbiologico (IDENTIFICAZIONE BIOCHIMICA).
   - 0.01 ml di **Sospensione batterica** nella fiala di **Suspension Medium** contenuta nel kit e distribuire 0.2 ml nei pozzetti da **11-AK** a **24-C** (ANTIBIOGRAMMA).
4. Coprire il sistema con l'apposito coperchio ed incubare a 36 °C ± 1°C per 18-24 ore.
5. Dopo l'incubazione, aggiungere:
   - 2 gocce di Kovac's Reagent al pozzetto **4-IND** (TEST DELL' INDOLO).
   - 1 disco di OXIDASE DISC al pozzetto **10-OX** (TEST DELL' OSSIDASI).

INTERPRETAZIONE DEI RISULTATI

IDENTIFICAZIONE BIOCHIMICA

Interpretare i risultati dei primi 10 pozzetti utilizzando la tabella n°3 e formare il codice numerico di 4 cifre seguendo le istruzioni riportate al paragrafo **FORMAZIONE DEL CODICE NUMERICO**. Risalire quindi all'identificazione batterica servendosi del tabulato dei codici numerici (tabella n°5).

ANTIBIOGRAMMA

Osservare il viraggio di colore dei pozzetti da **11-AK** a **23-SXT** ed interpretare i risultati servendosi della tabella n°3. Il pozzetto di controllo (**24-C**) deve risultare positivo (giallo). Nel caso in cui risultasse negativo (blu oppure grigio), è necessario verificare la vitalità dell'inoculo, la sua corretta preparazione e procedere alla ripetizione del test utilizzando un nuovo sistema.

Tabella n°3

Pozzetto	**IDENTIFICAZIONE BIOCHIMICA**	**Colore pozzetto**	
		Reazione positiva	**Reazione negativa**
1-LDC	Decarbossilazione della lisina	Viola	Giallo-Marrone
2-ODC	Decarbossilazione dell' ornitina	Viola	Giallo-Marrone
3-H_2S	Produzione di Idrogeno solforato	Nero	Giallo
4-IND	Test dell'indolo	Anello rosa-rosso	Giallo
5-LAC	Fermentazione del lattosio	Giallo	Blu-Verde
6-DUL	Fermentazione del dulcitolo	Giallo	Blu-Verde
7-PA	Deaminazione della fenilalanina	Nero-Marrone	Giallo
8-UR	Idrolisi dell'urea	Rosso-Fucsia	Giallo-Arancio
9-CIT	Utilizzazione del citrato	Blu-Verde scuro	Verde
10-OX	Test dell'ossidasi	Blu-Porpora (reazione immediata)	Incolore

ANTIBIOGRAMMA		
COLORE POZZETTO	**CRESCITA BATTERICA**	**INTERPRETAZIONE**
Blu	Inibita	S = Sensibile
Grigio	Intermedia	I = Sensibilità intermedia
Giallo	Buona	R = Resistente

FORMAZIONE DEL CODICE NUMERICO

1) I 10 test biochimici sono divisi in 3 gruppi contenenti 3 test ed un gruppo contenente 1 test, ed ognuno viene indicato con un valore di positività di 1,2,4.

- Valore 1 : primo test positivo di ogni gruppo
- (LDC, IND, PA,OX)
- Valore 2 : secondo test positivo di ogni gruppo
- (ODC,LAC,UR)
- Valore 4 : terzo test positivo di ogni gruppo
- (H_2S, DUL,CIT)
- Valore zero: reazioni negative di ogni gruppo

2) Addizionando in ogni gruppo i numeri delle reazioni positive, si ottiene un codice a 4 cifre che, servendosi del tabulato dei codici numerici, permette di identificare il microrganismo in esame come da esempio.

	Gruppo I			*Gruppo II*			*Gruppo III*			*Gruppo IV*
Pozzetto	**LDC**	**ODC**	**H_2S**	**IND**	**LAC**	**DUL**	**PA**	**UR**	**CIT**	**OX**
Codice di positività	1	2	4	1	2	4	1	2	4	1
Risultati	+	+	-	+	+	-	-	-	-	-
Somma dei codici		3			3			0		0
CODICE NUMERICO: 3300				*IDENTIFICAZIONE: E.coli*						

SCHEMA DELLE REAZIONI BIOCHIMICHE

Tabella n°4

Microrganismi	*Gruppo I*			*Gruppo II*			*Gruppo III*			*Gruppo IV*
	LDC	**ODC**	**H_2S**	**IND**	**LAC**	**DUL**	**PA**	**UR**	**CIT**	**OX**
E.coli	V	V	-	+	+	V	-	-	-	-
E.coli inactive	V	V	-	+	-	V	-	-	-	-
Shigella spp.	-	V	-	V	-	-	-	-	-	-
Edwardsiella spp.	+	+	+	+	-	-	-	-	-	-
Citrobacter spp.	-	V	+	V	V	V	-	V	V	-
Salmonella spp.	+	+	V	-	-	+*	-	-	V	-
Arizona spp.	+	+	+	V	V	-	-	-	+	-
K. pneumoniae	+	-	-	-	+	V	-	V	V	-
Klebsiella oxytoca	+	-	-	+	+	V	-	+	V	-
Enterobacter cloacae	V	+	-	-	V	-	-	V	+	-
E. aerogenes	+	+	-	-	+	-	-	-	+	-
Enterobacter hafnia	+	+	-	-	V	-	-	-	+	-
Serratia spp.	+	+	-	-	-	-	-	V	+	-
Proteus mirabilis	-	+	+	-	-	-	+	+	V	-
Proteus vulgaris	-	-	V	+	-	-	+	+	V	-
Morganella morganii	-	+	-	+	-	-	+	+	-	-
Providencia stuartii	-	-	-	+	-	-	+	-	+	-
Providencia rettgeri	-	-	-	+	-	-	+	+	+	-
Pseudomonas spp.	+	+	-	-	-	-	-	-	+	+

+ : Reazione Positiva - : Reazione Negativa V: Reazione Variabile

*****Dulcitolo: Alcuni ceppi di Salmonella fermentano lentamente il dulcitolo, pertanto possono essere inizialmente identificati come *Arizona spp*. E' necessario quindi eseguire una prova di conferma usando un antisiero polivalente per Salmonella.

TABULATO DEI CODICI NUMERICI

Tabella n°5

Codice	Microrganismo	Codice	Microrganismo	Codice	Microrganismo	Codice	Microrganismo
0000	*Shigella spp.*	2240	*Enterobacter cloacae*	4220	*Citrobacter spp.*	6160	*Citrobacter spp.*
0100	*E.coli inactive* 45%	2260	*Enterobacter cloacae*	4240	*Citrobacter spp.*	6200	*Citrobacter spp.*
	Shigella spp. 55%	2300	*E.coli*	4260	*Citrobacter spp.*	6220	*Citrobacter spp.*
0130	*Proteus vulgaris*	2500	*E.coli inactive*	4300	*Citrobacter spp.*	6240	*Citrobacter spp.*
0150	*Providencia stuartii*	2700	*E.coli*	4320	*Citrobacter spp.*	6260	*Citrobacter spp.*
0170	*Proteus vulgaris* 0.5%	3040	*Enterobacter hafniae* 60%	4340	*Citrobacter spp.*	6300	*Citrobacter spp.*
	Providencia rettgeri 99.5%		*Serratia spp.* 37%	4360	*Citrobacter freundii*	6320	*Citrobacter spp.*
0300	*E.coli*		*Enterobacter cloacae* 3%	4400	*Citrobacter spp.*	6340	*Citrobacter spp.*
0500	*E.coli inactive*	3041	*Pseudomonas spp.*	4420	*Citrobacter spp.*	6360	*Citrobacter spp.*
0700	*E.coli*	3060	*Serratia spp.* 98.9 %	4440	*Citrobacter spp.*	6400	*Citrobacter spp.*
1100	*E.coli inactive*		*Enterobacter cloacae* 1.1%	4460	*Citrobacter spp.*	6420	*Citrobacter spp.*
1200	*Klebsiella pneumoniae*	3100	*E.coli inactive*	4500	*Citrobacter spp.*	6440	*Citrobacter spp.*
1220	*Klebsiella pneumoniae*	3240	*Enterobacter cloacae* 7.7%	4520	*Citrobacter spp.*	6460	*Citrobacter spp.*
1240	*Klebsiella pneumoniae*		*Enterobacter aerogenes* 75%	4540	*Citrobacter spp.*	6500	*Citrobacter spp.*
1260	*Klebsiella pneumoniae*		*Enterobacter hafniae* 17.3%	4560	*Citrobacter spp.*	6520	*Citrobacter spp.*
1300	*E.coli*	3260	*Enterobacter cloacae*	4600	*Citrobacter spp.*	6540	*Citrobacter spp.*
1320	*Klebsiella oxytoca*	3300	*E.coli*	4620	*Citrobacter spp.*	6560	*Citrobacter spp.*
1360	*Klebsiella oxytoca*	3400	*Salmonella spp.*	4640	*Citrobacter spp.*	6600	*Citrobacter spp.*
1500	*E.coli inactive*	3440	*Salmonella spp.*	4660	*Citrobacter spp.*	6620	*Citrobacter spp.*
1600	*Klebsiella pneumoniae*	3500	*E.coli inactive*	4700	*Citrobacter spp.*	6640	*Citrobacter spp.*
1620	*Klebsiella pneumoniae*	3700	*E.coli*	4720	*Citrobacter spp.*	6660	*Citrobacter spp.*
1640	*Klebsiella pneumoniae*	4000	*Citrobacter spp.*	4740	*Citrobacter spp.*	6700	*Citrobacter spp.*
1660	*Klebsiella pneumoniae*	4020	*Citrobacter spp.*	4760	*Citrobacter spp.*	6720	*Citrobacter spp.*
1700	*E.coli*	4040	*Citrobacter spp.*	6000	*Citrobacter spp.*	6740	*Citrobacter spp.*
1720	*Klebsiella oxytoca*	4060	*Citrobacter spp.*	6020	*Citrobacter spp.*	6760	*Citrobacter spp.*
1760	*Klebsiella oxytoca*	4100	*Citrobacter spp.*	6030	*Proteus mirabilis*	7040	*Arizona spp.*
2000	*Shigella spp.*	4120	*Citrobacter spp.*	6040	*Citrobacter spp.*	7100	*Edwardsiella spp.*
2040	*Enterobacter cloacae*	4130	*Proteus vulgaris*	6060	*Citrobacter spp.*	7140	*Arizona spp.*
2060	*Enterobacter cloacae*	4140	*Citrobacter spp.*	6070	*Proteus mirabilis*	7240	*Arizona spp.*
2100	*E.coli inactive* 44.6%	4160	*Citrobacter spp.*	6100	*Citrobacter spp.*	7340	*Arizona spp.*
	Shigella spp. 55.4%	4170	*Proteus vulgaris*	6120	*Citrobacter spp.*	7400	*Salmonella spp.*
2130	*Morganella morganii*	4200	*Citrobacter spp.*	6140	*Citrobacter spp.*	7440	*Salmonella spp.*

CONTROLLO QUALITA'

Ogni lotto di INTEGRAL SYSTEM ENTEROBATTERI viene sottoposto al controllo qualità utilizzando microrganismi ATCC di riferimento.

Enterobacter cloacae…… ATCC 13047
Escherichia coli..... ATCC 25922
Klebsiella pneumoniae…… ATCC 13883
Proteus mirabilis…… ATCC 25933
Pseudomonas aeruginosa……... ATCC 27853
Salmonella typhimurium……... ATCC 14028

FATTORI CHE POSSONO INVALIDARE I RISULTATI

Non corretta standardizzazione dell'inoculo; applicazione del metodo a microrganismi non appartenenti al gruppo degli Enterobatteri; colture miste o inquinate; uso di sistemi o di reagenti scaduti; non corretta applicazione della tecnica d'uso.

LIMITI ED AVVERTENZE

L'identificazione di *Salmonella spp. e di Shigella spp* deve essere confermata utilizzando antisieri adatti. Per l'identificazione definitiva dei microrganismi in alcuni casi può essere necessario ricorrere a test supplementari di conferma.

PERFORMANCE

I risultati dell'identificazione batterica ottenuti con il sistema INTEGRAL SYSTEM ENTEROBATTERI concordano con quelli dei metodi di identificazione tradizionali come riportato da Piccolomini et al. [(4)]
I risultati dell'antibiogramma ottenuti con il sistema INTEGRAL SYSTEM ENTEROBATTERI concordano con quelli ottenuti con il metodo di diffusione radiale secondo Bauer et al.[(5)] raccomandato dalla Food and Drug Administration (FDA) e dalla National Committee for Clinical Laboratory Standards (NCCLS) U.S.A [(1,8)].

PRECAUZIONI

Il prodotto, INTEGRAL SYSTEM ENTEROBATTERI, non è classificabile come pericoloso ai sensi della legislazione vigente né contiene sostanze nocive in concentrazioni ≥1%, pertanto non richiede la disponibilità della Scheda di Sicurezza. INTEGRAL SYSTEM ENTEROBATTERI è un dispositivo monouso da usare solo per uso diagnostico *in vitro*, è destinato ad un ambito professionale e deve essere usato in laboratorio da operatori adeguatamente addestrati, con metodi approvati di asepsi e di sicurezza nei confronti degli agenti patogeni.

CONSERVAZIONE

Conservare a 2-8°C nella sua confezione originale. Non conservare vicino a fonti di calore ed evitare eccessive variazioni di temperatura. In queste condizioni il prodotto è valido fino alla data di scadenza indicata in etichetta. Non utilizzare oltre questa data. Eliminare se vi sono segni di deterioramento.

ELIMINAZIONE DEL MATERIALE USATO

Dopo l'utilizzazione INTEGRAL SYSTEM ENTEROBATTERI ed il materiale venuto a contatto con il campione devono essere decontaminati e smaltiti in accordo con le tecniche in uso in laboratorio per la decontaminazione e lo smaltimento di materiale potenzialmente infetto.

PRESENTAZIONE

Prodotto	Codice	Confezione
INTEGRAL SYSTEM ENTEROBATTERI	71714	20 *tests*

TABELLA DEI SIMBOLI

SIMBOLO	SIGNIFICATO	SIMBOLO	SIGNIFICATO
IVD	Dispositivo medico diagnostico *in vitro*		Non riutilizzare
	Fabbricante	Σ	Contenuto sufficiente per <n> saggi
REF	Numero di catalogo		Fragile, maneggiare con cura
	Utilizzare entro		Attenzione, vedere le istruzioni per l'uso
	Limiti di temperatura	LOT	Codice del lotto

CE IVD

ENGLISH

INTEGRAL SYSTEM ENTEROBATTERI

Colorimetric system for biochemical identification and susceptibility testing of enterobacteria

DESCRIPTION

INTEGRAL SYSTEM ENTEROBATTERI is a 24-well system containing desiccated biochemical and antibiotic substrates for the biochemical identification and susceptibility testing of enterobacteria.
The systyem is inoculated with the bacterial suspension of the micro-organism under examination and incubated at 36 °C ± 1°C for 18-24 hours. The tests for identification and for the susceptibility testing are interpreted by assessing the change in colour of the various wells.

KIT CONTENTS

The kit contains:
- 20 systems of INTEGRAL SYSTEM ENTEROBATTERI
- 20 Vials of Suspension Medium (7.0 ml/vial)
- 20 Oxidase Disc
- 1 Instruction sheet

ITEMS NECESSARY BUT NOT INCLUDED IN THE KIT

- Vaseline oil for microbiology use (Vaselin oil 2 bottles of 50 ml) .....cod. 80278
- Physiological Solution (20 vials of 7.0 ml/vial).....cod. 20095
- Kovac's Reagent (2 bottles of 25 ml).....cod. 80270
- Sundry microbiology laboratory materials

CONFIGURATION

The configuration of the system is shown in Table 1.

Table 1

Well	***BIOCHEMICAL IDENTIFICATION***
1-LDC	Decarboxylation of lysine
2-ODC	Decarboxylation of ornithine
3-H_2S	Production of hydrogen sulphide
4-IND	Indole test
5-LAC	Fermentation of lactose
6-DUL	Fermentation of dulcitol
7-PA	Deamination of phenylalanine
8-UR	Hydrolysis of urea
9-CIT	Utilisation of citrate
10-OX	Oxidase test
Well	***SUSCEPTIBILITY TESTING (*)***
11-AK	Amikacin - 32 µg/ml
12-CN	Gentamycin - 8 µg/ml
13-TOB	Tobramycin - 8 µg/ml
14-TZP	Piperacillin+Tazobactam - 128/4 µg/ml
15-FOS	Fosfomycin - 200 µg/ml
16-CFP	Cefoperazone - 64 µg/ml
17-CTX	Cefotaxime - 64 µg/ml
18-CAZ	Ceftazidime - 32 µg/ml
19-AMS	Ampicillin + Sulbactam – 32/16 µg/ml
20-NA	Nalidixic acid - 32 µg/ml
21-CIP	Ciprofloxacin - 4 µg/ml
22-LEV	Levofloxacin - 8 µg/ml
23-SXT	Co-Trimoxazole - 8 µg/ml
24-C	Growth control for the susceptibility testing

(*): The concentration of each antibiotic is according to the updated NCCLS-January 2004, Vol 24 N°1.[1]

PRINCIPLE OF THE METHOD

INTEGRAL SYSTEM ENTEROBATTERI makes possible simultaneous biochemical identification and assessment of the susceptibility testing of enterobacteria isolated from clinical samples.

- The identification is based on biochemical tests performed on culture media containing specific substrates in the wells from **1-LDC** to **10-OX**.
- The susceptibility testing is assessed on the basis of the growth or inhibition of the micro-organisms in the media containing the antibiotic and a growth indicator in the wells from **11-AK** to **23-SXT**. Well **24-C** does not contain antibiotics but only the culture medium and the indicator; it serves as a microbe growth control for assessment of the susceptibility testing.

COMPOSITION

Table n°2

Well	***Content***
1-LDC	Culture medium for showing lysine decarboxylase
2-ODC	Culture medium for showing ornithine decarboxylase
3-H_2S	Culture medium for showing production of hydrogen sulphide
4-IND	Culture medium for showing production of indole
5-LAC	Culture medium for showing fermentation of lactose
6-DUL	Culture medium for showing fermentation of dulcitol
7-PA	Culture medium for showing phenylalanine deamination
8-UR	Culture medium for showing hydrolysis of urea
9-CIT	Culture medium for showing utilisation of citrate
10-OX	Culture medium for showing enzyme cytochrome-oxidase
11-AK	Culture medium containing Amikacin - 32 µg/ml
12-CN	Culture medium containing Gentamycin - 8 µg/ml
13-TOB	Culture medium containing Tobramycin - 8 µg/ml
14-TZP	Culture medium containing Piperacillin+Tazobactam - 128/4 µg/ml
15-FOS	Culture medium containing Fosfomycin - 200 µg/ml
16-CFP	Culture medium containing Cefoperazone - 64 µg/ml
17-CTX	Culture medium containing Cefotaxime - 64 µg/ml
18-CAZ	Culture medium containing Ceftazidime - 32 µg/ml
19-AMS	Culture medium containing Ampicillin + Sulbactam - 32/16 µg/ml
20-NA	Culture medium containing Nalidixic acid - 32 µg/ml
21-CIP	Culture medium containing Ciprofloxacin - 4 µg/ml
22-LEV	Culture medium containing Levofloxacin - 8 µg/ml
23-SXT	Culture medium containing Co-Trimoxazole - 8 µg/ml
24-C	Medium without antibiotics

Suspension Medium (g/l): *Yeast extract **5 g**; Beef peptone **3 g**; Glucose **2 g**; Distilled water **1000.0 ml**; pH **6.8±0.2***

COLLECTION AND STORAGE OF THE SAMPLES

The colonies to be subjected to the biochemical identification test and susceptibility testing assessment with INTEGRAL SYSTEM ENTEROBATTERI must be taken from a culture medium – selective or non selective – used for the isolation of enterobacteria.

TEST PROCEDURE

1. Take a system from the kit.
2. After checking that the colonies that have developed on the culture media presumably belong to the enterobacteria group, take one or more morphologically similar, well isolated, colonies from a solid culture medium and suspend them in 5 ml of Physiological Solution for microbiological use in such a way as to obtain a turbidity equivalent to 0.5 McFarland **(Bacterial suspension)**.
3. Trasfer
   - 0.2 ml of **Bacterial suspension** into each of the first 10 wells and to wells **1-LDC**, **2-ODC**, **3-H_2S** and **8-UR** add 2 drops of Vaseline Oil for microbiological use (BIOCHEMICAL IDENTIFICATION).
   - 0.01 ml of **Bacterial suspension** to the vial of **Suspension Medium** contained into the kit and distribute 0.2 ml into the wells from **11-AK** to **24-C** (SUSCEPTIBILITY TESTING).
4. Cover the system with the lid provided and incubate at 36 °C ± 1°C for 18-24 hours.
5. After incubation. add:
   - 2 drops of Kovac's Reagent to well **4-IND** (INDOLE TEST).
   - 1 OXIDASE DISC to well **10-OX** (OXIDASE TEST).

INTERPRETATION OF THE RESULTS

BIOCHEMICAL IDENTIFICATION

Interpret the results of the first 10 wells using Table n° 3 and form the 4-digit code following the instructions given to the paragraph **FORMATION OF NUMERICAL CODE**. Then use the code and Table n°5 to identify the bacteria.

SUSCEPTIBILITY TESTING

Observe the changes of colour of the wells from **11-AK** to **23-SXT** and interpret the results using Table n°3. The control well (**24-C**) must be positive (yellow). If it is negative (blue or grey), it is necessary to check the vitality of the inoculum and whether it has been correctly prepared; then to repeat the tests using a new system.

Table n°3

Well	**BIOCHEMICAL IDENTIFICATION**	**Well colour**	
		Positive reaction	**Negative reaction**
1-LDC	Decarboxylation of lysine	Violet	Yellow-Brown
2-ODC	Decarboxylation of ornithine	Violet	Yellow-Brown
3 -H_2S	Production of hydrogen sulphide	Black	Yellow
4-IND	Indole test	Pink-Red ring	Yellow
5-LAC	Fermentation of lactose	Yellow	Blue-Green
6-DUL	Fermentation of dulcitol	Yellow	Blue-Green
7-PA	Deamination of phenylalanine	Black-Brown	Yellow
8-UR	Hydrolysis of urea	Red-Fuchsia	Yellow-Orange
9-CIT	Utilisation of citrate	Blue-Dark Green	Green
10-OX	Oxidase test	Blue-Purple (immediate reaction)	Colourless

SUSCEPTIBILITY TESTING		
WELL COLOUR	**BACTERIAL GROWTH**	**INTERPRETATION**
Blue	Inhibited	S = Sensitive
Grey	Intermediate	I = Intermediate sensitivity
Yellow	Good	R = Resistant

FORMATION OF THE NUMERICAL CODE

1) The 10 biochemical tests are divided into 3 groups each containing 3 tests and one group containing 1 test, each one is indicated with a positiveness value of 1,2,4.

- Value 1 : first test positive in each group
- (LDC, IND, PA,OX)
- Value 2 : second test positive in each group
- (ODC,LAC,UR)
- Value 4 : third test positive in each group
- (H_2S, DUL,CIT)
- Value zero: negative reaction in every group

2) Adding the number of positive reactions in each group, one obtains a 4-digit code which is used to identify the micro-organism from the table of numerical codes as in the example.

	Group I			*Group II*			*Group III*			*Group IV*
Well	**LDC**	**ODC**	**H_2S**	**IND**	**LAC**	**DUL**	**PA**	**UR**	**CIT**	**OX**
Positiveness code	1	2	4	1	2	4	1	2	4	1
Results	+	+	-	+	+	-	-	-	-	-
Codes addition		3			3			0		0
NUMERICAL CODE: 3300				*IDENTIFICATION: E.coli*						

TABLE OF BIOCHEMICAL REACTIONS

Table n°4

Micro-organisms	*Group I*			*Group II*			*Group III*			*Group IV*
	LDC	**ODC**	**H_2S**	**IND**	**LAC**	**DUL**	**PA**	**UR**	**CIT**	**OX**
E.coli	V	V	-	+	+	V	-	-	-	-
E.coli inactive	V	V	-	+	-	V	-	-	-	-
Shigella spp.	-	V	-	V	-	-	-	-	-	-
Edwardsiella spp.	+	+	+	+	-	-	-	-	-	-
Citrobacter spp.	-	V	+	V	V	V	-	V	V	-
Salmonella spp.	+	+	V	-	-	+*	-	-	V	-
Arizona spp.	+	+	+	V	V	-	-	-	+	-
K. pneumoniae	+	-	-	-	+	V	-	V	V	-
Klebsiella oxytoca	+	-	-	+	+	V	-	+	V	-
Enterobacter cloacae	V	+	-	-	V	-	-	V	+	-
E. aerogenes	+	+	-	-	+	-	-	-	+	-
Enterobacter hafnia	+	+	-	-	V	-	-	-	+	-
Serratia spp.	+	+	-	-	-	-	-	V	+	-
Proteus mirabilis	-	+	+	-	-	-	+	+	V	-
Proteus vulgaris	-	-	V	+	-	-	+	+	V	-
Morganella morganii	-	+	-	+	-	-	+	+	-	-
Providencia stuartii	-	-	-	+	-	-	+	-	+	-
Providencia rettgeri	-	-	-	+	-	-	+	+	+	-
Pseudomonas spp.	+	+	-	-	-	-	-	-	+	+

+ : Positive Reaction - : Negative Reaction V: Variable Reaction

*Dulcitol: Some strains of Salmonella ferment dulcitol slowly; they may therefore be identified initially as *Arizona spp*. A confirmation test is thus required using a polyvalent antiserum for Salmonella.

TABLE OF NUMERICAL CODES

Table n°5

Code	Micro-organism	Code	Micro-organism	Code	Micro-organism	Code	Micro-organism
0000	*Shigella spp.*	2240	*Enterobacter cloacae*	4220	*Citrobacter spp.*	6160	*Citrobacter spp.*
0100	*E.coli inactive* 45%	2260	*Enterobacter cloacae*	4240	*Citrobacter spp.*	6200	*Citrobacter spp.*
	Shigella spp. 55%	2300	*E.coli*	4260	*Citrobacter spp.*	6220	*Citrobacter spp.*
0130	*Proteus vulgaris*	2500	*E.coli inactive*	4300	*Citrobacter spp.*	6240	*Citrobacter spp.*
0150	*Providencia stuartii*	2700	*E.coli*	4320	*Citrobacter spp.*	6260	*Citrobacter spp.*
0170	*Proteus vulgaris* 0.5%	3040	*Enterobacter hafniae* 60%	4340	*Citrobacter spp.*	6300	*Citrobacter spp.*
	Providencia rettgeri 99.5%		*Serratia spp.* 37%	4360	*Citrobacter freundii*	6320	*Citrobacter spp.*
0300	*E.coli*		*Enterobacter cloacae* 3%	4400	*Citrobacter spp.*	6340	*Citrobacter spp.*
0500	*E.coli inactive*	3041	*Pseudomonas spp.*	4420	*Citrobacter spp.*	6360	*Citrobacter spp.*
0700	*E.coli*	3060	*Serratia spp.* 98.9 %	4440	*Citrobacter spp.*	6400	*Citrobacter spp.*
1100	*E.coli inactive*		*Enterobacter cloacae* 1.1%	4460	*Citrobacter spp.*	6420	*Citrobacter spp.*
1200	*Klebsiella pneumoniae*	3100	*E.coli inactive*	4500	*Citrobacter spp.*	6440	*Citrobacter spp.*
1220	*Klebsiella pneumoniae*	3240	*Enterobacter cloacae* 7.7%	4520	*Citrobacter spp.*	6460	*Citrobacter spp.*
1240	*Klebsiella pneumoniae*		*Enterobacter aerogenes* 75%	4540	*Citrobacter spp.*	6500	*Citrobacter spp.*
1260	*Klebsiella pneumoniae*		*Enterobacter hafniae* 17.3%	4560	*Citrobacter spp.*	6520	*Citrobacter spp.*
1300	*E.coli*	3260	*Enterobacter cloacae*	4600	*Citrobacter spp.*	6540	*Citrobacter spp.*
1320	*Klebsiella oxytoca*	3300	*E.coli*	4620	*Citrobacter spp.*	6560	*Citrobacter spp.*
1360	*Klebsiella oxytoca*	3400	*Salmonella spp.*	4640	*Citrobacter spp.*	6600	*Citrobacter spp.*
1500	*E.coli inactive*	3440	*Salmonella spp.*	4660	*Citrobacter spp.*	6620	*Citrobacter spp.*
1600	*Klebsiella pneumoniae*	3500	*E.coli inactive*	4700	*Citrobacter spp.*	6640	*Citrobacter spp.*
1620	*Klebsiella pneumoniae*	3700	*E.coli*	4720	*Citrobacter spp.*	6660	*Citrobacter spp.*
1640	*Klebsiella pneumoniae*	4000	*Citrobacter spp.*	4740	*Citrobacter spp.*	6700	*Citrobacter spp.*
1660	*Klebsiella pneumoniae*	4020	*Citrobacter spp.*	4760	*Citrobacter spp.*	6720	*Citrobacter spp.*
1700	*E.coli*	4040	*Citrobacter spp.*	6000	*Citrobacter spp.*	6740	*Citrobacter spp.*
1720	*Klebsiella oxytoca*	4060	*Citrobacter spp.*	6020	*Citrobacter spp.*	6760	*Citrobacter spp.*
1760	*Klebsiella oxytoca*	4100	*Citrobacter spp.*	6030	*Proteus mirabilis*	7040	*Arizona spp.*
2000	*Shigella spp.*	4120	*Citrobacter spp.*	6040	*Citrobacter spp.*	7100	*Edwardsiella spp.*
2040	*Enterobacter cloacae*	4130	*Proteus vulgaris*	6060	*Citrobacter spp.*	7140	*Arizona spp.*
2060	*Enterobacter cloacae*	4140	*Citrobacter spp.*	6070	*Proteus mirabilis*	7240	*Arizona spp.*
2100	*E.coli inactive* 44.6%	4160	*Citrobacter spp.*	6100	*Citrobacter spp.*	7340	*Arizona spp.*
	Shigella spp. 55.4%	4170	*Proteus vulgaris*	6120	*Citrobacter spp.*	7400	*Salmonella spp.*
2130	*Morganella morganii*	4200	*Citrobacter spp.*	6140	*Citrobacter spp.*	7440	*Salmonella spp.*

QUALITY CONTROL

Each batch of INTEGRAL SYSTEM ENTEROBATTERI is subjected to quality control using the following ATCC reference strains.

Enterobacter cloacae……..	*ATCC 13047*	*Proteus mirabilis*……	*ATCC 25933*
Escherichia coli.....….....	*ATCC 25922*	*Pseudomonas aeruginosa*……...	*ATCC 27853*
Klebsiella pneumoniae……	*ATCC 13883*	*Salmonella typhimurium*……..	*ATCC 14028*

FACTORS THAT MAY INVALIDATE THE RESULTS

Poor standardisation of the inoculum; application of the method to micro-organisms not in the Enterobacteria group; mixed or contaminated cultures; use of expired systems or expired supplementary reagents; incorrect application of the technique.

LIMITS AND WARNINGS

The identification of *Salmonella spp.* and of *Shigella spp* must be confirmed using appropriate antisera. In some cases, supplementary confirmation tests may be required for definitive identification of the micro-organisms.

PERFORMANCE

The bacterial identification results obtained with the INTEGRAL SYSTEM ENTEROBATTERI agree with those obtained using traditional methods as indicated by Piccolomini et al.[4].
The antibiogram results obtained with the INTEGRAL SYSTEM ENTEROBATTERI agree with those obtained using the radial diffusion methods according to Bauer et al.[5] recommended by the Food and Drug Administration (FDA) and by the National Committee for Clinical Laboratory Standards (NCCLS) U.S.A [1,8].

PRECAUTIONS

The product, INTEGRAL SYSTEM ENTEROBATTERI, cannot be classified as hazardous under current legislation nor does it contain harmful substances in concentrations ≥1%. It therefore does not require a Safety Data Sheet to be available. INTEGRAL SYSTEM ENTEROBATTERI is a disposable device to be used only for diagnostic use *in vitro;* it is intended for use in a professional environment and must be used in the laboratory by properly trained personnel, using approved asepsis and safety methods for handling pathogenic agents.

STORAGE

Store at 2-8°C in the original packaging. Keep away from sources of heat and avoid excessive changes in temperature. In such conditions, the product will remain valid until the expiry date indicated on the label. Do not use beyond that date. Eliminate without using if there are signs of deterioration.

DISPOSAL OF USED MATERIAL

After use, INTEGRAL SYSTEM ENTEROBATTERI and material that has come into contact with the sample must be decontaminated and disposed of in accordance with the techniques used in the laboratory for decontamination and disposal of potentially infected material.

PRESENTATION

Product	Code	Kit
INTEGRAL SYSTEM ENTEROBATTERI	71714	20 *tests*

TABLE OF SYMBOLS

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

CE IVD

FRANÇAIS

INTEGRAL SYSTEM ENTEROBATTERI

Système colorimétrique pour l'identification biochimique et l'antibiogramme des entérobactéries

DESCRIPTION

INTEGRAL SYSTEM ENTEROBATTERI est un système à 24 puits contenant des substrats biochimiques et antibiotiques séchés pour l'identification biochimique et l'antibiogramme des entérobactéries.
Le système est inoculé avec la suspension bactérienne du micro-organisme examiné et incubé à 36 °C ± 1°C pendant 18-24 heures. Les tests pour l'identification et pour l'antibiogramme sont interprétés en évaluant le virage de couleur des différents puits du système.

CONTENU DES EMBALLAGES

Chaque emballage contient:
· 20 Systèmes INTEGRAL SYSTEM ENTEROBATTERI
· 20 Ampoules de Suspension Medium (7,0 ml/ampoule)
· 20 Oxidase Disc
· 1 Notice

PRODUITS NÉCESSAIRES NON CONTENUS

- Huile de vaseline à usage microbiologique (Vaseline oil 2 flacons de 50 ml).....code 80278
- Physiological Solution (20 ampoules de 7.0 ml/ampoule).....code 20095
- Kovac's Reagent (2 flacons de 25 ml).....code 80270
- Matériel divers pour laboratoire de microbiologie

CONFIGURATION

Le système présente la configuration indiquée au tableau n°1.

Tableau n° 1

Puits	***IDENTIFICATION BIOCHIMIQUE***
1-LDC	Décarboxylation de la lysine
2-ODC	Décarboxylation de l'ornithine
3-H_2S	Production d'hydrogène sulfuré
4-IND	Test de l'indole
5-LAC	Fermentation du lactose
6-DUL	Fermentation du dulcitol
7-PA	Désamination de la phénylalanine
8-UR	Hydrolyse de l'urée
9-CIT	Utilisation du citrate
10-OX	Test de l'oxydase
Puits	***ANTIBIOGRAMME* (*)**
11-AK	Amikacine - 32 µg/ml
12-CN	Gentamicine - 8 µg/ml
13-TOB	Tobramicine - 8 µg/ml
14-TZP	Pipéracilline +Tazobactam - 128/4 µg/ml
15-FOS	Fosfomycine - 200 µg/ml
16-CFP	Céfopérazone - 64 µg/ml
17-CTX	Céfotaxime - 64 µg/ml
18-CAZ	Ceftazidime - 32 µg/ml
19-AMS	Ampicilline + Sulbactam – 32/16 µg/ml
20-NA	Acide nalidixique - 32 µg/ml
21-CIP	Ciprofloxacine - 4 µg/ml
22-LEV	Lévofloxacine - 8 µg/ml
23-SXT	Cotrimoxazole - 8 µg/ml
24-C	Contrôle de croissance pour l'antibiogramme

(*): La concentration de chaque antibiotique est conforme aux normes actualisées NCCLS-January 2004, Vol 24 N°1.[(1)]

PRINCIPE DE LA MÉTHODE

INTEGRAL SYSTEM ENTEROBATTERI permet d'effectuer simultanément l'identification biochimique et l'antibiogramme des Entérobactéries isolées à partir d'échantillons cliniques.

- L'identification se base sur des tests biochimiques effectués sur des milieux de culture contenant des substrats spécifiques dans les puits de **1-LDC** à **10-OX**.
- L'antibiogramme est évalué sur la base de la croissance ou de l'inhibition des micro-organismes dans les milieux contenant l'antibiotique et un indicateur de croissance dans les puits de **11-AK** à **23-SXT**.
  Le puits **24-C** ne contient pas d'antibiotiques, mais seulement le milieu de culture et l'indicateur, et il sert au contrôle de la croissance microbienne pour l'antibiogramme.

COMPOSITION

Tableau n° 2

Puits	***Contenu***
1-LDC	Milieu pour l'identification de la lysine décarboxylase
2-ODC	Milieu pour l'identification de l'ornithine décarboxylase
3-H_2S	Milieu pour l'identification de la production d'hydrogène sulfuré
4-IND	Milieu pour l'identification de la production de l'indole
5-LAC	Milieu pour l'identification de la fermentation du lactose
6-DUL	Milieu pour l'identification de la fermentation du dulcitol
7-PA	Milieu pour l'identification de la phénylalanine désaminase
8-UR	Milieu pour l'identification de l'hydrolyse de l'urée
9-CIT	Milieu pour l'identification de l'utilisation du citrate
10-OX	Milieu pour l'identification de l'enzyme cytochrome - oxydase
11-AK	Milieu contenant Amikacine - 32 µg/ml
12-CN	Milieu contenant Gentamicine - 8 µg/ml
13-TOB	Milieu contenant Tobramicine - 8 µg/ml
14-TZP	Milieu contenant Pipéracilline + Tazobactam - 128/4 µg/ml
15-FOS	Milieu contenant Fosfomycine - 200 µg/ml
16-CFP	Milieu contenant Céfopérazone - 64 µg/ml
17-CTX	Milieu contenant Céfotaxime - 64 µg/ml
18-CAZ	Milieu contenant Ceftazidime - 32 µg/ml
19-AMS	Milieu contenant Ampicilline + Sulbactam - 32/16 µg/ml
2-NA	Milieu contenant Acide nalidixique - 32 µg/ml
21-CIP	Milieu contenant Ciprofloxacine - 4 µg/ml
22-LEV	Milieu contenant Lévofloxacine - 8 µg/ml
23-SXT	Milieu contenant Cotrimoxazole - 8 µg/ml
24-C	Milieu sans antibiotiques

Suspension Medium (g/l): *Extrait de levure **5g**; Peptone de viande **3g**; Glucose **2g**; Eau distillée **1000.0 ml**; pH **6.8±0.2***

PRÉLÈVEMENT ET CONSERVATION DES ÉCHANTILLONS

Les colonies à soumettre au test d'identification biochimique et à l'antibiogramme avec INTEGRAL SYSTEM ENTEROBATTERI doivent être prélevées sur un milieu de culture, sélectif ou non sélectif, utilisé pour l'isolement des Entérobactéries.

PROCÉDURE DU TEST

1. Sortir un système du kit.
2. Après s'être assuré, à travers des examens appropriés, que les colonies développées sur les milieux de culture appartiennent présomptivement au groupe des Entérobactéries, prélever une ou plusieurs colonies morphologiquement semblables, bien isolées, sur un milieu de culture solide et les mettre en suspension dans 5 ml de solution physiologique à usage microbiologique afin d'obtenir une turbidité équivalente au standard 0,5 McFarland (**Suspension bactérienne**).
3. Transférer :
   - 0.2 ml de **Suspension bactérienne** dans les 10 premiers puits et ajouter aux puits **1-LDC**, **2-ODC**, **3-H_2S** et **8-UR** 2 gouttes d'huile de vaseline à usage microbiologique (IDENTIFICATION BIOCHIMIQUE).
   - 0.01 ml de **Suspension bactérienne** dans l'ampoule de **Suspension Medium** contenu dans le kit et distribuer 0,2 ml dans les puits de **11-AK** à **24-C** (ANTIBIOGRAMME).
4. Couvrir le système avec le couvercle et incuber à 36 °C ± 1°C pendant 18-24 heures.
5. Après l'incubation, ajouter :
   - 2 gouttes de Kovac's Reagent dans le puits **4-IND** (TEST DE L'INDOLE).
   - 1 disque de OXIDASE DISC dans le puits **10-OX** (TEST DE L'OXYDASE).

INTERPRÉTATION DES RÉSULTATS

IDENTIFICATION BIOCHIMIQUE

Interpréter les résultats des 10 premiers puits en utilisant le tableau n° 3 et former le code numérique à 4 chiffres en suivant les instructions du paragraphe **FORMATION DU CODE NUMÉRIQUE**. Remonter à l'identification bactérienne à l'aide du tableau des codes numériques (tableau n° 5).

ANTIBIOGRAMME

Observer le virage de couleur des puits de **11-AK** à **23-SXT** et interpréter les résultats à l'aide du tableau n° 3 . Le puits de contrôle (**24-C**) doit être positif (jaune). S'il est négatif (bleu ou gris), il est nécessaire de vérifier la vitalité de l'inoculum, sa préparation correcte, et de procéder à la répétition du test en utilisant un nouveau système.

Tableau n° 3

Puits	**IDENTIFICATION BIOCHIMIQUE**	**Couleur du puits**	
		Réaction positive	**Réaction négative**
1-LDC	Décarboxylation de la lysine	Violet	Jaune-Marron
2-ODC	Décarboxylation de l'ornithine	Violet	Jaune-Marron
3-H_2S	Production d'hydrogène sulfuré	Noir	Jaune
4-IND	Test de l'indole	Anneau rose-rouge	Jaune
5-LAC	Fermentation du lactose	Jaune	Bleu-Vert
6-DUL	Fermentation du dulcitol	Jaune	Bleu-Vert
7-PA	Désamination de la phénylalanine	Noir-Marron	Jaune
8-UR	Hydrolyse de l'urée	Rouge-Fuchsia	Jaune-Orange
9-CIT	Utilisation du citrate	Bleu-Vert foncé	Vert
10-OX	Test de l'oxydase	Bleu-Pourpre (réaction immédiate)	Incolore

ANTIBIOGRAMME		
COULEUR DU PUITS	**CROISSANCE BACTÉRIENNE**	**INTERPRÉTATION**
Bleu	Inhibée	S = Sensible
Gris	Intermédiaire	I = Sensibilité intermédiaire
Jaune	Bonne	R = Résistant

FORMATION DU CODE NUMÉRIQUE

1) Les 10 tests biochimiques sont divisés en 3 groupes contenant 3 tests et un groupe contenant 1 test ; chacun est indiqué avec une valeur de positivité de 1, 2, 4.

- Valeur 1 : premier test positif de chaque groupe
- (LDC, IND, PA, OX)
- Valeur 2 : deuxième test positif de chaque groupe
- (ODC, LAC, UR)
- Valeur 4 : troisième test positif de chaque groupe
- (H_2S, DUL, CIT)
- Valeur zéro : réactions négatives de chaque groupe

2) Additionner dans chaque groupe les nombres des réactions positives, pour obtenir un code à 4 chiffres qui, à l'aide du tableau des codes numériques, permet d'identifier le micro-organisme examiné comme dans l'exemple.

	Groupe I			*Groupe II*			*GroupeIII*			*Groupe IV*
Puits	**LDC**	**ODC**	**H_2S**	**IND**	**LAC**	**DUL**	**PA**	**UR**	**CIT**	**OX**
Code of positivité	1	2	4	1	2	4	1	2	4	1
Résultats	+	+	-	+	+	-	-	-	-	-
Somme des codes		3			3			0		0
CODE NUMÉRIQUE: 3300				*IDENTIFICATION: E.coli*						

TABLEAU DES RÉACTIONS BIOCHIMIQUES

Tableau n° 4

Micro-organismes	*Groupe I*			*Groupe II*			*Groupe III*			*Groupe IV*
	LDC	**ODC**	**H_2S**	**IND**	**LAC**	**DUL**	**PA**	**UR**	**CIT**	**OX**
E.coli	V	V	-	+	+	V	-	-	-	-
E.coli inactive	V	V	-	+	-	V	-	-	-	-
Shigella spp.	-	V	-	V	-	-	-	-	-	-
Edwardsiella spp.	+	+	+	+	-	-	-	-	-	-
Citrobacter spp.	-	V	+	V	V	V	-	V	V	-
Salmonella spp.	+	+	V	-	-	+*	-	-	V	-
Arizona spp.	+	+	+	V	V	-	-	-	+	-
K. pneumoniae	+	-	-	-	+	V	-	V	V	-
Klebsiella oxytoca	+	-	-	+	+	V	-	+	V	-
Enterobacter cloacae	V	+	-	-	V	-	-	V	+	-
E. aerogenes	+	+	-	-	+	-	-	-	+	-
Enterobacter hafnia	+	+	-	-	V	-	-	-	+	-
Serratia spp.	+	+	-	-	-	-	-	V	+	-
Proteus mirabilis	-	+	+	-	-	-	+	+	V	-
Proteus vulgaris	-	-	V	+	-	-	+	+	V	-
Morganella morganii	-	+	-	+	-	-	+	+	-	-
Providencia stuartii	-	-	-	+	-	-	+	-	+	-
Providencia rettgeri	-	-	-	+	-	-	+	+	+	-
Pseudomonas spp.	+	+	-	-	-	-	-	-	+	+

+ : Réaction Positive - : Réaction Négative V : Réaction Variable

*****Dulcitol : Certaines souches de Salmonella fermentent lentement le dulcitol, elles peuvent par conséquent être initialement identifiées comme *Arizona spp*. Il est donc nécessaire d'effectuer un test de confirmation en utilisant un antisérum polyvalent pour Salmonella.

TABLEAU DES CODES NUMÉRIQUES

Tableau n° 5

Code	Micro-organisme	Code	Micro-organisme	Code	Micro-organisme	Code	Micro-organisme
0000	*Shigella spp.*	2240	*Enterobacter cloacae*	4220	*Citrobacter spp.*	6160	*Citrobacter spp.*
0100	*E.coli inactive* 45%	2260	*Enterobacter cloacae*	4240	*Citrobacter spp.*	6200	*Citrobacter spp.*
	Shigella spp. 55%	2300	*E.coli*	4260	*Citrobacter spp.*	6220	*Citrobacter spp.*
0130	*Proteus vulgaris*	2500	*E.coli inactive*	4300	*Citrobacter spp.*	6240	*Citrobacter spp.*
0150	*Providencia stuartii*	2700	*E.coli*	4320	*Citrobacter spp.*	6260	*Citrobacter spp.*
0170	*Proteus vulgaris* 0.5%	3040	*Enterobacter hafniae* 60%	4340	*Citrobacter spp.*	6300	*Citrobacter spp.*
	Providencia rettgeri 99.5%		*Serratia spp.* 37%	4360	*Citrobacter freundii*	6320	*Citrobacter spp.*
0300	*E.coli*		*Enterobacter cloacae* 3%	4400	*Citrobacter spp.*	6340	*Citrobacter spp.*
0500	*E.coli inactive*	3041	*Pseudomonas spp.*	4420	*Citrobacter spp.*	6360	*Citrobacter spp.*
0700	*E.coli*	3060	*Serratia spp.* 98.9 %	4440	*Citrobacter spp.*	6400	*Citrobacter spp.*
1100	*E.coli inactive*		*Enterobacter cloacae* 1.1%	4460	*Citrobacter spp.*	6420	*Citrobacter spp.*
1200	*Klebsiella pneumoniae*	3100	*E.coli inactive*	4500	*Citrobacter spp.*	6440	*Citrobacter spp.*
1220	*Klebsiella pneumoniae*	3240	*Enterobacter cloacae* 7.7%	4520	*Citrobacter spp.*	6460	*Citrobacter spp.*
1240	*Klebsiella pneumoniae*		*Enterobacter aerogenes* 75%	4540	*Citrobacter spp.*	6500	*Citrobacter spp.*
1260	*Klebsiella pneumoniae*		*Enterobacter hafniae* 17.3%	4560	*Citrobacter spp.*	6520	*Citrobacter spp.*
1300	*E.coli*	3260	*Enterobacter cloacae*	4600	*Citrobacter spp.*	6540	*Citrobacter spp.*
1320	*Klebsiella oxytoca*	3300	*E.coli*	4620	*Citrobacter spp.*	6560	*Citrobacter spp.*
1360	*Klebsiella oxytoca*	3400	*Salmonella spp.*	4640	*Citrobacter spp.*	6600	*Citrobacter spp.*
1500	*E.coli inactive*	3440	*Salmonella spp.*	4660	*Citrobacter spp.*	6620	*Citrobacter spp.*
1600	*Klebsiella pneumoniae*	3500	*E.coli inactive*	4700	*Citrobacter spp.*	6640	*Citrobacter spp.*
1620	*Klebsiella pneumoniae*	3700	*E.coli*	4720	*Citrobacter spp.*	6660	*Citrobacter spp.*
1640	*Klebsiella pneumoniae*	4000	*Citrobacter spp.*	4740	*Citrobacter spp.*	6700	*Citrobacter spp.*
1660	*Klebsiella pneumoniae*	4020	*Citrobacter spp.*	4760	*Citrobacter spp.*	6720	*Citrobacter spp.*
1700	*E.coli*	4040	*Citrobacter spp.*	6000	*Citrobacter spp.*	6740	*Citrobacter spp.*
1720	*Klebsiella oxytoca*	4060	*Citrobacter spp.*	6020	*Citrobacter spp.*	6760	*Citrobacter spp.*
1760	*Klebsiella oxytoca*	4100	*Citrobacter spp.*	6030	*Proteus mirabilis*	7040	*Arizona spp.*
2000	*Shigella spp.*	4120	*Citrobacter spp.*	6040	*Citrobacter spp.*	7100	*Edwardsiella spp.*
2040	*Enterobacter cloacae*	4130	*Proteus vulgaris*	6060	*Citrobacter spp.*	7140	*Arizona spp.*
2060	*Enterobacter cloacae*	4140	*Citrobacter spp.*	6070	*Proteus mirabilis*	7240	*Arizona spp.*
2100	*E.coli inactive* 44.6%	4160	*Citrobacter spp.*	6100	*Citrobacter spp.*	7340	*Arizona spp.*
	Shigella spp. 55.4%	4170	*Proteus vulgaris*	6120	*Citrobacter spp.*	7400	*Salmonella spp.*
2130	*Morganella morganii*	4200	*Citrobacter spp.*	6140	*Citrobacter spp.*	7440	*Salmonella spp.*

CONTRÔLE QUALITÉ

Chaque lot de INTEGRAL SYSTEM ENTÉROBATTÉRI est soumis au contrôle de qualité en utilisant les micro-organismes ATCC de référence .

Enterobacter cloacae……..	*ATCC 13047*	*Proteus mirabilis*……	*ATCC 25933*
Escherichia coli.....….....	*ATCC 25922*	*Pseudomonas aeruginosa*……...	*ATCC 27853*
Klebsiella pneumoniae……	*ATCC 13883*	*Salmonella typhimurium*……..	*ATCC 14028*

FACTEURS POUVANT INVALIDER LES RÉSULTATS

Standardisation incorrecte de l'inoculum ; application de la méthode à des micro-organismes n'appartenant pas au groupe des Entérobactéries ; cultures mixtes ou polluées ; utilisation de systèmes ou de réactifs après leur date limite d'utilisation ; application incorrecte de la technique d'utilisation.

IMITES ET AVERTISSEMENTS

L'identification de *Salmonella spp.* et de *Shigella spp* doit être confirmée en utilisant les antisérums appropriés. Pour l'identification définitive des micro-organismes, il peut être nécessaire, dans certains cas, de recourir à des tests supplémentaires de confirmation.

PERFORMANCES

Les résultats de l'identification bactérienne obtenus avec le système INTEGRAL SYSTEM ENTEROBATTERI concordent avec ceux des méthodes d'identification traditionnelles (Piccolomini el at.[(4)]).
Les résultats de l'antibiogramme obtenus avec le système INTEGRAL SYSTEM ENTEROBATTERI concordent avec ceux obtenus avec la méthode de diffusion radiale selon Bauer et al.[(5)] recommandée par la Food and Drug Administration (FDA) et par le National Committee for Clinical Laboratory Standards (NCCLS) U.S.A [(1,8)].

PRÉCAUTIONS

Le produit, INTEGRAL SYSTEM ENTÉROBATTERI, n'est pas classé comme dangereux aux termes de la législation en vigueur, ni ne contient de substances nocives dans des concentrations ≥ 1 %, il ne requiert donc pas la disponibilité de la Fiche de données de sécurité. INTEGRAL SYSTEM ENTÉROBATTERI est un dispositif à usage unique, il est destiné exclusivement à un usage diagnostique *in vitro* et à un usage professionnel ; il doit être utilisé en laboratoire par des opérateurs correctement formés, avec des méthodes approuvées d'asepsie et de sécurité à l'égard des agents pathogènes.

CONSERVATION

Conserver à 2-8°C dans son emballage d'origine. Ne pas conserver à proximité de sources de chaleur et éviter toute variation excessive de température. Dans ces conditions, le produit est valable jusqu'à la date limite d'utilisation indiquée sur l'étiquette. Ne pas utiliser au-delà de cette date. Éliminer en présence de signes de détérioration.

ÉLIMINATION DU MATÉRIEL UTILISÉ

Après utilisation, INTEGRAL SYSTEM ENTÉROBATTERI et le matériel ayant été au contact de l'échantillon doivent être décontaminés et éliminés conformément aux techniques utilisées en laboratoire pour la décontamination et l'élimination de matériel potentiellement infecté.

PRÉSENTATION

Produit	Code	Emballage
INTEGRAL SYSTEM ENTÉROBATTERI	71714	20 *tests*

TABLEAU DES SYMBOLES

SYMBOLE	SIGNIFICATION	SYMBOLE	SIGNIFICATION
IVD	Dispositif médical diagnostic *in vitro*		Ne pas réutiliser
	Fabricant		Contenu suffisant pour <n> tests
REF	Numéro de catalogue		Fragile, manipuler avec soin
	Utiliser avant		Attention, voir les instructions pour l'utilisation
	Limites de température	LOT	Code du lot

CE IVD

ESPAÑOL

INTEGRAL SYSTEM ENTEROBATTERI

Sistema colorimétrico para la identificación bioquímica y el antibiograma de las enterobacterias

DESCRIPCIÓN

INTEGRAL SYSTEM ENTEROBATTERI es un sistema de 24 pozos que contienen substratos bioquímicos y antibióticos desecados para la identificación bioquímica y el antibiograma de las enterobacterias.
El sistema se inocula con la suspensión bacteriana del microorganismo en examen e incubado a 36 °C ± 1°C por 18-24 horas. Los tests para la identificación y para el antibiograma se interpretan evaluando el viraje de color de los distintos pozos del sistema.

CONTENIDO DE LOS ESTUCHES

El estuche contiene:
- 20 Sistemas INTEGRAL SYSTEM ENTEROBATTERI
- 20 Ampollas de Suspension Medium (7.0 ml/ampolla)
- 20 Oxidase Disc
- 1 Hoja instrucciones

PRODUCTOS NECESARIOS NO CONTENIDOS

- Aceite de vaselina para uso microbiológico (Vaseline oil 2 frascos de 50 ml).....cód. 80278
- Physiological Solution (20 ampollas de 7.0 ml/ampolla)…..cód. 20095
- Kovac's Reagent (2 frascos de 25 ml).....cód. 80270
- Material vario para laboratorio de microbiología

CONFIGURACIÓN

El sistema presenta la configuración indicada en la tabla n°1.

Tabla n°1

Pozo	***IDENTIFICACIÓN BIOQUÍMICA***
1-LDC	Descarboxilación de la lisina
2-ODC	Descarboxilación de la ornitina
3-H_2S	Producción de hidrógeno sulfurado
4-IND	Test del indol
5-LAC	Fermentación de la lactosa
6-DUL	Fermentación del dulcitol
7-PA	Desaminción de la fenilalanina
8-UR	Hidrólisis de la urea
9-CIT	Utilización del citrato
10-OX	Test de la oxidasa
Pozo	***ANTIBIOGRAMA (*)***
11-AK	Amikacina - 32 µg/ml
12-CN	Gentamicina - 8 µg/ml
13-TOB	Tobramicina - 8 µg/ml
14-TZP	Piperacillina+Tazobactam - 128/4 µg/ml
15-FOS	Fosfomicina - 200 µg/ml
16-CFP	Cefoperazona - 64 µg/ml
17-CTX	Cefotaxima - 64 µg/ml
18-CAZ	Ceftazidima - 32 µg/ml
19-AMS	Ampicillina + Sulbactam – 32/16 µg/ml
20-NA	Ácido Nalidixico - 32 µg/ml
21-CIP	Ciprofloxacina - 4 µg/ml
22-LEV	Levofloxacin - 8 µg/ml
23-SXT	Co-Trimoxazolo - 8 µg/ml
24-C	Control de crecimiento para el antibiograma

(*): La concentración de cada antibiótico es conforme a las normas actualizadas NCCLS-January 2004, Vol 24 N°1.[(1)]

PRINCIPIO DEL MÉTODO

INTEGRAL SYSTEM ENTEROBATTERI permite realizar simultáneamente la identificación bioquímica y el antibiograma de las enterobacterias aisladas de muestras clínicas.

- La identificación se basa en pruebas bioquímicas realizadas en terrenos de cultivo que contienen substratos específicos en los pozos de **1-LDC** a **10-OX**.
- El antibiograma se evalúa en base al crecimiento o a la inhibición de los microorganismos en terrenos que contienen el antibiótico y un indicador de crecimiento en los pozos de **11-AK** a **23-SXT**. El pozo **24-C** no contiene antibióticos, sino solamente el terreno de cultivo y el indicador y sirve como control del crecimiento microbiano para el antibiograma.

COMPOSICIÓN

Tabla n°2

Pozo	***Contenido***
1-LDC	Terreno para la evidenciación de la lisina descarboxilasa
2-ODC	Terreno para la evidenciación de la ornitina descarboxilasa
3-H_2S	Terreno para la evidenciación de la producción de hidrógeno sulfurado
4-IND	Terreno para la evidenciación del indol
5-LAC	Terreno para la evidenciación de la fermentación de la lactosa
6-DUL	Terreno para la evidenciación de la fermentación del dulcitol
7-PA	Terreno para la evidenciación de la fenilalanina desaminasa
8-UR	Terreno para la evidenciación de la hidrólisis de la urea
9-CIT	Terreno para la evidenciación de la utilización del citrato
10-OX	Terreno para la evidenciación de la oxidasa
11-AK	Terreno que contiene Amikacina - 32 µg/ml
12-CN	Terreno que contiene Gentamicina - 8 µg/ml
13-TOB	Terreno que contiene Tobramicina - 8 µg/ml
14-TZP	Terreno que contiene Piperacillina+Tazobactam - 128/4 µg/ml
15-FOS	Terreno que contiene Fosfomicina - 200 µg/ml
16-CFP	Terreno que contiene Cefoperazona - 64 µg/ml
17-CTX	Terreno que contiene Cefotaxima - 64 µg/ml
18-CAZ	Terreno que contiene Ceftazidima - 32 µg/ml
19-AMS	Terreno que contiene Ampicillina + Sulbactam - 32/16 µg/ml
2-NA	Terreno que contiene Ácido Nalidixico - 32 µg/ml
21-CIP	Terreno que contiene Ciprofloxacina - 4 µg/ml
22-LEV	Terreno que contiene Levofloxacin - 8 µg/ml
23-SXT	Terreno que contiene Co-Trimoxazolo - 8 µg/ml
24-C	Terreno sin antibióticos

Suspension Medium(g/l): *Extracto de levadura **5g**; Peptona de carne **3g**;Glucosa **2g**;Agua destilada **1000,0 ml** pH **6.8±0.2***

RECOLECCIÓN Y CONSERVACIÓN DE LAS MUESTRAS

Las colonias a someter al test de identificación bioquímica y al antibiograma con INTEGRAL SYSTEM ENTEROBATTERI se tienen que tomar de un terreno de cultivo, selectivo o no selectivo, utilizado para el aislamiento de las Enterobacterias.

PROCEDIMIENTO DEL TEST

1. Tomar un sistema del kit.

2. Tras asegurarse, con oportunas investigaciones, que las colonias desarrolladas en terrenos de cultivo pertenecen presuntivamente al grupo de los Enterobacterias, tomar una o más colonias morfológicamente similares, bien aisladas, de un terreno de cultivo sólido y ponerlas en suspensión en 5 ml de solución fisiológica para uso microbiológico a fin de obtener una turbidez equivalente a 0,5 McFarland **(Suspensión bacteriana).**

3. Transferir:
   - 0,2 ml de **Suspensión bacteriana** en los primeros 10 pozos y añadir a los pozos **1-LDC**, **2-ODC**, **3-H_2S** y **8-UR** 2 gotas de aceite de vaselina para uso microbiológico (IDENTIFICACIÓN BIOQUÍMICA).
   - 0,01 ml de **Suspensión bacteriana** en la ampolla de **Suspension Medium** contenido en le kit y distribuir 0,2 ml en los pozos de **11-AK** a **24-C**. (ANTIBIOGRAMA).

4. Cubrir el sistema con la tapa al efecto e incubar a 36 °C ± 1°C por 18-24 horas.

5. Después de la incubación, añadir:
   - 2 gotas de Kovac's Reagent al pozo **4-IND** (TEST DEL INDOL).
   - 1 disco de OXIDASE DISC al pozo **10-OX** (TEST DE LA OXIDASA).

INTERPRETACIÓN DE LOS RESULTADOS

IDENTIFICACIÓN BIOQUÍMICA

Interpretar los resultados de los primeros 10 pozos utilizando la tabla n°3 y formar el código numérico de 4 cifras siguiendo las instrucciones indicadas en el párrafo **FORMACIÓN DEL CÓDIGO NUMÉRICO**. Luego remontar a la identificación bacteriana sirviéndose del tabulado de los códigos numéricos (tabla n°5).

ANTIBIOGRAMA

Observar el viraje de color de los pozos de **11-AK** a **23-SXT** e interpretar los resultados sirviéndose de la tabla n°3 . El pozo de control (**24-C**) tiene que resultar positivo (amarillo). En caso de que resultara negativo (azul o gris), es necesario verificar la vitalidad de la inoculación, su correcta preparación y proceder a la repetición del test utilizando un nuevo sistema.

Tabla n°3

Pozo	**IDENTIFICACIÓN BIOQUÍMICA**	**Color pozo**	
		Reacción positiva	**Reacción negativa**
1-LDC	Descarboxilación de la lisina	Violeta	Amarillo-Marrón
2-ODC	Descarboxilación de la ornitina	Violeta	Amarillo-Marrón
3 -H_2S	Producción de hidrógeno sulfurado	Negro	Amarillo
4-IND	Test del indol	Anillo rosa-rojo	Amarillo
5-LAC	Fermentación de la lactosa	Amarillo	Azul-Verde
6-DUL	Fermentación del dulcitol	Amarillo	Azul-Verde
7-PA	Desaminación de la fenilalanina	Negro-Marrón	Amarillo
8-UR	Hidrólisis de la urea	Rojo-Fucsia	Amarillo-Anaranjado
9-CIT	Utilización del citrato	Azul-Verde oscuro	Verde
10-OX	Test de la oxidasa	Azul-Púrpura (reacción immediata)	Incoloro

ANTIBIOGRAMA		
COLOR POZO	**CRECIMIENTO BACTERIANO**	**INTERPRETACIÓN**
Azul	Inhibida	S = Sensible
Gris	Intermedia	I = Sensibilidad intermedia
Amarillo	Buena	R = Resistente

FORMACIÓN DEL CÓDIGO NUMÉRICO

1) Los 10 tests bioquímicos están divididos en 3 grupos que contienen 3 tests y un grupo que contiene 1 test, y cada uno es indicado por un valor de resultado positivo de 1,2,4.

- Valor 1: primer test positivo de cada grupo
- (LDC, IND, PA,OX)
- Valor 2: segundo test positivo de cada grupo
- (ODC,LAC,UR)
- Valor 4: tercer test positivo de cada grupo
- (H_2S, DUL,CIT)
- Valor cero: reacciones negativas de cada grupo

2) Añadiendo en cada grupo los números de las reacciones positivas, se obtiene un código de 4 cifras que, sirviéndose del tabulado de los códigos numéricos, permite identificar el microorganismo en examen, como en el ejemplo.

	Grupo I			*Grupo II*			*Grupo III*			*Grupo IV*
	LDC	**ODC**	**H_2S**	**IND**	**LAC**	**DUL**	**PA**	**UR**	**CIT**	**OX**
Codigo de resultado positivo	1	2	4	1	2	4	1	2	4	1
Resultados	+	+	-	+	+	-	-	-	-	-
Suma de los códigos	3			3			0			0
CÓDIGO NUMÉRICO: 3300				*IDENTIFICACIÓN: E.coli*						

ESQUEMA DE LAS REACCIONES BIOQUÍMICAS

Tabla n°4

Microorganismos	*Grupo I*			*Grupo II*			*Grupo III*			*Grupo IV*
	LDC	**ODC**	**H_2S**	**IND**	**LAC**	**DUL**	**PA**	**UR**	**CIT**	**OX**
E.coli	V	V	-	+	+	V	-	-	-	-
E.coli inactive	V	V	-	+	-	V	-	-	-	-
Shigella spp.	-	V	-	V	-	-	-	-	-	-
Edwardsiella spp.	+	+	+	+	-	-	-	-	-	-
Citrobacter spp.	-	V	+	V	V	V	-	V	V	-
Salmonella spp.	+	+	V	-	-	+*	-	-	V	-
Arizona spp.	+	+	+	V	V	-	-	-	+	-
K. pneumoniae	+	-	-	-	+	V	-	V	V	-
Klebsiella oxytoca	+	-	-	+	+	V	-	+	V	-
Enterobacter cloacae	V	+	-	-	V	-	-	V	+	-
E. aerogenes	+	+	-	-	+	-	-	-	+	-
Enterobacter hafnia	+	+	-	-	V	-	-	-	+	-
Serratia spp.	+	+	-	-	-	-	-	V	+	-
Proteus mirabilis	-	+	+	-	-	-	+	+	V	-
Proteus vulgaris	-	-	V	+	-	-	+	+	V	-
Morganella morganii	-	+	-	+	-	-	+	+	-	-
Providencia stuartii	-	-	-	+	-	-	+	-	+	-
Providencia rettgeri	-	-	-	+	-	-	+	+	+	-
Pseudomonas spp.	+	+	-	-	-	-	-	-	+	+

+ : Reacción Positiva - : Reacción Negativa V: Reacción Variable

*Dulcitol: Algunas cepas de Salmonella fermentan lentamente el dulcitol, por lo tanto inicialmente se pueden identificar como *Arizona spp*. Es necesario, pues, realizar una prueba de confirmación usando un antisuero polivalente para Salmonella.

TABULADO DE LOS CÓDIGOS NUMÉRICOS

Tabla n°5

Código	Microrganismo	Código	Microrganismo	Código	Microrganismo	Código	Microrganismo
0000	*Shigella spp.*	2240	*Enterobacter cloacae*	4220	*Citrobacter spp.*	6160	*Citrobacter spp.*
0100	*E.coli inactive* 45%	2260	*Enterobacter cloacae*	4240	*Citrobacter spp.*	6200	*Citrobacter spp.*
	Shigella spp. 55%	2300	*E.coli*	4260	*Citrobacter spp.*	6220	*Citrobacter spp.*
0130	*Proteus vulgaris*	2500	*E.coli inactive*	4300	*Citrobacter spp.*	6240	*Citrobacter spp.*
0150	*Providencia stuartii*	2700	*E.coli*	4320	*Citrobacter spp.*	6260	*Citrobacter spp.*
0170	*Proteus vulgaris* 0.5%	3040	*Enterobacter hafniae* 60%	4340	*Citrobacter spp.*	6300	*Citrobacter spp.*
	Providencia rettgeri 99.5%		*Serratia spp.* 37%	4360	*Citrobacter spp.*	6320	*Citrobacter spp.*
0300	*E.coli*		*Enterobacter cloacae* 3%	4400	*Citrobacter spp.*	6340	*Citrobacter spp.*
0500	*E.coli inactive*	3041	*Pseudomonas spp.*	4420	*Citrobacter spp.*	6360	*Citrobacter spp.*
0700	*E.coli*	3060	*Serratia spp.* 98.9 %	4440	*Citrobacter spp.*	6400	*Citrobacter spp.*
1100	*E.coli inactive*		*Enterobacter cloacae* 1.1%	4460	*Citrobacter spp.*	6420	*Citrobacter spp.*
1200	*Klebsiella pneumoniae*	3100	*E.coli inactive*	4500	*Citrobacter spp.*	6440	*Citrobacter spp.*
1220	*Klebsiella pneumoniae*	3240	*Enterobacter cloacae* 7.7%	4520	*Citrobacter spp.*	6460	*Citrobacter spp.*
1240	*Klebsiella pneumoniae*		*Enterobacter aerogenes* 75%	4540	*Citrobacter spp.*	6500	*Citrobacter spp.*
1260	*Klebsiella pneumoniae*		*Enterobacter hafniae* 17.3%	4560	*Citrobacter spp.*	6520	*Citrobacter spp.*
1300	*E.coli*	3260	*Enterobacter cloacae*	4600	*Citrobacter spp.*	6540	*Citrobacter spp.*
1320	*Klebsiella oxytoca*	3300	*E.coli*	4620	*Citrobacter spp.*	6560	*Citrobacter spp.*
1360	*Klebsiella oxytoca*	3400	*Salmonella spp.*	4640	*Citrobacter spp.*	6600	*Citrobacter spp.*
1500	*E.coli inactive*	3440	*Salmonella spp.*	4660	*Citrobacter spp.*	6620	*Citrobacter spp.*
1600	*Klebsiella pneumoniae*	3500	*E.coli inactive*	4700	*Citrobacter spp.*	6640	*Citrobacter spp.*
1620	*Klebsiella pneumoniae*	3700	*E.coli*	4720	*Citrobacter spp.*	6660	*Citrobacter spp.*
1640	*Klebsiella pneumoniae*	4000	*Citrobacter spp.*	4740	*Citrobacter spp.*	6700	*Citrobacter spp.*
1660	*Klebsiella pneumoniae*	4020	*Citrobacter spp.*	4760	*Citrobacter spp.*	6720	*Citrobacter spp.*
1700	*E.coli*	4040	*Citrobacter spp.*	6000	*Citrobacter spp.*	6740	*Citrobacter spp.*
1720	*Klebsiella oxytoca*	4060	*Citrobacter spp.*	6020	*Citrobacter spp.*	6760	*Citrobacter spp.*
1760	*Klebsiella oxytoca*	4100	*Citrobacter spp.*	6030	*Proteus mirabilis*	7040	*Arizona spp.*
2000	*Shigella spp.*	4120	*Citrobacter spp.*	6040	*Citrobacter spp.*	7100	*Edwardsiella spp.*
2040	*Enterobacter cloacae*	4130	*Citrobacter spp.*	6060	*Citrobacter spp.*	7140	*Arizona spp.*
2060	*Enterobacter cloacae*	4140	*Citrobacter spp.*	6070	*Proteus mirabilis*	7240	*Arizona spp.*
2100	*E.coli inactive* 44.6%	4160	*Citrobacter spp.*	6100	*Citrobacter spp.*	7340	*Arizona spp.*
2130	*Shigella spp.* 55.4%	4170	*Proteus vulgaris*	6120	*Citrobacter spp.*	7400	*Salmonella spp.*
	Morganella morganii	4200	*Citrobacter spp.*	6140	*Citrobacter spp.*	7440	*Salmonella spp.*

CONTROL CALIDAD

Cada lote de INTEGRAL SYSTEM ENTEROBATTERI se somete al control calidad utilizando los siguientes microorganismos ATCC de referencia.

Enterobacter cloacae…….. *ATCC 13047*
Escherichia coli..... *ATCC 25922*
Klebsiella pneumoniae…… *ATCC 13883*
Proteus mirabilis…… *ATCC 25933*
Pseudomonas aeruginosa……... *ATCC 27853*
Salmonella typhimurium…….. *ATCC 14028*

FACTORES QUE PUEDEN INVALIDAR LOS RESULTADOS

No correcta estandarización de la inoculación; aplicación del método a microorganismos no pertenecientes al grupo de las Enterobacterias; cultivos mixtos o contaminadas; uso de sistemas o de reactivos caducados; no correcta aplicación de la técnica de uso.

LÍMITES Y ADVERTENCIAS

La identificación de *Salmonella spp. y de Shigella spp* se tiene que confirmar utilizando antisueros adecuados. Para la identificación definitiva de los microorganismos en algunos casos puede ser necesario recurrir a tests suplementarios de confirmación.

PERFORMANCE

Los resultados de la identificación bacteriana obtenidos con el sistema INTEGRAL SYSTEM ENTEROBATTERI concuerdan con los de los métodos de identificación tradicionales (Piccolomini et al.[4]).
Los resultados del antibiograma obtenidos con el sistema INTEGRAL SYSTEM ENTEROBATTERI concuerdan con los obtenidos con el método de difusión radial según Bauer et al.[5] recomendado por Food and Drug Administration (FDA) y por National Committee for Clinical Laboratory Standards (NCCLS) U.S.A [1,8].

PRECAUCIONES

El producto, INTEGRAL SYSTEM ENTEROBATTERI, no se clasifica como peligroso según la legislación vigente ni contiene sustancias nocivas en concentraciones ≥1%, por lo tanto no requiere la disponibilidad de la Ficha de Seguridad. INTEGRAL SYSTEM ENTEROBATTERI es un dispositivo desechable a utilizar sólo para uso diagnóstico *in vitro*, está destinado a un ámbito profesional y tiene que ser usado en laboratorio por operadores adecuadamente formados, con métodos aprobados de asepsia y seguridad con respecto a los agentes patógenos.

CONSERVACIÓN

Conservar a 2-8°C en su estuche original. No conservar cerca de fuentes de calor y evitar excesivas variaciones de temperatura. En estas condiciones el producto es válido hasta la fecha de caducidad indicada en la etiqueta. No utilizar después de esta fecha. Eliminar si hay signos de deterioro.

ELIMINACIÓN DEL MATERIAL UTILIZADO

Después de la utilización INTEGRAL SYSTEM ENTEROBATTERI y el material que ha entrado en contacto con la muestra tienen que ser descontaminados y eliminados de acuerdo con las técnicas en uso en laboratorio para la descontaminación y la eliminación de material potencialmente infecto.

PRESENTACIÓN

Producto	Código	Estuche
INTEGRAL SYSTEM ENTEROBATTERI	71714	20 *tests*

TABLA DE LOS SÍMBOLOS

SÍMBOLO	SIGNIFICADO	SÍMBOLO	SIGNIFICADO
IVD	Producto sanitario para diagnóstico *in vitro*		No reutilizar
	Fabricante		Contenido suficiente para <n> ensayos
REF	Referência de catálogo		Frágil, manipular con precaución
	Fecha de caducidad		Atencion, ver instrucciones de uso
	Limite de temperatura	LOT	Código de lote

CE IVD

INTEGRAL SYSTEM ENTEROBATTERI

Sistema calorimetrico para a identificação bioquímica e o antibiograma das enterobactérias

DESCRIÇÃO

INTEGRAL SYSTEM ENTEROBATTERI é um sistema com 24 cavidades que contêm substratos bioquímicos e antibióticos exsicados para a identificação bioquímica e o antibiograma das enterobactérias.
O sistema é inoculado com a suspensão bactérica do microrganismo em exame e incubado a 36 °C ± 1°C por 18-24 horas. Os testes para a identificação e para o antibiograma são interpretados avaliando a viragem da cor das várias cavidades do sistema.

CONTEÚDO DAS CONFECÇÕES

A confecção contém:
· 20 Sistemas INTEGRAL SYSTEM ENTEROBATTERI
· 20 Ampolas de Suspension Medium (7.0 ml/ampola)
· 20 Oxidase Disc
· 1 Folha de instruções

PRODUTOS NECESSÁRIOS NÃO CONTIDOS

- Óleo de vaselina para uso microbiológico (Vaseline oil 2 frascos de 50 ml).....cód. 80278
- Physiological Solution (20 ampolas de 7.0 ml/ampola).....cód. 20095
- Kovac's Reagent (2 frascos de 25 ml).....cód. 80270
- Material vário para laboratório de microbiologia

CONFIGURAÇÃO

O sistema apresenta a configuração indicada na tabela n°1.

Tabela n°1

Placa multi-cavidades	***IDENTIFICAÇÃO BIOQUÍMICA***
1-LDC	Decarboxilação da lisina
2-ODC	Decarboxilação da ornitina
3-H_2S	Produção de hidrogeno sulfatado
4-IND	Teste indologenéo
5-LAC	Fermentação da lactose
6-DUL	Fermentação do dulcitol
7-PA	Desaminação da fenilalanina
8-UR	Hidrólise da ureia
9-CIT	Utilização do citrato
10-OX	Teste da oxidase
Placa multi-cavidades	***ANTIBIOGRAMA* (*)**
11-AK	Amikacina - 32 µg/ml
12-CN	Gentamicina - 8 µg/ml
13-TOB	Tobramicina - 8 µg/ml
14-TZP	Piperacilina+Tazobactam - 128/4 µg/ml
15-FOS	Fosfomicina - 200 µg/ml
16-CFP	Cefoperazona - 64 µg/ml
17-CTX	Cefotaxima - 64 µg/ml
18-CAZ	Ceftazidima - 32 µg/ml
19-AMS	Ampiciline + Sulbactam – 32/16 µg/ml
20-NA	Acido Nalidíxico - 32 µg/ml
21-CIP	Ciprofloxacino - 4 µg/ml
22-LEV	Levofloxacin - 8 µg/ml
23-SXT	Cotrimoxazol - 8 µg/ml
24-C	Controle do crescimento por antibiograma

(*): A concentração de cada antibiótico é conforme as normas actualizadas NCCLS-January 2004, Vol 24 N°1.[(1)]

PRINCÍPIO DO MÉTODO

INTEGRAL SYSTEM ENTEROBATTERI permite de realizar simultaneamente a identificação bioquímica e o antibiograma das enterobactérias isoladas das amostras clínicas.

- A identificação se baseia em provas bioquímicas realizadas em terrenos culturais que contêm substratos específicos nas cavidades de **1-LDC** a **10-OX**.
- O antibiograma é avaliado em base ao crescimento ou a inibição dos microrganismos em terrenos que contêm o antibiótico e um indicador de crescimento nas cavidades de **11-AK** a **23-SXT**. A cavidade ***24-C*** não contém antibióticos, mas, somente o terreno cultural e o indicador e serve como controlo do crescimento microbial para o antibiograma.

COMPOSIÇÃO

Tabela n°2

Placa multi-cavidades	***Conteúdo***
1-LDC	Terreno para a evidencia da lisina decarboxilase
2-ODC	Terreno para a evidencia da ornitina decarboxilase
3-H_2S	Terreno para a evidencia de produção de hidrogeno sulfatado
4-IND	Terreno para a evidencia de indologéneo
5-LAC	Terreno para a evidencia da fermentação da lactose
6-DUL	Terreno para a evidencia da fermentação do dulcitol
7-PA	Terreno para a evidencia da fenilalanina desaminase
8-UR	Terreno para a evidencia da hidrolise da ureia
9-CIT	Terreno para a evidencia da utilização do citrato
10-OX	Terreno para a evidencia da oxidase
11-AK	Terreno que contém Amikacina - 32 µg/ml
12-CN	Terreno que contém Gentamicina - 8 µg/ml
13-TOB	Terreno que contém Tobramicina - 8 µg/ml
14-TZP	Terreno que contém Piperacilina+Tazobactam - 128/4 µg/ml
15-FOS	Terreno que contém Fosfomicina - 200 µg/ml
16-CFP	Terreno que contém Cefoperazona - 64 µg/ml
17-CTX	Terreno que contém Cefotaxima - 64 µg/ml
18-CAZ	Terreno que contém Ceftazidima - 32 µg/ml
19-AMS	Terreno que contém Ampiciline + Sulbactam - 32/16 µg/ml
2-NA	Terreno que contém Ácido Nalidixico - 32 µg/ml
21-CIP	Terreno que contém Ciprofloxacino - 4 µg/ml
22-LEV	Terreno que contém Levofloxacin - 8 µg/ml
23-SXT	Terreno que contém Cotrimoxazol - 8 µg/ml
24-C	Terreno sem antibióticos

Suspension Medium (g/l): *Extracto de fermento **5g**;Peptona de carne **3g**;Glicose **2g**;Água destilada **1000.0 ml***
*pH **6.8±0.2***

RECOLHIMENTO E CONSERVAÇÃO DAS AMOSTRAS

As colónias que devem ser submetidas ao teste de identificação bioquímica e ao antibiograma com INTEGRAL SYSTEM ENTEROBATTERI devem ser retomados de um terreno cultural, selectivo ou não selectivo, utilizado para o isolamento das Enterobactérias.

PROCEDIMENTO DO TESTE

1. Pegar um sistema do kit.
2. Depois de ter-se certificado com adequadas pesquisas que as colónias desenvolvidas nos terrenos culturais presumivelmente pertencem ao grupo das Enterobactérias, pegar uma ou mais colónias morfologicamente semelhantes, bem isoladas, de um terreno de cultura sólido e suspende-las em 5 ml de solução fisiológica para uso microbiológico de modo a obter uma aparência túrbida equivalente a 0,5 McFarland **(Suspensão bactérica)**.
3. Passar:
   - 0,2 ml de **Suspensão bactérica** nas primeiras 10 cavidades e adicionar nas cavidades **1-LDC**, **2-ODC**, **3-H_2S** e **8-UR** 2 gotas de óleo de vaselina para uso microbiológico (IDENTIFICAÇÃO BIOQUÍMICA).
   - 0,01 ml de **Suspensão bactérica** na ampola de **Suspension Medium** contido do kit e distribuir 0,2 ml nas cavidades de **11-AK** a **24-C**. (ANTIBIOGRAMA).
4. Cobrir o sistema com a adequada tampa e incubar a 36 °C ± 1°C por 18-24 horas.
5. Depois da incubação, adicionar:
   - 2 gotas de Kovac's Reagent na cavidade **4-IND** (TESTE INDOLOGÉNEO).
   - 1 disco de OXIDASE DISC na cavidade **10-OX** (TESTE DA OXIDASE).

INTERPRETAÇÃO DOS RESULTADOS

IDENTIFICAÇÃO BIOQUÍMICA

Interpretar os resultados das primeiras 10 cavidades utilizando a tabela n°3 e formar o código numérico de 4 dígitos seguindo as instruções indicadas ao parágrafo **FORMAÇÃO DO CÓDIGO NUMÉRICO**. Portanto, efectuar a identificação bactérica servindo-se da tabela dos códigos numéricos (tabela n° 5).

ANTIBIOGRAMA

Observar a viragem da cor das cavidades de **11-AK** a **23-SXT** e interpretar os resultados servindo-se da tabela n°3. A cavidade de controlo (**24-C**) deve resultar positiva (amarelo). No caso em que resultasse negativa (azul ou cinzento), é necessário verificar a vitalidade do inoculo, a sua correcta preparação e proceder com a repetição do teste utilizando um novo sistema.

Tabela n°3

Placa multi-cavidades	**IDENTIFICAÇÃO BIOQUÍMICA**	**Cor da placa multi-cavidades**	
		Reacção positiva	**Reacção negativa**
1-LDC	Decarboxilação da lisina	Viola	Amarelo-Marrom
2-ODC	Decarboxilação da ornitina	Viola	Amarelo-Marrom
3 -H_2S	Produção de Hidrogeno sulfatado	Preto	Amarelo
4-IND	Teste indologéneo	Anel cor-de-rosa/vermelho	Amarelo
5-LAC	Fermentação da lactose	Amarelo	Azul-Verde
6-DUL	Fermentação do dulcitol	Amarelo	Azul-Verde
7-PA	Desaminação da fenilalanina	Preto-Marrom	Amarelo
8-UR	Hidrolise da ureia	Vermelho-púrpura	Amarelo/Cor-de-laranja
9-CIT	Utilização do citrato	Azul-Verde escuro	Verde
10-OX	Teste da oxidase	Azul-turquesa (reação imediata)	Incolor

ANTIBIOGRAMA		
COR DA PLACA MULTI-CAVIDADES	**CRESCIMENTO BACTÉRICO**	**INTERPRETAÇÃO**
Azul	Inibido	S = Sensível
Cinzento	Intermédio	I = Sensibilidade intermédia
Amarelo	Bom	R = Resistente

FORMAÇÃO DO CÓDIGO NUMÉRICO

1) Os 10 testes bioquímicos são divididos em 3 grupos que contêm 3 testes e um grupo que contêm 1 teste, cada um é indicado de um valor de positividade de 1,2,4.

- Valor 1: primeiro teste positivo de cada grupo
- (LDC, IND, PA,OX)
- Valor 2: segundo teste positivo de cada grupo
- (ODC,LAC,UR)
- Valor 4: terceiro teste positivo de cada grupo
- (H_2S, DUL,CIT)
- Valor zero: reacções negativas de cada grupo

2) Ao adicionar em cada grupo os números das reacções positivas, se obtém um código com 4 dígitos que, servindo-se da tabela dos códigos numéricos, permite de identificar o microrganismo em exame, como no exemplo.

	Grupo I			*Grupo II*			*Grupo III*			*Grupo IV*
	LDC	**ODC**	**H_2S**	**IND**	**LAC**	**DUL**	**PA**	**UR**	**CIT**	**OX**
Código de positividade	1	2	4	1	2	4	1	2	4	1
Resultados	+	+	-	+	+	-	-	-	-	-
Soma dos códigos	3			3			0			0
CÓDIGO NUMÉRICO: 3300				*IDENTIFICAÇÃO: E.coli*						

ESQUEMA DAS REACÇÕES BIOQUÍMICAS

Tabela n° 4

Microrganismos	*Grupo I*			*Grupo II*			*Grupo III*			*Grupo IV*
	LDC	**ODC**	**H_2S**	**IND**	**LAC**	**DUL**	**PA**	**UR**	**CIT**	**OX**
E. coli	V	V	-	+	+	V	-	-	-	-
E. coli inactive	V	V	-	+	-	V	-	-	-	-
Shigella spp.	-	V	-	V	-	-	-	-	-	-
Edwardsiella spp.	+	+	+	+	-	-	-	-	-	-
Citrobacter spp.	-	V	+	V	V	V	-	V	V	-
Salmonela spp.	+	+	V	-	-	+*	-	-	V	-
Arizona spp.	+	+	+	V	V	-	-	-	+	-
K. pneumoniae	+	-	-	-	+	V	-	V	V	-
Klebsiella oxytoca	+	-	-	+	+	V	-	+	V	-
Enterobacter cloacae	V	+	-	-	V	-	-	V	+	-
E. aerogenes	+	+	-	-	+	-	-	-	+	-
Enterobacter hafnia	+	+	-	-	V	-	-	-	+	-
Serratia spp.	+	+	-	-	-	-	-	V	+	-
Proteus mirabilis	-	+	+	-	-	-	+	+	V	-
Proteus vulgaris	-	-	V	+	-	-	+	+	V	-
Morganella morganii	-	+	-	+	-	-	+	+	-	-
Providencia stuartii	-	-	-	+	-	-	+	-	+	-
Providencia rettgeri	-	-	-	+	-	-	+	+	+	-
Pseudomonas spp.	+	+	-	-	-	-	-	-	+	+

+ : Reacção Positiva - : Reacção Negativa V: Reacção Variável

*****Dulcitol: Algumas estirpes de Salmonela fermentam lentamente o dulcitol, portanto, podem ser inicialmente identificados como *Arizona spp*. É necessário realizar uma prova de confirmação utilizando um anti-soro polivalente para a Salmonela.

TABELA DOS CÓDIGOS NUMÉRICOS

Tabela n° 5

Código	Microorganisme	Código	Microorganisme	Código	Microorganisme	Código	Microorganisme
0000	*Shigella spp.*	2240	*Enterobacter cloacae*	4220	*Citrobacter spp.*	6160	*Citrobacter spp.*
0100	*E.coli inactive* 45%	2260	*Enterobacter cloacae*	4240	*Citrobacter spp.*	6200	*Citrobacter spp.*
	Shigella spp. 55%	2300	*E.coli*	4260	*Citrobacter spp.*	6220	*Citrobacter spp.*
0130	*Proteus vulgaris*	2500	*E.coli inactive*	4300	*Citrobacter spp.*	6240	*Citrobacter spp.*
0150	*Providencia stuartii*	2700	*E.coli*	4320	*Citrobacter spp.*	6260	*Citrobacter spp.*
0170	*Proteus vulgaris* 0.5%	3040	*Enterobacter hafniae* 60%	4340	*Citrobacter spp.*	6300	*Citrobacter spp.*
	Providencia rettgeri 99.5%		*Serratia spp.* 37%	4360	*Citrobacter spp.*	6320	*Citrobacter spp.*
0300	*E.coli*		*Enterobacter cloacae* 3%	4400	*Citrobacter spp.*	6340	*Citrobacter spp.*
0500	*E.coli inactive*	3041	*Pseudomonas spp.*	4420	*Citrobacter spp.*	6360	*Citrobacter spp.*
0700	*E.coli*	3060	*Serratia spp.* 98.9 %	4440	*Citrobacter spp.*	6400	*Citrobacter spp.*
1100	*E.coli inactive*		*Enterobacter cloacae* 1.1%	4460	*Citrobacter spp.*	6420	*Citrobacter spp.*
1200	*Klebsiella pneumoniae*	3100	*E.coli inactive*	4500	*Citrobacter spp.*	6440	*Citrobacter spp.*
1220	*Klebsiella pneumoniae*	3240	*Enterobacter cloacae* 7.7%	4520	*Citrobacter spp.*	6460	*Citrobacter spp.*
1240	*Klebsiella pneumoniae*		*Enterobacter aerogenes* 75%	4540	*Citrobacter spp.*	6500	*Citrobacter spp.*
1260	*Klebsiella pneumoniae*		*Enterobacter hafniae* 17.3%	4560	*Citrobacter spp.*	6520	*Citrobacter spp.*
1300	*E.coli*	3260	*Enterobacter cloacae*	4600	*Citrobacter spp.*	6540	*Citrobacter spp.*
1320	*Klebsiella oxytoca*	3300	*E.coli*	4620	*Citrobacter spp.*	6560	*Citrobacter spp.*
1360	*Klebsiella oxytoca*	3400	*Salmonella spp.*	4640	*Citrobacter spp.*	6600	*Citrobacter spp.*
1500	*E.coli inactive*	3440	*Salmonella spp.*	4660	*Citrobacter spp.*	6620	*Citrobacter spp.*
1600	*Klebsiella pneumoniae*	3500	*E.coli inactive*	4700	*Citrobacter spp.*	6640	*Citrobacter spp.*
1620	*Klebsiella pneumoniae*	3700	*E.coli*	4720	*Citrobacter spp.*	6660	*Citrobacter spp.*
1640	*Klebsiella pneumoniae*	4000	*Citrobacter spp.*	4740	*Citrobacter spp.*	6700	*Citrobacter spp.*
1660	*Klebsiella pneumoniae*	4020	*Citrobacter spp.*	4760	*Citrobacter spp.*	6720	*Citrobacter spp.*
1700	*E.coli*	4040	*Citrobacter spp.*	6000	*Citrobacter spp.*	6740	*Citrobacter spp.*
1720	*Klebsiella oxytoca*	4060	*Citrobacter spp.*	6020	*Citrobacter spp.*	6760	*Citrobacter spp.*
1760	*Klebsiella oxytoca*	4100	*Citrobacter spp.*	6030	*Proteus mirabilis*	7040	*Arizona spp.*
2000	*Shigella spp.*	4120	*Citrobacter spp.*	6040	*Citrobacter spp.*	7100	*Edwardsiella spp.*
2040	*Enterobacter cloacae*	4130	*Citrobacter spp.*	6060	*Citrobacter spp.*	7140	*Arizona spp.*
2060	*Enterobacter cloacae*	4140	*Citrobacter spp.*	6070	*Proteus mirabilis*	7240	*Arizona spp.*
2100	*E.coli inactive* 44.6%	4160	*Citrobacter spp.*	6100	*Citrobacter spp.*	7340	*Arizona spp.*
2130	*Shigella spp.* 55.4%	4170	*Proteus vulgaris*	6120	*Citrobacter spp.*	7400	*Salmonella spp.*
	Morganella morganii	4200	*Citrobacter spp.*	6140	*Citrobacter spp.*	7440	*Salmonella spp.*

CONTROLO DA QUALIDADE

Cada lote de INTEGRAL SYSTEM ENTEROBATTÉRI é submetido ao controlo de qualidade utilizando os microrganismos ATCC de referência.

Enterobacter cloacae……..... *ATCC 13047*
Escherichia coli.....….. *ATCC 25922*
Klebsiella pneumoniae……..... *ATCC 13883*
Proteus mirabilis……..... *ATCC 25933*
Pseudomonas aeruginosa……..... *ATCC 27853*
Salmonella typhimurium……..... *ATCC 14028*

FACTORES QUE PODEM INVALIDAR OS RESULTADOS

Não correcta padronização do inoculo; aplicação do método em microrganismos que não pertence ao grupo das Enterobactérias; culturas mistas ou poluídas; uso de sistemas ou de reagentes vencidos; não correcta aplicação da técnica de uso.

LIMITES E ADVERTÊNCIAS

A identificação de *Salmonela spp. e de Shigella spp* deve ser confirmada utilizando anti-soros adequados. Para a identificação definitiva dos microrganismos em alguns casos pode ser necessário recorrer aos testes suplementares de confirmação.

PERFORMANCE

Os resultados da identificação bactérica obtidos com o sistema INTEGRAL SYSTEM ENTEROBATTERI concordam com aqueles dos métodos de identificação tradicionais (Piccolomini et al [4])
Os resultados do antibiograma obtidos com o sistema INTEGRAL SYSTEM ENTEROBATTERI concordam com aqueles obtidos com o método de difusão radial conforme Bauer et al.[5] recomendado pela Food and Drug Administration (FDA) e pela National Committee for Clinical Laboratory Standards (NCCLS) U.S.A [1,8].

PRECAUÇÕES

O produto, INTEGRAL SYSTEM ENTEROBATTÉRI, não pode ser classificado como perigoso nos termos da legislação em vigor, nem contém substâncias nocivas em concentrações ≥1%, portanto, não necessita da disponibilidade da Ficha de Segurança. O INTEGRAL SYSTEM ENTEROBATTERI é um dispositivo de uso único que deve ser utilizado somente para uso diagnóstico *in vitro*, é destinado a um âmbito profissional e deve ser utilizado em laboratório por operadores adequadamente treinados, com métodos aprovados de assepsia e de segurança nos confrontos dos agentes patogénicos.

CONSERVAÇÃO

Conserve a 2-8°C na sua confecção original. Não conserve próximo a fontes de calor e evite excessivas variações de temperatura. Nestas condições o produto é válido até a data de vencimento indicada na etiqueta. Não utilize além desta data. Elimine caso sejam presentes sinais de deterioração.

ELIMINAÇÃO DO MATERIAL UTILIZADO

Depois de utilizar o INTEGRAL SYSTEM ENTEROBATTERI e o material que entrou em contacto com a amostra devem ser descontaminados e eliminados em acordo com as técnicas em uso em laboratório para a descontaminação e a eliminação de material potencialmente infecto.

APRESENTAÇÃO

Produto	Código	Confecção
INTEGRAL SYSTEM ENTEROBATTERI	71714	20 *tests*

TABELA DOS SÍMBOLOS

SÍMBOLO	SIGNIFICADO	SÍMBOLO	SIGNIFICADO
IVD	Dispositivo médico para diagnóstico *in vitro*		Não reutilizar
	Fabricante	Σ	Conteúdo suficiente para “n” ensaios
REF	Referência de catálogo		Frágil, manusear com cuidado
	Prazo de validade		Atenção, consulte a documentação incluída
	Limites de temperatura	LOT	Código do lote

CE IVD

ΕΛΛΗΝΙΚΑ

INTEGRAL SYSTEM ENTEROBATTERI

Χρωματικό σύστημα για τον βιοχημικό προσδιορισμό και το αντιβιόγραμμα των εντεροβακτηριδίων

ΠΕΡΙΓΡΑΦΗ

Το INTEGRAL SYSTEM ENTEROBATTERI είναι ένα σύστημα με 24 υποδοχείς που περιέχουν βιοχημικά υποστρώματα και ξηραμένα αντιβιοτικά για τον βιοχημικό προσδιορισμό και το αντιβιόγραμμα των εντεροβακτηριδίων. Το σύστημα εμβολιάζεται με εναιώρημα βακτηριδίων του εξεταζόμενου μικροοργανισμού και επωάζεται στους 36°C. ± 1°C για 18-24 ώρες. Τα τεστ προσδιορισμού και για αντιβιογράμματα ερμηνεύονται αξιολογώντας την μεταβολή χρώματος των διαφόρων υποδοχέων στο σύστημα.

ΠΕΡΙΕΧΟΜΕΝΟ ΤΗΣ ΣΥΣΚΕΥΑΣΙΑΣ

Η συσκευασία περιλαμβάνει:
- 20 Συστήματα INTEGRAL SYSTEM ENTEROBATTERI
- 20 Φιάλη Suspension Medium (7.0 ml/φιάλη)
- 20 Oxidase Disc
- 1 Φύλλο οδηγιών

ΑΠΑΡΑΙΤΗΤΑ ΠΡΟΪΟΝΤΑ ΠΟΥ ΔΕΝ ΠΕΡΙΕΧΟΝΤΑΙ

- Λάδι βαζελίνης για μικροβιολογική χρήση (Vaseline oil 2 φιαλίδια των 50 ml).....κωδ. 80278
- Physiological Solution (20 φιάλη 7.0 ml/φιάλη).....κωδ. 20095
- Kovac's Reagent (2 φιάλες των 25 ml).....κωδ. 80270
- Υλικά διάφορα για το μικροβιολογικό εργαστήριο

ΔΙΑΜΟΡΦΩΣΗ

Η διαμόρφωση του συστήματος παρουσιάζεται στον πίνακα 1.

Πίνακας 1

Υποδοχέας	***ΒΙΟΧΗΜΙΚΟΣ ΠΡΟΣΔΙΟΡΙΣΜΟΣ***
1-LDC	Αποκαρβοξυλίωση λυσίνης
2-ODC	Αποκαρβοξυλίωση ορνιθίνης
3-H_2S	Παραγωγή θειώδους οξέως
4-IND	Τεστ ινδόλου
5-LAC	Ζύμωση λακτόζης
6-DUL	Ζύμωση γλυκιτόλης
7-PA	Απαμίνωση φαινυλαλανίνης
8-UR	Υδρόλυση ουρίας
9-CIT	Χρήση κιτρικού
10-OX	Τεστ οξειδάσης
Υποδοχέας	***ΑΝΤΙΒΙΟΓΡΑΜΜΑ* (*)**
11-AK	Αμικασίνη - 32 μg/ml
12-CN	Γενταμικίνη - 8 μg/ml
13-TOB	Τομπραμυκίνη - 8 μg/ml
14-TZP	Piperacillin+Tazobactam - 128/4 μg/ml
15-FOS	Fosfomicin - 200 μg/ml
16-CFP	Κεφοπεραζόνη - 64 μg/ml
17-CTX	Cefotaxim - 64 μg/ml
18-CAZ	Ceftazidim - 32 μg/ml
19-AMS	Ampicillin + Sulbactam – 32/16 μg/ml
20-NA	Ναλιδιξικό οξύ - 32 μg/ml
21-CIP	Σιπροφλοξακίνη - 4 μg/ml
22-LEV	Levofloxacin - 8 μg/ml
23-SXT	Co-Trimossazolo - 8 μg/ml
24-C	Έλεγχος ανάπτυξης για το αντιβιόγραμμα

(*):Η συγκέντρωση κάθε αντιβιοτικού συμμορφώνεται με τα ενημερωμένα πρότυπα NCCLS-Ιανουάριος 2004, Τόμ. 24 N°1.[1]

ΑΡΧΗ ΤΗΣ ΜΕΘΟΔΟΥ

Το INTEGRAL SYSTEM ENTEROBATTERI επιτρέπει την ταυτόχρονη εκτέλση βιοχημικού προσδιορισμού και αντιβιογράμματος των εντεροβακτηριδίων που έχουν απομονωθεί από κλινικά δείγματα.

- Ο προσδιορισμός βασίζεται σε βιοχημικές δοκιμές που έγιναν σε υλικά καλλιέργειας που περιείχαν ειδικά υποστρώματα στους υποδοχείς **1-LDC** έως **10-OX**.
- Το αντιβιόγραμμα αξιολογείται με βάση την ανάπτυξη ή την αναστολή των μικροοργανισμών σε υλικά που περιέχουν αντιβιοτικό και έναν δείκτη ανάπτυξης στους υποδοχείς **11-AK** έως **23-SXT**. Ο υποδοχέας **24-C** δεν περιέχει αντιβιοτικά, αλλά μόνο υλικό καλλιέργειας και ένα δείκτη ο οποίος χρησιμεύει ως έλεγχος της μικροβιακής ανάπτυξης για το αντιβιόγραμμα.

ΣΥΝΘΕΣΗ

Πίνακας 2

Υποδοχέας	***Περιεχόμενο***
1-LDC	Υπόστρωμα για τον τονισμό της λυσίνης δεκαρβοξυλάση
2-ODC	Υπόστρωμα για τον τονισμό αποκαρβοξυλάσης της ορνιθίνης
3-H_2S	Υλικό καλλιέργειας με υπόστρωμα για την παραγωγή και τον τονισμό θειώδους οξέως
4-IND	Υλικό καλλιέργειας με υπόστρωμα για το τεστ ινδόλου
5-LAC	Υλικό καλλιέργειας για τον τονισμό της ζύμωσης λακτόζης
6-DUL	Υλικό καλλιέργειας για τον τονισμό ζύμωσης γλυκιτόλης
7-PA	Υλικό καλλιέργειας για τον τονισμό απαμίνωσης φαινυλαλανίνης
8-UR	Υλικό καλλιέργειας για τον τονισμό υδρόλυσης της ουρίας
9-CIT	Υλικό καλλιέργειας για τον τονισμό της χρήσης κιτρικού
10-OX	Υλικό καλλιέργειας για τον τονισμό οξειδάσης
11-AK	Υπόστρωμα που περιέχει Αμικασίνη - 32 μg/ml
12-CN	Υπόστρωμα που περιέχει Γενταμικίνη - 8 μg/ml
13-TOB	Υπόστρωμα που περιέχει Τομπραμυκίνη - 8 μg/ml
14-TZP	Υπόστρωμα που περιέχει Piperacillin+Tazobactam - 128/4 μg/ml
15-FOS	Υπόστρωμα που περιέχει Fosfomicin - 200 μg/ml
16-CFP	Υπόστρωμα που περιέχει Κεφοπεραζόνη - 64 μg/ml
17-CTX	Υπόστρωμα που περιέχει Cefotaxim - 64 μg/ml
18-CAZ	Υπόστρωμα που περιέχει Ceftazidim - 32 μg/ml
19-AMS	Υπόστρωμα που περιέχει Ampicillina + Sulbactam - 32/16 μg/ml
2-NA	Υπόστρωμα που περιέχει Ναλιδιξικό οξύ - 32 μg/ml
21-CIP	Υπόστρωμα που περιέχει Σιπροφλοξακίνη - 4 μg/ml
22-LEV	Υπόστρωμα που περιέχει Levofloxacin - 8 μg/ml
23-SXT	Υπόστρωμα που περιέχει Co-Trimossazol - 8 μg/ml
24-C	Υπόστρωμα χωρίς αντιβιοτικά

Εναιώρημα (g/l): *Απόσταγμα ζύμης **5g**, Πεπτόνη κρέατος **3 g**, Γλυκόζη **2 g** , Απεσταγμένο νερό **1000.0 ml** , pH **6.8± 0.2***

ΣΥΛΛΟΓΗ ΚΑΙ ΔΙΑΤΗΡΗΣΗ ΔΕΙΓΜΑΤΩΝ

Οι αποικίες που θα υποστούν το τεστ βιοχημικού προσδιορισμού και το αντιβιόγραμμα με INTEGRAL SYSTEM ENTEROBATTERI πρέπει να παραλαμβάνονται από ένα επιλεκτικό ή μη επιλεκτικό υπόστρωμα καλλιέργειας, που χρησιμοποιείται για την απομόνωση των Εντεροβακτηριδίων.

ΔΙΑΔΙΚΑΣΙΑ ΤΕΣΤ

1. Παραλάβετε ένα σύστημα από το κιτ
2. Αφού βεβαιωθείτε με την κατάλληλη έρευνα ότι οι αποικίες που έχουν αναπτυχθεί στα υποστρώματα καλλιέργειας ανήκουν πιθανότατα στην ομάδα εντεροβακτηριδίων, παραλάβετε μία ή περισσότερες αποικίες παρόμοιες από μορφολογικής πλευράς, καλά απομονωμένες, από ένα στερεό υπόστρωμα καλλιέργειας και αραιώστε σε 5 ml φυσιολογικού διαλύματος για μικροβιλογική χρήση με τρόπο ώστε να επιτύχετε θολότητα ισοδύναμη με 0,5 McFarland **(Βακτηριακού εναιωρήματος)**.
3. Μεταφέρετε:
   - 0,2 ml **Βακτηριακού εναιωρήματος** στους 10 πρώτους υποδοχείς και προσθέστε στους υποδοχείς **1-LDC**, **2-ODC**, **3-H_2S** και **8-UR** 2 σταγόνες λαδιού βαζελίνης για μικροβιολογική χρήση (ΒΙΟΧΗΜΙΚΟΣ ΠΡΟΣΔΙΟΡΙΣΜΟΣ).
   - 0,01 ml **Βακτηριακού εναιωρήματος** στη φιάλη **Suspension Medium** και κατανέμετε 0,2 ml στους υποδοχείς από **11-AK** έως **24-C**. (ΑΝΤΙΒΙΟΓΡΑΜΜΑ).
4. Καλύψτε το σύστημα με το κατάλληλο καπάκι και επωάστε στους 36 °C ± 1°C για 18-24 ώρες.
5. Μετά την επώαση προσθέστε:
   - 2 σταγόνες Kovac's Reagent στον υποδοχέα **4-IND (**ΤΕΣΤ ΙΝΔΟΛΟΥ)
   - 1 δισκίο OXIDASE DISC στον υποδοχέα **10-OX** (ΤΕΣΤ ΟΞΕΙΔΑΣΗΣ)

ΕΡΜΗΝΕΙΑ ΤΩΝ ΑΠΟΤΕΛΕΣΜΑΤΩΝ

ΒΙΟΧΗΜΙΚΟΣ ΠΡΟΣΔΙΟΡΙΣΜΟΣ

Ερμηνεύστε τα αποτελέσματα των πρώτων 10 υποδοχέων χρησιμοποιώντας τον πίνακα αρ. 3 και σχηματίστε τον αριθμητικό κωδικό 4 ψηφίων ακολουθώντας τις οδηγίες που αναφέρονται εδώ στην παράγραφο **ΣΧΗΜΑΤΙΣΜΟΣ ΑΡΙΘΜΗΤΙΚΟΥ ΚΩΔΙΚΟΥ**. Κάντε λοιπόν τον βακτηριδιακό προσδιορισμό με τη βοήθεια της λίστας αριθμητικών κωδικών (πίνακας 5).

ΑΝΤΙΒΙΟΓΡΑΜΜΑ

Παρατηρήστε τη μεταβολή χρώματος στους υποδοχείς από **11-AK** έως **23-SXT** και ερμηνεύστε τα αποτελέσματα με τη βοήθεια του πίνακα 3 . Ο υποδοχέας ελέγχου (**24-C**) πρέπει να είναι θετικός (κίτρινος). Στην περίπτωση που παρουσιάζεται αρνητικός (μπλε ή γκρι), είναι αναγκαίο να γίνει έλεγχος της ζωτικότητας επώασης, της σωστής προετοιμασίας και να προχωρήσετε στην επανάληψη του τεστ χρησιμοποιώντας ένα καινούργιο σύστημα.

Πίνακας 3

Υποδοχέας	**ΒΙΟΧΗΜΙΚΟΣ ΠΡΟΣΔΙΟΡΙΣΜΟΣ**	**Χρώμα υποδοχέα**	
		Θετική αντίδραση	**Αρνητική αντίδραση**
1-LDC	Αποκαρβοξυλίωση λυσίνης	Μωβ	Κίτρινο-Καφέ
2-ODC	Αποκαρβοξυλίωση ορνιθίνης	Μωβ	Κίτρινο-Καφέ
3 -H_2S	Παραγωγή θειώδους οξέως	Μαύρο	Κίτρινο
4-IND	Τεστ ινδόλου	Δακτύλιος ροζ-κόκκινος	Κίτρινο
5-LAC	Ζύμωση λακτόζης	Κίτρινο	Μπλε-Πράσινο
6-DUL	Ζύμωση γλυκιτόλης	Κίτρινο	Μπλε-Πράσινο
7-PA	Απαμίνωση φαινυλαλανίνης	Μαύρο-Καφέ	Κίτρινο
8-UR	Υδρόλυση ουρίας	Κόκκινο-Φούξια	Κίτρινο-Πορτοκαλί
9-CIT	Χρήση κιτρικού	Μπλε-Πράσινο σκούρο	Πράσινο
10-OX	Τεστ οξειδάσης	Μπλε-Πορφυρό (άμεση αντίδραση)	Άχρωμο

ΑΝΤΙΒΙΟΓΡΑΜΜΑ		
ΧΡΏΜΑ ΥΠΟΔΟΧΈΑ	**ΒΑΚΤΗΡΙΑΚΗ ΑΝΑΠΤΥΞΗ**	**ΕΡΜΗΝΕΙΑ**
Μπλε	Αναστέλλεται	S = Ευαίσθητο
Γκρι	Ενδιάμεση	I = Ενδιάμεση ευαισθησία
Κίτρινο	καλή	R = Ανθεκτικό

ΣΧΗΜΑΤΙΣΜΟΣ ΑΡΙΘΜΗΤΙΚΟΥ ΚΩΔΙΚΟΥ

1) Τα 10 βιοχημικά τεστ χωρίζονται σε 3 ομάδες που περιέχουν 3 τεστ και μία ομάδα που περιέχει 1 τεστ, ενώ κάθε ένα από αυτά υποδεικνύονται με έναν αριθμό θετικότητας 1,2,4.

- Αξία 1: πρώτο θετικό τεστ κάθε ομάδας
- (LDC, IND, PA,OX)
- Αξία 2: δεύτερο θετικό τεστ κάθε ομάδας
- (ODC,LAC,UR)
- Αξία 4: τρίτο θετικό τεστ κάθε ομάδας
- (H_2S, DUL,CIT)
- Αξία μηδέν: αρνητικές αντιδράσεις κάθε ομάδας

2) Προσθέτωντας σε κάθε ομάδα τους αριθμούς των θετικών αντιδράσεων, θα έχουμε έναν κωδικό με 4 ψηφία ο οποίος, χρησιμοποιώντας τη λίστα αριθμητικών κωδικών, μας επιτρέπει να προσδιορίσουμε τον υπό εξέταση μικροοργανισμό όπως στο παράδειγμα.

	Ομάδα I			*Ομάδα II*			*Ομάδα III*			*Ομάδα IV*
	LDC	**ODC**	**H_2S**	**IND**	**LAC**	**DUL**	**PA**	**UR**	**CIT**	**OX**
Κωδικός θετικότητας	1	2	4	1	2	4	1	2	4	1
Αποτελέσματα	+	+	-	+	+	-	-	-	-	-
Άθροισμα κωδικών	3			3			0			0
ΑΡΙΘΜΗΤΙΚΟΣ ΚΩΔΙΚΟΣ: 3300				*ΠΡΟΣΔΙΟΡΙΣΜΟΣ: E.coli*						

ΣΧΗΜΑ ΒΙΟΧΗΜΙΚΩΝ ΑΝΤΙΔΡΑΣΕΩΝ

Πίνακας 4

Μικροοργανισμοί	*Ομάδα I*			*Ομάδα II*			*Ομάδα III*			*Ομάδα IV*
	LDC	**ODC**	**H_2S**	**IND**	**LAC**	**DUL**	**PA**	**UR**	**CIT**	**OX**
E.coli	V	V	-	+	+	V	-	-	-	-
E.coli inactive	V	V	-	+	-	V	-	-	-	-
Shigella spp.	-	V	-	V	-	-	-	-	-	-
Edwardsiella spp.	+	+	+	+	-	-	-	-	-	-
Citrobacter spp.	-	V	+	V	V	V	-	V	V	-
Salmonella spp.	+	+	V	-	-	+*	-	-	V	-
Arizona spp.	+	+	+	V	V	-	-	-	+	-
K. pneumoniae	+	-	-	-	+	V	-	V	V	-
Klebsiella oxytoca	+	-	-	+	+	V	-	+	V	-
Enterobacter cloacae	V	+	-	-	V	-	-	V	+	-
E. aerogenes	+	+	-	-	+	-	-	-	+	-
Enterobacter hafnia	+	+	-	-	V	-	-	-	+	-
Serratia spp.	+	+	-	-	-	-	-	V	+	-
Proteus mirabilis	-	+	+	-	-	-	+	+	V	-
Proteus vulgaris	-	-	V	+	-	-	+	+	V	-
Morganella morganii	-	+	-	+	-	-	+	+	-	-
Providencia stuartii	-	-	-	+	-	-	+	-	+	-
Providencia rettgeri	-	-	-	+	-	-	+	+	+	-
Pseudomonas spp.	+	+	-	-	-	-	-	-	+	+

+ : Θετική αντίδραση - : Αρνητική αντίδραση V: Αντίδραση μεταβλητή

*Γλυκιτόλη : Ορισμένοι βρόχοι Salmonella ζυμώνουν αργά τη γλυκιτόλη, ως εκ τούτου ενδέχεται αρχικά να προσδιοριστούν ως *Arizona spp*. Είναι λοιπόν αναγκαίο να γίνει μια δοκιμή επιβεβαίωσης χρησιμοποιώντας πολυδύναμο αντιορό για Salmonella.

ΛΙΣΤΑ ΑΡΙΘΜΗΤΙΚΩΝ ΚΩΔΙΚΩΝ

Πίνακας 5

Κωδικός	*Μικροοργανισμοί*	*Κωδικός*	*Μικροοργανισμοί*	*Κωδικός*	*Μικροοργανισμοί*	*Κωδικός*	*Μικροοργανισμοί*
0000	*Shigella spp.*	2240	*Enterobacter cloacae*	4220	*Citrobacter spp.*	6160	*Citrobacter spp.*
0100	*E.coli inactive* 45%	2260	*Enterobacter cloacae*	4240	*Citrobacter spp.*	6200	*Citrobacter spp.*
	Shigella spp. 55%	2300	*E.coli*	4260	*Citrobacter spp.*	6220	*Citrobacter spp.*
0130	*Proteus vulgaris*	2500	*E.coli inactive*	4300	*Citrobacter spp.*	6240	*Citrobacter spp.*
0150	*Providencia stuartii*	2700	*E.coli*	4320	*Citrobacter spp.*	6260	*Citrobacter spp.*
0170	*Proteus vulgaris* 0.5%	3040	*Enterobacter hafniae* 60%	4340	*Citrobacter spp.*	6300	*Citrobacter spp.*
	Providencia rettgeri 99.5%		*Serratia spp.* 37%	4360	*Citrobacter freundii*	6320	*Citrobacter spp.*
0300	*E.coli*		*Enterobacter cloacae* 3%	4400	*Citrobacter spp.*	6340	*Citrobacter spp.*
0500	*E.coli inactive*	3041	*Pseudomonas spp.*	4420	*Citrobacter spp.*	6360	*Citrobacter spp.*
0700	*E.coli*	3060	*Serratia spp.* 98.9 %	4440	*Citrobacter spp.*	6400	*Citrobacter spp.*
1100	*E.coli inactive*		*Enterobacter cloacae* 1.1%	4460	*Citrobacter spp.*	6420	*Citrobacter spp.*
1200	*Klebsiella pneumoniae*	3100	*E.coli inactive*	4500	*Citrobacter spp.*	6440	*Citrobacter spp.*
1220	*Klebsiella pneumoniae*	3240	*Enterobacter cloacae* 7.7%	4520	*Citrobacter spp.*	6460	*Citrobacter spp.*
1240	*Klebsiella pneumoniae*		*Enterobacter aerogenes* 75%	4540	*Citrobacter spp.*	6500	*Citrobacter spp.*
1260	*Klebsiella pneumoniae*		*Enterobacter hafniae* 17.3%	4560	*Citrobacter spp.*	6520	*Citrobacter spp.*
1300	*E.coli*	3260	*Enterobacter cloacae*	4600	*Citrobacter spp.*	6540	*Citrobacter spp.*
1320	*Klebsiella oxytoca*	3300	*E.coli*	4620	*Citrobacter spp.*	6560	*Citrobacter spp.*
1360	*Klebsiella oxytoca*	3400	*Salmonella spp.*	4640	*Citrobacter spp.*	6600	*Citrobacter spp.*
1500	*E.coli inactive*	3440	*Salmonella spp.*	4660	*Citrobacter spp.*	6620	*Citrobacter spp.*
1600	*Klebsiella pneumoniae*	3500	*E.coli inactive*	4700	*Citrobacter spp.*	6640	*Citrobacter spp.*
1620	*Klebsiella pneumoniae*	3700	*E.coli*	4720	*Citrobacter spp.*	6660	*Citrobacter spp.*
1640	*Klebsiella pneumoniae*	4000	*Citrobacter spp.*	4740	*Citrobacter spp.*	6700	*Citrobacter spp.*
1660	*Klebsiella pneumoniae*	4020	*Citrobacter spp.*	4760	*Citrobacter spp.*	6720	*Citrobacter spp.*
1700	*E.coli*	4040	*Citrobacter spp.*	6000	*Citrobacter spp.*	6740	*Citrobacter spp.*
1720	*Klebsiella oxytoca*	4060	*Citrobacter spp.*	6020	*Citrobacter spp.*	6760	*Citrobacter spp.*
1760	*Klebsiella oxytoca*	4100	*Citrobacter spp.*	6030	*Proteus mirabilis*	7040	*Arizona spp.*
2000	*Shigella spp.*	4120	*Citrobacter spp.*	6040	*Citrobacter spp.*	7100	*Edwardsiella spp.*
2040	*Enterobacter cloacae*	4130	*Proteus vulgaris*	6060	*Citrobacter spp.*	7140	*Arizona spp.*
2060	*Enterobacter cloacae*	4140	*Citrobacter spp.*	6070	*Proteus mirabilis*	7240	*Arizona spp.*
2100	*E.coli inactive* 44.6%	4160	*Citrobacter spp.*	6100	*Citrobacter spp.*	7340	*Arizona spp.*
	Shigella spp. 55.4%	4170	*Proteus vulgaris*	6120	*Citrobacter spp.*	7400	*Salmonella spp.*
2130	*Morganella morganii*	4200	*Citrobacter spp.*	6140	*Citrobacter spp.*	7440	*Salmonella spp.*

ΕΛΕΓΧΟΣ ΠΟΙΟΤΗΤΑΣ

Κάθε παρτίδα INTEGRAL SYSTEM ENTEROBATTERI υπόκειται σε έλεγχο ποιότητας χρησιμοποιώντας τους παρακάτω μικροοργανισμούς αναφοράς.

Enterobacter cloacae……..	*ATCC 13047*	*Proteus mirabilis*……	*ATCC 25933*
Escherichia coli.....….....	*ATCC 25922*	*Pseudomonas aeruginosa*……...	*ATCC 27853*
Klebsiella pneumoniae……	*ATCC 13883*	*Salmonella typhimurium*……..	*ATCC 14028*

ΠΑΡΑΓΟΝΤΕΣ ΠΟΥ ΕΝΔΕΧΕΤΑΙ ΝΑ ΑΚΥΡΩΣΟΥΝ ΤΟ ΑΠΟΤΕΛΕΣΜΑ

Όχι σωστή τυποποίηση επώασης, εφαρμογή της μέθοδος σε μικροοργανισμούς που δεν ανήκουν στην ομάδα των Εντεροβακτηριδίων, μικτές ή μολυσμένες καλλιέργειες, χρήση συστημάτων ή αντιδραστηρίων που έχουν λήξει, όχι σωστή εφαρμογή της τεχνικής χρήσης.

ΠΕΡΙΟΡΙΣΜΟΙ ΚΑΙ ΠΡΟΕΙΔΟΠΟΙΗΣΕΙΣ

Ο προσδιορισμός *Salmonella spp. και Shigella spp* πρέπει να επιβεβαιώνεται χρησιμοποιώντας τους κατάλληλους αντιορούς. Για τον οριστικό προσδιορισμό των μικροοργανισμών σε ορισμένες περιπτώσεις πρέπει να ανατρέξετε σε συμπληρωματικά τεστ επιβεβαίωσης.

ΑΠΟΔΟΣΗ

Τα αποτελέσματα βακτηριακού προσδιορισμού που επιτυγχάνονται με το σύστημα INTEGRAL SYSTEM ENTEROBATTERI συμφωνούν με τα αποτελέσματα των παραδοσιακών μεθόδων προσδιορισμού σε δοκιμαστικό σωλήνα (Piccolomini et al.[4])Τα αποτελέσματα αντιβιογράμματος που επιτυγχάνονται με το σύστημα INTEGRAL SYSTEM ENTEROBATTERI συμφωνούν με τα αποτελέσματα της μεθόδου ακτινικής διάχυσης κατά Bauer et al.[5] που συνιστάται από την Food and Drug Administration (FDA) και από την National Committee for Clinical Laboratory Standards (NCCLS) U.S.A [1,8].

ΠΡΟΦΥΛΑΞΕΙΣ

Το προϊόν INTEGRAL SYSTEM ENTEROBATTERI , δεν ταξινομείται ως επικίνδυνο σύμφωνα με την ισχύουσα νομοθεσία ούτε περιέχει βλαβερές ουσίες σε συγκεντρώσεις ≥1%, γι'αυτό δεν απαιτείται η διαθεσιμότητα της Κάρτας Ασφαλείας. Το INTEGRAL SYSTEM ENTEROBATTERI είναι μια συσκευή μιας χρήσης που πρέπει να χρησιμοποιείται μόνο για διαγνωστική χρήση *in vitro*, προορίζεται για επαγγελματική χρήση και πρέπει να χρησιμοποιείται στο εργαστήριο από κατάλληλα εκπαιδευμένο προσωπικό και με εγκεκριμένες ασηπτικές και ασφαλείς μεθόδους σε σχέση με τις παθογόνες ουσίες.

ΦΥΛΑΞΗ

Φυλάξτε το σε θερμοκρασία 2-8°C στην αρχική του συσκευασία. Δεν πρέπει να φυλάσσεται κοντά σε πηγές θερμότητας και πρέπει να αποφεύγονται οι μεταβολές θερμοκρασίας. Υπό αυτές τις συνθήκες το προϊόν ισχύει μέχρι την ημερομηνία λήξης που αναγράφεται στην ετικέτα. Μην το χρησιμοποιείτε πέραν αυτής της ημερομηνίας. Μην τα χρησιμοποιείτε εάν παρουσιάζουν σημεία αλλοίωσης.

ΑΠΟΡΡΙΨΗ ΤΟΥ ΧΡΗΣΙΜΟΠΟΙΗΜΕΝΟΥ ΥΛΙΚΟΥ

Μετά τη χρήση το INTEGRAL SYSTEM ENTEROBATTERI και τα υλικά που ήρθαν σε επαφή με το δείγμα πρέπει να απολυμαίνονται και να απορρίπτονται σύμφωνα με τις συνήθεις τεχνικές εργαστηρίου για την απολύμανση και την απόρριψη πιθανώς μολυσμένου υλικού.

ΠΑΡΟΥΣΙΑΣΗ

Προϊόν	**Κωδικός**	**Συσκευασία**
INTEGRAL SYSTEM ENTEROBATTERI	71714	20 *tests*

ΠΙΝΑΚΑΣ ΣΥΜΒΟΛΩΝ

ΣΥΜΒΟΛΟ	**ΣΗΜΑΣΙΑ**	**ΣΥΜΒΟΛΟ**	**ΣΗΜΑΣΙΑ**
IVD	In Vitro Διαγνωστικό Ιατροτεχνολογικό προϊόν		Μην κάνετε επαναληπτική χρήση
	Κατασκευαστής	Σ	Περιεχόμενο επαρκές για «ν» εξετάσεις
REF	Αριθμός καταλόγου		Εύθραυστο, να χρησιμοποιείται με προσοχή
	Ημερομηνία λήξης		Προειδοποίηση, συμβουλευτείτε τα συνοδά έντυπα
	Περιορισμοί θερμοκρασίας	LOT	Αριθμός Παρτίδας

CE IVD

BIBLIOGRAFIA / BIBLIOGRAPHY / BIBLIOGRAPHIE
BIBLIOGRAFIA / BIBLIOGRAFIA / ΒΙΒΛΙΟΓΡΑΦΊΑ

1.
2. NCCLS – Performance Standards for antimicrobial Susceptibility Testing, eleventh Informational Supplement- January 2004, Vol 24 N°1.
3. Ewing H.E., 1986 Edwards and Ewings “Identification of Enterobacteriaceae”, 4th ed. Burgess Publishing co.Minneapolis
4. Kelly M.T., D.J. Brenne and J.J. Farmer,1985. “Enterobacteriaceae”, p.263-277 E.H.Lennette, A.Balows, W.J. Hauster,Jr.and H.J. Shadomy (ed.) Manual of Clinical Microbiology, 4th ed. American Society for Microbiology Washington, D.C.
5. Piccolomini R., Di Girolamo A., Catamo G., Cellini L., Allocati N. and Ravagnan G.’ Enterosistem 18-R: description and Comparative Evaluation with Conventional Methods for Identification of Members of the Family *Enterobacteriaceae.* 1991 *J.Clin.Microbiol*. **29**(10), 2300-2304.
6. Baker C. et al. Inoculum Standardization in Antimicrobial susceptibility testing:evaluation of overnight agar cultures and the rapid inoculum standardization system”. 1983 *J.Clin.Microbiol*. **17**, 450-457.
7. Lampe M/F et al. Relationship of early readings of minimal inhibitory concentrations to the results of overnight tests. 1975. *Ant. Agents Chernoth*. **8**, 429-433.
8. Bauer, A.W., W.M.M. Kirby, J.C. Sherris and M. Turk. 1966. Antimicrobic susceptibility testing by a standardized single disk method. *AM. J. Clin. Pathol*. 45:493-496.
9. Dati di archivio Liofilchem s.r.l. “Integral System Enterobatteri“ (Giugno 2003).

LIOFILCHEM Bacteriology Products

64026 ROSETO D.A. (TE) ITALY- Via Scozia- Zona Ind.le

Tel.+39 085 8930745 - Fax +39 085 8930330

Sito Web: http://www.liofilchem.net E-Mail: liofilchem@liofilchem.net