O.K.N.V.I. RESIST-5

Manufacturer:

Coris BioConcept
CREALYS Science Park
Rue Guillaume Fouquet, 11
5032 GEMBLOUX
BELGIUM
Tel.: +32(0)81.719.917
Fax: +32(0)81.719.919
info@corisbio.com
Produced in BELGIUM

www.corisbio.com
IFU-58R11/EN/06

***In vitro* rapid diagnostic test for the detection of OXA-48, KPC, NDM, VIM and IMP carbapenemases in bacterial culture**

FOR IN VITRO DIAGNOSTIC USE **EN**
FOR PROFESSIONAL USE ONLY
References: K-15R11, 2x20 cassettes, buffer, 20 tubes and transfer pipets

I. INTRODUCTION

Carbapenemase-producing Organisms (CPO), and more specifically, Carbapenem-resistant Enterobacteriaceae (CRE) represent a major public health concern worldwide due to their broad spectrum of resistance to antibiotics including, besides carbapenems, most classes of antimicrobial agents, and thus leaving very few options for the management of infected patients. Besides CREs, CPOs also include nonfermenting Gram-negative bacilli (NFGNB), such as *Pseudomonas aeruginosa* and *Acinetobacter baumannii* that exhibit resistance not only to beta lactam and other groups of antibiotics, but also to carbapenems. The rapid spread of CPOs and genes encoding these resistances has led to nosocomial outbreaks and endemic situations worldwide.
Development of new rapid diagnostic tests to track antimicrobial resistance patterns is considered as one of the priority core actions by international experts and health authorities. NDM and KPC represent two of the most increasing and prevalent carbapenemases in many countries. On the other hand, class D OXA-48 type carbapenemases are the most challenging resistance mechanisms to be detected by clinical laboratories. VIM is not only present in Enterobacteriaceae but is also highly prevalent in non-fermenting bacteria. IMP should be regarded as a potential problem since they degrade not only C3G but also carbapenem antimicrobial drug like Imipenem. IMP prevalence is the lowest, apart from Japan where it is more prevalent.
Inhibitor-based phenotypic confirmatory tests exist for the confirmation of class A (KPC) and class B (VIM, IMP, NDM) carbapenemases, Nowadays, definitive confirmation of CPO resistance mechanism relies on molecular assays. These tests are expensive and can only be performed in dedicated environment and by skilled personnel, hence limiting their more generalized usage.
O.K.N.V.I. RESIST-5 test is part of Coris BioConcept RESIST range of antimicrobial resistance diagnostic tests.

II. PRINCIPLE OF THE TESTS

These tests are ready to use and are based on a membrane technology with colloidal gold nanoparticles. Our kit is aimed to detect and identify the carbapenemases from a bacterial colony isolate of Enterobacteriaceae or NFGNB growing on agar plate. Each pouch contains: 2 lateral-flow cassettes for the identification of (i) OXA-48, KPC, NDM and (ii) VIM and IMP.
Identification of OXA-48, KPC and NDM. A nitrocellulose membrane is sensitised with:
(1) a monoclonal antibody directed against OXA-48 carbapenemase and variants (except OXA-163-like enzymes) (“O” line)
(2) a monoclonal antibody directed against KPC carbapenemase (“K” line)
(3) a monoclonal antibody directed against NDM carbapenemase (“N” line)
(4) a control capture reagent (upper “C” line).
Four different colloidal gold nanoparticles conjugates are dried on a membrane: a conjugate directed against a second epitope of the OXA-48 carbapenemase, a conjugate directed against a second epitope of the KPC carbapenemase, a third conjugate specific to NDM carbapenemase and a control conjugate to validate the test conditions.
Identification of VIM and IMP. A nitrocellulose membrane is sensitised with:
(1) a monoclonal antibody directed against VIM carbapenemase (“V” line),
(2) a monoclonal antibody directed against IMP carbapenemase (“I” line)
(3) a control capture reagent (upper “C” line).
Three different colloidal gold nanoparticles conjugates are dried on a membrane: a conjugate directed against VIM carbapenemase, a conjugate directed against IMP carbapenemase and a control conjugate.
When the provided buffer containing the resuspended bacteria comes into contact with the membrane, the solubilised conjugates migrate with the sample by passive diffusion, while conjugates and sample material come into contact with the immobilised respective antibodies that are adsorbed onto the nitrocellulose strip. If the sample contains an OXA-48, KPC, NDM, VIM or IMP carbapenemase, the respective complexes made of the conjugates and either OXA-48, or KPC, or NDM or VIM or IMP will remain bound to their respective specific lines (OXA-48 : “O” line; KPC : “K” line; NDM : “N” line, VIM : “V” line, IMP : “I” line). The migration continues by passive diffusion and both conjugates and sample material come into contact with the (upper) line control reagent that binds a control conjugate (“C” line), thereby producing a red line.
The result is visible within 15 minutes in the form of red lines on the strip.

III. REAGENTS AND MATERIALS

1. O.K.N.V.I. RESIST-5 (2x20 cassettes)
20 sealed pouches containing two lateral-flow cassettes and one desiccant.
Each cassette contains one sensitised strip.
2. LY-D buffer vial (7 mL)
Tris-EDTA solution containing NaN_3 (<0.1%) and a detergent.
3. Instruction for use (1)
4. Disposable collection tubes (20)
5. Disposable transfer pipettes (20)

Materials to be ordered separately:
- RESIST-BC (S-1001): reagents kit for use with blood culture
- ReSCape (S-1002): reagents kits for use with rectal swab

IV. SPECIAL PRECAUTIONS

- All operations linked to the use of the test must be performed in accordance with good laboratory practices.
- All reagents are for *in vitro* diagnostic use only.
- Pouch must be opened with care.
- Avoid touching nitrocellulose with your fingers.
- Wear gloves when handling samples.
- Never use reagents from another kit.
- Green lines indicate immunoreagents adsorption sites. Green colour disappears during the test.
- The quality of the reagents cannot be guaranteed beyond their shelf-life dates or if reagents are not stored under required conditions as indicated in the insert.

V. WASTE DISPOSAL

- Dispose of gloves, swabs, test tubes and used devices in accordance with GLP.
- Each user is responsible for the management of any waste produced, and must ensure that it is disposed of in accordance with the applicable legislation.

VI. STORAGE

- An unopened pouch may be kept at between 4 and 30°C and used until the shelf-life date indicated on the packaging. Once the pouch is opened, run the test immediately.
- Avoid freezing devices and buffer.

VII. SPECIMEN HANDLING AND COLLECTION

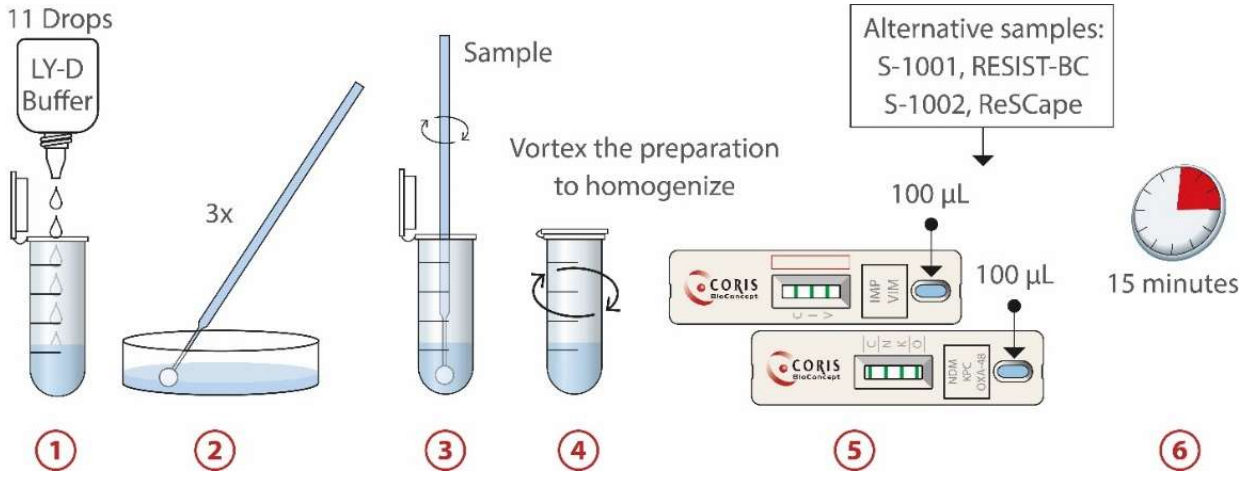
Specimens to be tested should be obtained and handled by standard microbiological methods.
Make sure that the specimens are not treated with solutions containing formaldehyde or its derivatives.
Culture media tested and validated with Coris BioConcept RESIST kits are listed on the website: https://www.corisbio.com/products/oknvi-resist-5/faq

VIII. PROCEDURE

PREPARATIONS OF THE TEST:
Allow kit components, in unopened packaging, and specimens (in the event that the plate containing colony to be tested was kept at 4°C) to equilibrate at room temperature (15-30°C) before performing a test.
Open the pouch and remove the device. Once opened, run the test immediately. Indicate the patient’s name or specimen number on the device (one device per sample).

SPECIMEN PREPARATION PROCEDURE:
Performance claims with regard to sample types other than bacterial colonies have been established for rectal swabs and blood cultures.
With rectal swabs and blood cultures, the preparation procedure has to be followed as described in the respective kits (S-1002, ReSCape and S-1001, RESIST-BC)
With bacterial colonies, we recommend the use of fresh agar cultures for optimal test performance and as followed:

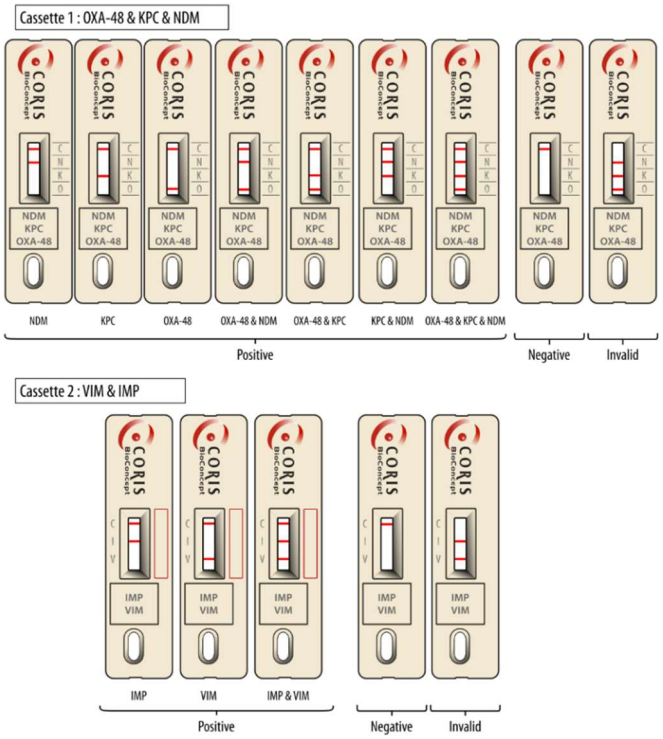
1. Prepare one collection tube and add **11 drops** of LY-D buffer in the tube
2. Harvest bacteria by taking **3 colonies** with a disposable bacteriological loop and dip the loop in the bottom of the tube containing the buffer. The same bacteriological loop can be used to collect the 3 colonies.
3. Stir thoroughly before removing the loop.
4. Close de tube and vortex the preparation to homogenize.
5. Use the transfer pipette provided in the kit and add 100 µL of diluted sample into the sample well of each of the two cassettes labelled (i) NDM, KPC and OXA-48 and (ii) IMP and VIM (**diluted sample must reach the black line indicated on the transfer pipette to accurately aspirate 100 µL**).
6. Allow to react for 15 minutes and read the result.



Positive results may be reported as soon as the test and control lines become visible.
Do not take the appearance of new lines into account after the reaction time has passed.
The result must be read on still wet strip.

IX. INTERPRETING RESULTS

The results are to be interpreted as follows for each of the two cassettes:
Negative test result: a reddish-purple line appears across the central reading window at the Control line (C) position. No other line is present.
Positive test result: in addition to a reddish-purple line at the Control line (C), a visible reddish-purple line appears at one of the Test lines position ("N" or "K" or "O") on cassette labelled (i) NDM, KPC, OXA-48 or at one of the Test lines position ("I" or "V") on cassette labelled (ii) IMP and VIM. Intensity of the test line may vary according to the quantity of antigens as well as of the variant type present in the sample. Any reddish-purple test line (OXA-48, KPC, NDM, VIM and IMP), even weak, should be considered as a positive result.
If a positive test line appears beside of the "O" mark, the sample contains OXA-48 or OXA-48-like variants. If it appears beside the "K" mark, the sample contains KPC variants; beside the "N" mark, the sample contains NDM; the "V" mark, the sample contains VIM; and beside of the "I" mark, IMP is present in the sample. Combinations of positive test lines can occur.
In this case the sample contains several carbapenemases.
Invalid test result: The absence of a Control line indicates a failure in the test procedure. Repeat invalid tests with a new test device.
Note: during the drying process, a very faint shadow may appear at the Test line positions. It should not be regarded as a positive result.



X. PERFORMANCE

A. Detection Limit

The detection limit determined with purified recombinant proteins of OXA-48, KPC, NDM, VIM and IMP have been evaluated at 0.25 ng/mL, 0.5 ng/mL, 0.0625 ng/mL, 0.23 ng/mL and 0.781 ng/mL, respectively.

B. Retrospective study

The test cassettes were validated by comparison with reference molecular method (validated in house multiplex PCR including sequencing) in a retrospective study performed on 180 non duplicated, consecutive suspected CPE clinical isolates collected between 2012 and 2021 from Belgian hospitals.

Molecular method / OXA-48 test	Positive	Negative	Total
Positive	41	0	41
Negative	0	139	139
Total	41	139	180

95 % Confidence Interval [1]

Sensitivity:	100 %	(89.3 to 100 %)
Specificity:	100 %	(96.6 to 100 %)
Positive Predictive value:	100 %	(89.3 to 100 %)
Negative predictive value:	100 %	(96.7 to 100 %)
Agreement:	100 %	(180/180)

Molecular method / KPC test	Positive	Negative	Total
Positive	24	0	24
Negative	0	156	156
Total	24	156	180

95 % Confidence Interval [1]

Sensitivity:	100 %	(82.8 to 100 %)
Specificity:	100 %	(97.0 to 100 %)
Positive Predictive value:	100 %	(82.8 to 100 %)
Negative predictive value:	100 %	(97.0 to 100 %)
Agreement:	100 %	(180/180)

Molecular method / NDM test	Positive	Negative	Total
Positive	40	0	40
Negative	0	140	140
Total	40	140	180

95 % Confidence Interval [1]

Sensitivity:	100 %	(89.1 to 100 %)
Specificity:	100 %	(96.7 to 100 %)
Positive Predictive value:	100 %	(89.1 to 100 %)
Negative predictive value:	100 %	(96.7 to 100 %)
Agreement:	100 %	(180/180)

Molecular method / VIM test	Positive	Negative	Total
Positive	43	0	43
Negative	3	134	137
Total	46	134	180

95 % Confidence Interval [1]

Sensitivity:	93.5 %	(81.1 to 98.3 %)
Specificity:	100 %	(96.5 to 100 %)
Positive Predictive value:	100 %	(89.8 to 100 %)
Negative predictive value:	97.8 %	(93.2 to 99.4 %)
Agreement:	98.3 %	(177/180)

Molecular method / IMP test	Positive	Negative	Total
Positive	19	0	19
Negative	0	161	161
Total	19	161	180

95 % Confidence Interval [1]

Sensitivity:	100 %	(79.1 to 100 %)
Specificity:	100 %	(97.1 to 100 %)
Positive Predictive value:	100 %	(79.1 to 100 %)
Negative predictive value:	100 %	(97.1 to 100 %)
Agreement:	100 %	(180/180)

The O.K.N.V.I. RESIST-5 kit was also validated with rectal swabs and blood cultures.

C. Repeatability and reproducibility

To check intra-batch accuracy (repeatability), the same positive samples and a buffer solution were processed 15 times on kits of the same production batch in the same experimental conditions. All observed results were confirmed as expected.
To check inter-batch accuracy (reproducibility), some samples (positive and buffer) were processed on kits from three different production batches. All results were confirmed as expected.

XI. LIMITS OF THE KIT

The test is qualitative and cannot predict the quantity of antigens present in the sample. Clinical presentation and other test results must be taken into consideration to establish diagnosis. A positive test does not rule out the possibility that other antibiotic resistance mechanisms may be present.

XII. TECHNICAL PROBLEMS / COMPLAINTS

If you face a technical problem or if performances do not correspond with those indicated in this package insert:

1. Record the lot number of the kit concerned.
2. If possible, keep the sample in the appropriate storage condition during the complaint management.
3. Contact Coris BioConcept (client.care@corisbio.com) or your local distributor.

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

XIII. BIBLIOGRAPHIC REFERENCES

A. **J. Wesley MacDonald and V. Chibabhai Evaluation of the RESIST-4 O.K.N.V immunochromatographic lateral flow assay for the rapid detection of OXA-48, KPC, NDM and VIM carbapenemases from cultured isolates** Access Microbiology 2019;1
B. T. Pilate, S. Desmet Detection of carbapenemase production in pseudomonas aeruginosa in a tertiary care centre Annual Meeting of the Royal Belgian Society of Laboratory Medicine November 15th, 2019 Belgium
C. **Oueslati S, Iorga BI, Tlili L, Exilie C, Zavala A, Dortet L, Jousset AB, Bernabeu S, Bonnin RA, Naas T.** Unravelling ceftazidime/avibactam resistance of KPC-28, a KPC-2 variant lacking carbapenemase activity. **J Antimicrob Chemother. 2019 Aug 1;74(8):2239-2246**
D. **Brolund A, Lagerqvist N, Byfors S, Struelens MJ, Monnet DL, Albiger B, Kohlenberg A.** Worsening epidemiological situation of carbapenemase-producing Enterobacteriaceae in Europe, **assessment by national experts from 37 countries, July 2018. Euro Surveill. 2019 Feb; 24 (9) 1560-7917**
E. **Oliveira J, Reygaert WC.** Gram Negative Bacteria. **StatPearls Publishing; 2019 Jan-2019**
F. **Baeza LL, Pfennigwerth N, Greissl C, Göttig S, Saleh A, Stelzer Y, Gatermann SG, Hamprecht A.** Comparison of five methods for detection of carbapenemases in Enterobacterales with proposal of a new algorithm. **Clin Microbiol Infect. 2019 Mar 18. pii: S1198-743X(19)30104-1**
G. **Rösner S, Kamalanabhaiah S, Küsters U, Kolbert M, Pfennigwerth N, Mack D.** Evaluation of a novel immunochromatographic lateral flow assay for rapid detection of OXA-48, NDM, KPC and VIM carbapenemases in multidrug-resistant Enterobacteriaceae. **J Med Microbiol. 2019 Mar;68(3):379-381.**
H. **Glupczynski Y, Evrard S, Huang TD, Bogaerts P.** Evaluation of the RESIST-4 K-SeT assay, a multiplex immunochromatographic assay for the rapid detection of OXA-48-like, KPC, VIM and NDM carbapenemases. **J Antimicrob Chemother. 2019 Feb 6. doi: 10.1093**

Last update : 20 FEBRUARY 2023

REF	Catalogue number		Manufacturer
IVD	In vitro diagnostic medical device		Temperature limits
Σ	Contains sufficient for <n> tests	LOT	Batch code
	Consult instructions for use		Do not reuse
	Keep dry		Use by
DIL SPE	Diluent specimen	CONT NaN_3	Contains Sodium azide
UDI	Unique device identifier		

[1] Newcombe, Robert G. "Two-Sided Confidence Intervals for the Single Proportion: Comparison of Seven Methods," *Statistics in Medicine*, **17**, 857-872 (1998).

Amoxicillin Ezy MIC™ Strip (AMX) (0.016-256 mcg/ml) EM002

Antimicrobial Susceptibility Testing
For *In Vitro* Diagnostic use

It is a unique MIC determination paper strip which is coated with Amoxicillin on a single paper strip in a concentration gradient manner, capable of showing MICs in the range of 0.016 mcg/ml to 256 mcg/ml, on testing against the test organism.

Introduction:
Ezy MIC™ strip is useful for quantitative determination of susceptibility of bacteria to antibacterial agents. The system comprises of a predefined quantitative gradient which is used to determine the Minimum Inhibitory Concentration (MIC) in mcg/ml of different antimicrobial agents against microorganisms as tested on appropriate agar media, following overnight incubation.

Ezy MIC™ Strip FEATURES AND ADVANTAGES

Ezy MIC™ strip exhibits several advantages over existing plastic strip.

1. Ezy MIC™ strip is made up of porous paper material unlike plastic non-porous material
2. Ezy MIC™ strip has MIC values printed on both sides identically.
3. The antimicrobial agent is evenly distributed on either side of the Ezy MIC™ strip and hence it can be placed by any side on the agar surface.
4. For Ezy MIC™ strips, MIC values can be read without opening the lid of the plate as most commonly translucent medium such as Mueller Hinton Agar is employed.
5. Once placed, Ezy MIC™ strip is adsorbed within 60 seconds and firmly adheres to the agar surface.
6. Unlike the plastic material, it does not form air bubbles underneath and hence there is no need to press the strip once placed.

METHOD AND USE OF EZY MIC™ STRIPS

- **Type of specimen**

Pure cultures should be derived from specimens obtained from patients prior to the initiation of antimicrobial therapy. Specimens can be of bacterial or fungal isolates derived from blood, urine, faeces, pus, CSF etc. Direct specimens should not be employed in this test. Refer procedure, which includes preparation of inoculum (1,3).

- **Clinical specimen collection, handling and processing**

Follow appropriate techniques for handling specimens as per established guidelines. After use, contaminated materials must be sterilized by autoclaving before discarding (1,3).

- **Guidelines for preparation of the medium**

Prepare the medium of choice from dehydrated powder according to the directions specified on the label. Cool the sterilized molten medium to 45-50°C and pour in sterile, dry Petri plates on a leveled surface, to a depth of 4 ± 0.2 mm and allow to solidify. Few droplets appearing on the surface of the medium following cooling do not matter. Hence, once poured, Petri plates containing media should not be dried on laminar flow and can be used immediately for swabbing.

- **Preparation of Inoculum**

Use only pure cultures. Confirm by Gram-staining before starting susceptibility test. Transfer 4-5 similar colonies with a wire, needle or loop to 5 ml Tryptone Soya Broth (M011) and incubate at 35-37°C for 2-8 hours until light to moderate turbidity develops. Compare the inoculum turbidity with that of standard 0.5 McFarland. Alternatively, the inoculum can be standardized by other appropriate optical method (0.08 - 0.13 OD turbid suspension at 620 nm).
Also direct colony suspension method can be used. Prepare a direct colony suspension, from 18-24 hour old non-selective media agar plate in broth or saline. Adjust the turbidity to that of standard 0.5 McFarland .This method is recommended for testing fastidious organisms like *Haemophilus* spp., *Neisseria* spp., *Bacteroides* spp, streptococci and for testing staphylococci for potential Methicillin or Oxacillin resistance.

- **Test Procedure**

1. Prepare plates with suitable make of Mueller Hinton Agar, supplemented with 5% sterile, defibrinated sheep blood for fastidious organism such as streptococci.
2. Dip a sterile non-toxic cotton swab on a wooden applicator into the standardized inoculum and rotate the soaked swab firmly against the upper inside wall of the tube to express excess fluid. Streak the entire agar surface of the plate with the swab three times, turning the plate at 60° angle between each streaking.
3. Remove Ezy MIC™ strip container from cold and keep it at room temperature for 15 minutes before opening.
4. Remove one applicator from the self sealing bag stored at room temperature.
5. Hold the applicator in the middle and gently press its broader sticky side on the centre of Ezy MIC™ strip.
6. Lift the applicator along with attached Ezy MIC™ strip.
7. Place the strip at a desired position on agar plate swabbed with test culture. Gently turn the applicator clockwise with fingers. With this action, the applicator will detach from the strip.
8. DO NOT PRESS EZY MIC™ STRIP. Within 60 seconds, Ezy MIC™ strip will be adsorbed and will firmly adhere to the agar surface.
9. Ezy MIC™ strip should not be repositioned or adjusted once placed.
10. Transfer plates in the incubator under appropriate conditions.

MIC Reading:

1. Read the plates only when sufficient growth is seen.
2. Read the MIC where the ellipse intersects the MIC scale on the strip.
3. For bactericidal drugs such as Amoxicillin and other members of β-lactams class of drugs, Amikacin, Vancomycin, Gentamicin, Carbapenems always read the MIC at the point of complete inhibition of all growth, including hazes, microcolonies and isolated colonies. If necessary, use magnifying glass.
4. Isolated colonies, microcolonies and hazes appearing in the zone of inhibition are indicative of hetero nature of the culture having resistant subpopulation in it. In such cases, consider reading for MIC determination at a point on the scale above which no resistant colonies are observed close to MIC strip (within 1-3 mm distance from the strip).
5. Since Ezy MIC™ strip has continuous gradient, MIC values “in-between” two fold dilutions can be obtained.
6. Always round up these values to the next two-fold dilution before categorization. For example: Amoxicillin showing reading of 0.38 mcg/ml should be rounded up to next concentration ie. 0.5 mcg/ml.
7. If the ellipse intersects the strip in between 2 dilutions, read the MIC as the value which is nearest to the intersection.
8. When growth occurs along the entire strip, report the MIC as $\geq$ the highest values on the MIC strip. When the inhibition ellipse is below the strip (does not intersect the strip), report the MIC < the lowest value on the MIC scale.

Warning and Precautions:

1. Ezy MIC™ Strip is intended for *In vitro* diagnostic use only.
2. Although based on simple procedure, Ezy MIC™ Strip should only be used by at least semi-trained personnel.
3. This strip is intended only for agar diffusion method and not for broth dilution method.
4. Ezy MIC™ Strip should be used strictly according to procedures described herein.
5. Performance of Ezy MIC™ Strips depends on use of proper inoculum and control cultures, recommended test medium and proper storage temperature.
6. Follow aseptic techniques and precautions against microbiological hazards should be used when handling bacterial or fungal specimen throughout the testing procedure.
7. Before using Ezy MIC™ Strips, ensure that the strip is at room temperature.
8. When applying strips be steady. Do not move the strip once in contact with agar surface, since the antibiotic instantaneously diffuse on contact with agar.
9. Place the unused strips back to recommended temperature.

INTERPRETATION & QUALITY CONTROL :

Interpretation

Table 1: Use following interpretive criteria for susceptibility categorization as per CLSI.

When testing	Incubation	Interpretive Criteria		
		≤ S	I	≥ R
S.pneumoniae (non meningitis)	35-37°C for 20-24hrs at 5% CO_2	2	4	8

Quality Control

Quality control of Ezy MIC™ Strip is carried out by testing the strips with standard ATCC cultures recommended by CLSI on suitable medium incubated appropriately.

Table 2: Following are the reference MIC values (mcg/ml) range for Amoxicillin.

Organism	Medium used	Incubation	Std. Quality Control limits (mcg/ml)
K. pneumoniae ATCC 700603	Mueller Hinton Agar	35-37°C for 18 hrs.	>128
S. pneumoniae ATCC 49619	Mueller Hinton Agar w/ 5% Sheep Blood	35-37°C for 20-24hrs at 5% CO_2	0.03 – 0.06 – 0.12

Storage & Shelf Life:

1. Once the consignment is received, store applicators at Room Temperature and Ezy MIC™ strips container at -20°C or below.
2. Use before expiry date on the label.
3. Ezy MIC™ Strip left over from opened package must be kept dry.
4. Moisture should be prevented from penetrating into or forming within the package or storage container.
5. Check whether the batch number and expiry date are marked on the storage container.
6. Product performance is best within stated expiry period if correctly stored and handled.

Disposal:

After use, Ezy MIC™ Strips and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (2,3).

Limitation of Test

Ezy MIC™ Strips provides *In vitro* MIC values, which provides only a possible insinuation of pathogens potential in *In vivo* susceptibility. These values can be considered as a guide to therapy selection only after taking into consideration several other factors; and must be the sole decision and responsibility of the physician along with the clinical experience in treating the infection. These tests are comparable to the standards as per the given specifications and set of experiment standards as far as possible. Please refer to CLSI standards for detailed limitation of susceptibility test on the clinical use of an antibiotic in various therapeutic conditions.

References:

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2. Isenberg, H.D. Clinical Microbiology Procedures Handbook. 2nd Edition, Vol. 3, Section 15.
3. Jorgensen,J.H., Pfaller , M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.
4. Performance Standards of Antimicrobial Susceptibility Testing; 34th Edition. M100-Ed34, Vol.44, No.5, Jan-2024.

Packing:
Each Pack contains following material packed in sealed glass vial with a desiccator capsule.

1) Amoxicillin Ezy MIC™ strips (10/30/60/90/120/150 Strips per pack)
2) Applicator sticks
3) Package insert

Revision: 05/2024

CE

On receipt store at -20°C

IVD In vitro diagnostic medical device

Plot No. C-40,
Road No. 21Y, MIDC,
Wagle Industrial Estate,
Thane (W) - 400604,
Maharashtra, India

Indicates a single sterile barrier system

Do not re-use

EC REP CEpartner4U,
ESDOORNLAAN 13,
3951DB MAARN, NL
www.cepartner 4u.eu

Do not use if package is damaged

Disclaimer :

CE

User must ensure suitability of the product(s) in their application prior to use. Products conform solely to the information contained in this and other related HiMedia™ publications. The information contained in this publication is based on our research and development work and is to the best of our knowledge true and accurate. HiMedia™ Laboratories Pvt Ltd reserves the right to make changes to specifications and information related to the products at any time. Products are not intended for human or animal or therapeutic use but for laboratory,diagnostic, research or further manufacturing use only, unless otherwise specified. Statements contained herein should not be considered as a warranty of any kind, expressed or implied, and no liability is accepted for infringement of any patents.

Clarithromycin Ezy MIC™ Strip (CLR) (0.016-256 mcg/ml) EM018

Antimicrobial Susceptibility Testing
For *In Vitro* Diagnostic use

It is a unique MIC determination paper strip which is coated with Clarithromycin on a single paper strip in a concentration gradient manner, capable of showing MICs in the range of 0.016 mcg/ml to 256 mcg/ml, on testing against the test organism.

Introduction:
Ezy MIC™ strip is useful for quantitative determination of susceptibility of bacteria to antibacterial agents. The system comprises of a predefined quantitative gradient which is used to determine the Minimum Inhibitory Concentration (MIC) in mcg/ml of different antimicrobial agents against microorganisms as tested on appropriate agar media, following overnight incubation.

Ezy MIC™ Strip FEATURES AND ADVANTAGES

Ezy MIC™ strip exhibits several advantages over existing plastic strip.

1. Ezy MIC™ strip is made up of porous paper material unlike plastic non-porous material
2. Ezy MIC™ strip has MIC values printed on both sides identically.
3. The antimicrobial agent is evenly distributed on either side of the Ezy MIC™ strip and hence it can be placed by any side on the agar surface.
4. For Ezy MIC™ strips, MIC values can be read without opening the lid of the plate as most commonly translucent medium such as Mueller Hinton Agar is employed.
5. Once placed, Ezy MIC™ strip is adsorbed within 60 seconds and firmly adheres to the agar surface.
6. Unlike the plastic material, it does not form air bubbles underneath and hence there is no need to press the strip once placed.

METHOD AND USE OF EZY MIC™ STRIPS

- **Type of specimen**

Pure cultures should be derived from specimens obtained from patients prior to the initiation of antimicrobial therapy. Specimens can be of bacterial or fungal isolates derived from blood, urine, faeces, pus, CSF etc. Direct specimens should not be employed in this test. Refer procedure, which includes preparation of inoculum (1,3).

- **Clinical specimen collection, handling and processing**

Follow appropriate techniques for handling specimens as per established guidelines. After use, contaminated materials must be sterilized by autoclaving before discarding (1,3).

- **Guidelines for preparation of the medium**

Prepare the medium of choice from dehydrated powder according to the directions specified on the label. Cool the sterilized molten medium to 45 - 50°C and pour in sterile, dry Petri plates on a leveled surface, to a depth of 4 ± 0.2 mm and allow to solidify. Few droplets appearing on the surface of the medium following cooling do not matter. Hence, once poured, Petri plates containing media should not be dried on laminar flow and can be used immediately for swabbing.

- **Preparation of Inoculum**

Use only pure cultures. Confirm by Gram-staining before starting susceptibility test. Transfer 4-5 similar colonies with a wire, needle or loop to 5 ml Tryptone Soya Broth (M011) and incubate at 35-37°C for2-8 hours until light to moderate turbidity develops. Compare the inoculum turbidity with that of standard 0.5 McFarland. Alternatively, the inoculum can be standardized by other appropriate optical method (0.08 - 0.13 OD turbid suspension at 620 nm).

Also, direct colony suspension method can be used. Prepare a direct colony suspension, from 18-24 hour old non-selective media agar plate in broth or saline. Adjust the turbidity to that of standard 0.5 McFarland. This method is recommended for testing fastidious organisms like *Haemophilus* spp., *Neisseria* spp, *Bacteroides* spp, streptococci and for testing staphylococci for potential Methicillin or Oxacillin resistance.

Please refer disclaimer Overleaf

- **Test Procedure**

1. Prepare plates with suitable make of Mueller Hinton Agar for rapidly growing aerobic organisms as mentioned above. For fastidious organisms such as Streptococci, Mueller Hinton Agar is supplemented with 5% sterile, defibrinated blood is recommended. For *Haemophilus* spp, Haemophilus Test Agar Base (M1259) with added supplement (FD117) is to be used.
2. Dip a sterile non-toxic cotton swab on a wooden applicator into the standardized inoculum and rotate the soaked swab firmly against the upper inside wall of the tube to express excess fluid. Streak the entire agar surface of the plate with the swab three times, turning the plate at 60° angle between each streaking.
3. Remove Ezy MIC™ strip container from cold and keep it at room temperature for 15 minutes before opening.
4. Remove one applicator from the self sealing bag stored at room temperature.
5. Hold the applicator in the middle and gently press its broader sticky side on the centre of Ezy MIC™ strip.
6. Lift the applicator along with attached Ezy MIC™ strip.
7. Place the strip at a desired position on agar plate swabbed with test culture. Gently turn the applicator clockwise with fingers. With this action, the applicator will detach from the strip.
8. DO NOT PRESS EZY MIC™ STRIP. Within 60 seconds, Ezy MIC™ strip will be adsorbed and will firmly adhere to the agar surface.
9. Ezy MIC™ strip should not be repositioned or adjusted once placed.
10. Transfer plates in the incubator under appropriate conditions.

MIC Reading:

1. Read the plates only when sufficient growth is seen.
2. Read the MIC where the ellipse intersects the MIC scale on the strip.
3. For bacteriostatic drugs such as Clarithromycin, Chloramphenicol, Tetracycline, Azithromycin, Fluconazole, Linezolid and Trimethoprim/ sulphamethoxazole, read MICs at 80% inhibition for homogenously sensitive strains such as QC control strains.
4. Isolated colonies, microcolonies and hazes appearing in the zone of inhibition are indicative of hetero nature of the culture having resistant subpopulation in it. In such cases, consider reading for MIC determination at a point on the scale above which no resistant colonies are observed close to MIC strip (within 1-3 mm distance from the strip).
5. Since Ezy MIC™ strip has continuous gradient, MIC values “in-between” two fold dilutions can be obtained.
6. Always round up these values to the next two-fold dilution before categorization. For example: Clarithromycin showing reading of 0.38 mcg/ml should be rounded up to next concentration ie. 0.5 mcg/ml.
7. If the ellipse intersects the strip in between 2 dilutions, read the MIC as the value which is nearest to the intersection.
8. When growth occurs along the entire strip, report the MIC as $\geq$ the highest values on the MIC strip. When the inhibition ellipse is below the strip (does not intersect the strip), report the MIC < the lowest value on the MIC scale.

Warning and Precautions:

1. Ezy MIC™ Strip is intended for *In vitro* diagnostic use only.
2. Although based on simple procedure, Ezy MIC™ Strip should only be used by at least semi-trained personnel.
3. This strip is intended only for agar diffusion method and not for broth dilution method.
4. Ezy MIC™ Strip should be used strictly according to procedures described herein.
5. Performance of Ezy MIC™ Strips depends on use of proper inoculum and control cultures, recommended test medium and proper storage temperature.
6. Follow aseptic techniques and precautions against microbiological hazards should be used when handling bacterial or fungal specimen throughout the testing procedure.
7. Before using Ezy MIC™ Strips, ensure that the strip is at room temperature.
8. When applying strips be steady. Do not move the strip once in contact with agar surface, since the antibiotic instantaneously diffuse on contact with agar.
9. Place the unused strips back to recommended temperature

INTERPRETATION & QUALITY CONTROL:

Interpretation

Table 1: Use following interpretive criteria for susceptibility categorization as per CLSI.

When testing	Incubation	Interpretative Criteria		
		≤ S	I	≥ R
Staphylococcus spp.	35-37°C for 18 hrs.	2	4	8
Haemophilus spp.	35-37°C for 20-24hrs at 5% CO_2	8	16	32
S.pneumoniae, Streptococcus spp. Beta haemolytic group, *Streptococcus* spp. Viridans group	35-37°C for 20-24hrs at 5% CO_2	0.25	0.5	1

Quality Control

Quality control of Ezy MIC™ Strip is carried out by testing the strips with standard ATCC Cultures recommended by CLSI on suitable medium incubated appropriately.

Table 2: Following are the reference MIC values (mcg/ml) range for Clarithromycin.

Organism	Medium used	Incubation	Std. Quality Control limits (mcg/ml)
S.aureus ATCC 29213	Mueller Hinton Agar	35-37°C for 18 hrs.	0.12 – 0.25 - 0.5
S. pneumoniae ATCC 49619	Mueller Hinton Agar w/ 5% Sheep Blood	35-37°C for 20-24hrs at 5% CO_2	0.03 - 0.06 - 0.12
H.influenzae ATCC 49247	Haemophilus Test Medium	35-37°C for 20-24hrs at 5% CO_2	4.0 - 8.0 - 16.0

Storage & Shelf Life:

1. Once the consignment is received, store applicators at Room Temperature and Ezy MIC™ strips container at 2-8°C , for prolonged use store at -20°C.
2. Use before expiry date on the label.
3. Ezy MIC™ Strip left over from opened package must be kept dry.
4. Moisture should be prevented from penetrating into or forming within the package or storage container.
5. Check whether the batch number and expiry date are marked on the storage container.
6. Product performance is best within stated expiry period if correctly stored and handled.

Disposal:

After use, Ezy MIC™ Strips and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (2,3).

Limitation of Test

Ezy MIC™ Strips provides *In vitro* MIC values, which provides only a possible insinuation of pathogens potential in *In vivo* susceptibility. These values can be considered as a guide to therapy selection only after taking into consideration several other factors; and must be the sole decision and responsibility of the physician along with the clinical experience in treating the infection. These tests are comparable to the standards as per the given specifications and set of experiment standards as far as possible. Please refer to CLSI standards for detailed limitation of susceptibility test on the clinical use of an antibiotic in various therapeutic conditions.

References:

1. Isenberg, H.D. Clinical Microbiology Procedures Handbook. 2nd Edition, Vol. 1, Section 2.
2. Isenberg, H.D. Clinical Microbiology Procedures Handbook. 2nd Edition, Vol. 3, Section 15.
3. Jorgensen, J. H., Pfaller., M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.
4. Performance Standards of Antimicrobial Susceptibility Testing; 34th Edition. M100-Ed34, Vol.44, No.5, Jan-2024.

Packing:

Each Pack contains following material packed in air-tight plastic container with a desiccator capsule.

1) Clarithromycin Ezy MIC™ strips (10/30/60/90/120/150 Strips per pack)
2) Applicator sticks
3) Package insert

Revision: 05/2024

CE

On receipt store at -20°C 8°C

IVD In vitro diagnostic medical device

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Maharashtra, India

Indicates a single sterile barrier system with protective packaging outside

Do not re-use

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Do not use if package is damaged

Disclaimer :

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Levofloxacin Ezy MIC™ (LEV) (0.002-32 mcg/ml) EM027

Antimicrobial Susceptibility Testing
For *In Vitro* Diagnostic use

It is a unique MIC determination paper strip which is coated with Levofloxacin on a single paper strip in a concentration gradient manner, capable of showing MICs in the range of 0.002 mcg/ml to 32 mcg/ml, on testing against the test organism.

Introduction:
Ezy MIC™ strip is useful for quantitative determination of susceptibility of bacteria to antibacterial agents. The system comprises of a predefined quantitative gradient which is used to determine the Minimum Inhibitory Concentration (MIC) in mcg/ml of different antimicrobial agents against microorganisms as tested on appropriate agar media, following overnight incubation.

Ezy MIC™ Strip FEATURES AND ADVANTAGES

Ezy MIC™ strip exhibits several advantages over existing plastic strip.

1. Ezy MIC™ strip is made up of porous paper material unlike plastic non-porous material.
2. Ezy MIC™ strip has MIC values printed on both sides identically.
3. The antimicrobial agent is evenly distributed on either side of the Ezy MIC™ strip and hence it can be placed by any side on the agar surface.
4. For Ezy MIC™ strips, MIC values can be read without opening the lid of the plate as most commonly translucent medium such as Mueller Hinton Agar is employed.
5. Once placed, Ezy MIC™ strip is adsorbed within 60 seconds and firmly adheres to the agar surface.
6. Unlike the plastic material, it does not form air bubbles underneath and hence there is no need to press the strip once placed.

METHOD AND USE OF EZY MIC™ STRIPS

- **Type of specimen**

Pure cultures should be derived from specimens obtained from patients prior to the initiation of antimicrobial therapy. Specimens can be of bacterial or fungal isolates derived from blood, urine, faeces, pus, CSF etc. Direct specimens should not be employed in this test. Refer procedure, which includes preparation of inoculum (1,3).

- **Clinical specimen collection, handling and processing**

Follow appropriate techniques for handling specimens as per established guidelines. After use, contaminated materials must be sterilized by autoclaving before discarding (1,3).

- **Guidelines for preparation of the medium**

Prepare the medium of choice from dehydrated powder according to the directions specified on the label. Cool the sterilized molten medium to 45-50°C and pour in sterile, dry Petri plates on a leveled surface, to a depth of 4 ± 0.2 mm and allow to solidify. Few droplets appearing on the surface of the medium following cooling do not matter. Hence, once poured, Petri plates containing media should not be dried on laminar flow and can be used immediately for swabbing.

- **Preparation of Inoculum**

Use only pure cultures. Confirm by Gram-staining before starting susceptibility test. Transfer 4-5 similar colonies with a wire, needle or loop to 5 ml Tryptone Soya Broth (M011) and incubate at 35-37°C for2-8 hours until light to moderate turbidity develops. Compare the inoculum turbidity with that of standard 0.5 McFarland. Alternatively, the inoculum can be standardized by other appropriate optical method (0.08 - 0.13 OD turbid suspension at 620 nm).
Also direct colony suspension method can be used. Prepare a direct colony suspension, from 18-24 hour old non-selective media agar plate in broth or saline. Adjust the turbidity to that of standard 0.5 McFarland .This method is recommended for testing fastidious organisms like *Haemophilus* spp., *Neisseria* spp, streptococci and for testing staphylococci for potential Methicillin or Oxacillin resistance.

- **Test Procedure**

1. Prepare plates with suitable make of Mueller Hinton Agar for rapidly growing aerobic organisms as mentioned above. For fastidious organisms such as Streptococci, Mueller Hinton Agar is supplemented with 5% sterile, defibrinated blood. For *Haemophilus* spp, Haemophilus Test Agar Base (M1259) with added supplement (FD117) is to be used.
2. Dip a sterile non-toxic cotton swab on a wooden applicator into the standardized inoculum and rotate the soaked swab firmly against the upper inside wall of the tube to express excess fluid. Streak the entire agar surface of the plate with the swab three times, turning the plate at 60° angle between each streaking.
3. Remove Ezy MIC™ strip container from cold and keep it at room temperature for 15 minutes before opening.
4. Remove one applicator from the self-sealing bag stored at room temperature.
5. Hold the applicator in the middle and gently press its broader sticky side on the centre of Ezy MIC™ strip.
6. Lift the applicator along with attached Ezy MIC™ strip.
7. Place the strip at a desired position on agar plate swabbed with test culture. Gently turn the applicator clockwise with fingers. With this action, the applicator will detach from the strip.
8. DO NOT PRESS EZY MIC™ STRIP. Within 60 seconds, Ezy MIC™ strip will be adsorbed and will firmly adhere to the agar surface.
9. Ezy MIC™ strip should not be repositioned or adjusted once placed.
10. Transfer plates in the incubator under appropriate conditions.

MIC Reading:

1. Read the plates only when sufficient growth is seen.
2. Read the MIC where the ellipse intersects the MIC scale on the strip.
3. For bactericidal drugs such as Levofloxacin and other members of quinolones class of drugs, Amikacin, Vancomycin, Gentamicin and members of β-lactams class of drugs always read the MIC at the point of complete inhibition of all growth, including hazes, microcolonies and isolated colonies. If necessary, use magnifying glass.
4. Isolated colonies, microcolonies and hazes appearing in the zone of inhibition are indicative of hetero nature of the culture having resistant subpopulation in it. In such cases, consider reading for MIC determination at a point on the scale above which no resistant colonies are observed close to MIC strip (within 1-3 mm distance from the strip).
5. Since Ezy MIC™ strip has continuous gradient, MIC values "in-between" two fold dilutions can be obtained.
6. Always round up these values to the next two-fold dilution before categorization. For example: Levofloxacin showing reading of 0.75 mcg/ml should be rounded up to next concentration ie. 1.0 mcg/ml.
7. If the ellipse intersects the strip in between 2 dilutions, read the MIC as the value which is nearest to the intersection.
8. When growth occurs along the entire strip, report the MIC as $\geq$ the highest values on the MIC strip. When the inhibition ellipse is below the strip (does not intersect the strip), report the MIC < the lowest value on the MIC scale.

Warning and Precautions:

1. Ezy MIC™ Strip is intended for *In vitro* diagnostic use only.
2. Although based on simple procedure, Ezy MIC™ Strip should only be used by at least semi-trained personnel.
3. This strip is intended only for agar diffusion method and not for broth dilution method.
4. Ezy MIC™ Strip should be used strictly according to procedures described herein.
5. Performance of Ezy MIC™ Strips depends on use of proper inoculum and control cultures, recommended test medium and proper storage temperature.
6. Follow aseptic techniques and precautions against microbiological hazards should be used when handling bacterial or fungal specimen throughout the testing procedure.
7. Before using Ezy MIC™ Strips, ensure that the strip is at room temperature.
8. When applying strips be steady. Do not move the strip once in contact with agar surface, since the antibiotic instantaneously diffuse on contact with agar.
9. Place the unused strips back to recommended temperature.

INTERPRETATION & QUALITY CONTROL:

Interpretation

Table 1: Use following interpretive criteria for susceptibility categorization as per CLSI.

When testing	Incubation	Interpretive Criteria		
		≤ S	I	≥ R
Enterobacterales (Excluding *Salmonella/Shigella* spp)	35-37°C for 18 hrs.	0.5	1	2
Other non *Enterobacterales, Acinetobacter* spp., *B.cepecia, S. maltophilia, Enterococcus* spp.	35-37°C for 18 hrs.	2	4	8
Staphylococcus spp, *Pseudomonas aeruginosa*	35-37°C for 18 hrs.	1	2	4
S.Typhi, S. Paratyphi A-C, Salmonella spp.	35-37°C for 18 hrs.	0.12	0.25 -1	2
Shigella spp.	35-37°C for 18 hrs.	0.5	1	2
Haemophilus spp.	35-37°C for 20-24hrs at 5% CO_2	2	-	-
S.pneumoniae, Streptococcus spps. Beta haemolytic group, *Streptococcus* spps. Viridians group.	35-37°C for 20-24hrs at 5% CO_2	2	4	8
N.meningitidis	35-37°C for 20-24hrs at 5% CO_2	0.03	0.06	0.12

Quality control

Quality control of Ezy MIC™ Strip is carried out by testing the strips with standard ATCC Cultures recommended by CLSI on suitable medium incubated appropriately.

Table 2: Following are the reference MIC values (mcg/ml) range for Levofloxacin

Organism	Medium used	Incubation	Std. Quality Control limits (mcg/ml)
S.aureus ATCC 29213	Mueller Hinton Agar	35-37°C for 18 hrs.	0.06 - 0.12 - 0.25 - 0.5
E. faecalis ATCC 29212	Mueller Hinton Agar	35-37°C for 18 hrs.	0.25 - 0.5 - 1.0 - 2.0
E.coli ATCC 25922	Mueller Hinton Agar	35-37°C for 18 hrs.	0.008 - 0.016 - 0.03 - 0.06
P. aeruginosa ATCC 27853	Mueller Hinton Agar	35-37°C for 18 hrs.	0.5 - 1.0 - 2.0 - 4.0
S. pneumoniae ATCC 49619	Mueller Hinton Agar w/ 5% Sheep Blood	35-37°C for 20-24hrs at 5% CO_2	0.5 - 1.0 - 2.0
H.influenzae ATCC 49247	Haemophilus Test Medium	35-37°C for 20-24hrs at 5% CO_2	0.008 - 0.016 - 0.03

Storage & Shelf Life:

1. Once the consignment is received, store applicators at Room Temperature and Ezy MIC™ strips container at 2-8°C, for prolonged use store below -20°C.
2. Use before expiry date on the label.
3. Ezy MIC™ Strip left over from opened package must be kept dry.
4. Moisture should be prevented from penetrating into or forming within the package or storage container.
5. Check whether the batch number and expiry date are marked on the storage container.
6. Product performance is best within stated expiry period if correctly stored and handled.

Disposal:

After use, Ezy MIC™ Strips and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (2, 3).

Limitation of Test

Ezy MIC™ Strips provides *in vitro* MIC values, which provides only a possible insinuation of pathogens potential in *In vivo* susceptibility. These values can be considered as a guide to therapy selection only after taking into consideration several other factors; and must be the sole decision and responsibility of the physician along with the clinical experience in treating the infection. These tests are comparable to the standards as per the given specifications and set of experiment standards as far as possible. Please refer to CLSI standards for detailed limitation of susceptibility test on the clinical use of an antibiotic in various therapeutic conditions.

References:

1. Isenberg, H.D. Clinical Microbiology Procedures Handbook. 2nd Edition, Vol. 1, Section 2.
2. Isenberg, H.D. Clinical Microbiology Procedures Handbook. 2nd Edition, Vol. 3, Section 15.
3. Jorgensen,J.H., Pfaller , M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.
4. Performance Standards of Antimicrobial Susceptibility Testing; 34th Edition. M100-Ed34, Vol.44, No.5, Jan-2024.

Packing:

Each Pack contains following material packed in air-tight plastic container with a desiccator capsule.

1) Levofloxacin Ezy MIC™ strips (10/30/60/90/120/150 Strips per pack)
2) Applicator sticks
3) Package insert

Revision: 05/2024

CE

On receipt store at -20°C / 8°C

IVD — In vitro diagnostic medical device

Plot No. C-40,
Road No. 21Y, MIDC,
Wagle Industrial Estate,
Thane (W) - 400604,
Maharashtra, India

Indicates a single sterile barrier system with protective packaging outside

Do not re-use

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www.cepartner 4u.eu

Do not use if package is damaged

Technical Data

Bos Selective Supplement

FD004

An antibiotic supplement for the selective isolation of *Bordetella pertussis*.

Composition

Per vial sufficient for 500 ml/ 1000 ml medium

Ingredients	Concentration
Cephalexin	20mg

Directions:

Rehydrate the content of 1 vial aseptically with 2 ml of sterile distilled water. Mix well and aseptically add it to 500 ml of sterile, molten, cooled (45-50°C) Bordet Gengou Agar Base M175/ M175A/ Bordet Gengou HiVeg™ Agar Base MV175 / MV175A or 1000 ml of sterile, molten Charcoal Agar Base w/Niacin M1053/ Charcoal HiVeg™ Agar Base w/Niacin MV1053 together with 10% v/v defibrinated horse blood. Mix well and pour into sterile petri plates. The vial content may be added to 500 ml of sterile half strength Charcoal Agar Base M344/ Charcoal HiVeg™ Agar Base MV344 with 10% v/ v defibrinated horse blood for use as a transport medium for *Bordetella pertussis*.

Type of specimen

Clinical samples -Pharyngeal extracts, nasopharyngeal secretions and pre-nasal swabs.

Specimen Collection and Handling

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (1,2). After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning & Precautions

In Vitro diagnostic use only. For professional use only. Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling clinical specimens. Safety guidelines may be referred in individual safety data sheets.

Storage and Shelf Life

Store at 2 - 8°C. Use before expiry date on the label.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (1,2).

Reference

1. Isenberg (Ed.),2004, Clinical Microbiology Procedures Handbook, Vol.3, American Society for Microbiology, Washington. D.C.
2. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology,11th Edition. Vol. 1.

*** Not For Medicinal Use**

Revision : 03/2022

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IVD ***In vitro* diagnostic medical device**

CE **CE Marking**

2°C 8°C **Storage temperature**

Do not use if package is damaged

MeRS Selective Supplement

FD229

An antimicrobial supplement recommended for the selective isolation of Methicillin Resistant *Staphylococcus aureus* from clinical specimens.

Composition

Per vial sufficient for 500 ml medium

*Ingredients	Concentration
Methicillin	2mg

Directions:

Rehydrate the contents of one vial with 5 ml of sterile distilled water and aseptically add to 500 ml of sterile, molten, cooled (45-50ºC) MeReSa Agar Base M1594/ HiCrome™ MeReSa Agar, Base M1674/ HiCrome™ MeReSa HiVeg™ Agar Base MV1674/ HiCrome™ MeReSa HiCynth™ Agar Base MCD1674/ HiCrome™ MRSA Agar Base, Modified M1953. This supplement can either be used individually or in combination with FD259 Cf Selective Supplement II for more selectivity. Mix well and pour into sterile Petri plates. Mix well and pour into sterile petri plates.

Type of specimen

Clinical samples - Mouth, skin, intestine, upper respiratory tract of humans, urine, pus, wound samples etc.

Specimen Collection and Handling

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (1,2). After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning & Precautions

In Vitro diagnostic use only. For professional use only. Read the label before opening the container. Wear protective gloves/ protective clothing/eye protection/face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling clinical specimens. Safety guidelines may be referred in individual safety data sheets.

Storage and Shelf Life

Store at 2 - 8°C. Use before expiry date on the label.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (1,2).

Reference

1. Isenberg (Ed.),2004, Clinical Microbiology Procedures Handbook, Vol.3, American Society for Microbiology, Washington. D.C.
2. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology,11th Edition. Vol. 1.

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IVD ***In vitro* diagnostic medical device**

CE Marking

2°C – 8°C **Storage temperature**

Do not use if package is damaged

*** Not For Medicinal Use**

Revision : 02/2022

VF Selective Supplement

FD277

Intended use:

Recommended for selective isolation of Vancomycin Resistant Enterococci (VRE).

Composition

Per vial sufficient for 500 ml medium

*Ingredients	Concentration
Vancomycin	4mg
Fluconazole	5mg

Directions:

1. This product is available in one size containing 5 vials.
2. The bottles when supplied are intact. Ensure that bottles do not have any cracks or defects.
3. User may remove the desired number of bottles from the box as per their requirement.
4. It should be handled by trained person wearing appropriate personal protective equipment (PPE) and sterile gloves.
5. Place the bottles on sterile surfaces such as laminar air flow or sterile working bench.
6. Label them accordingly.
7. Disinfect the outer surface of cap or closures with suitable disinfectant example 70% IPA.
8. Rehydrate the contents of 1 vial aseptically with 5 ml of 50% v/v aqueous ethanol.
9. Mix well and aseptically add to 500ml of sterile, molten, cooled (45-50°C) HiCrome™ VRE Agar Base M1830/ HiCrome™ VRE Agar Base, Modified M1925. Mix well and pour into sterile Petri plates.
10. Follow good lab practices for procedures and disposal.

Type of specimen

Clinical samples - faecal sample

Specimen Collection and Handling

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (1,2). After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning & Precautions

In Vitro diagnostic use only. For professional use only. Read the label before opening the container. Wear protective gloves/ protective clothing/eye protection/face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling clinical specimens. Safety guidelines may be referred in individual safety data sheets.

Storage and Shelf Life

Store at 2 - 8°C. Use before expiry date on the label.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (1,2).

Reference

1. Isenberg (Ed.),2004, Clinical Microbiology Procedures Handbook, Vol.3, American Society for Microbiology, Washington. D.C.
2. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology,11th Edition. Vol. 1.

*** Not For Medicinal Use**

Revision : 00/2024

Packaging

FD277-5VL - VF Selective Supplement

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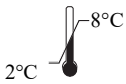
IVD ***In vitro* diagnostic medical device**



Storage temperature

EC REP CEpartner4U,Esdoornlaan13,
3951DB Maarn, NL
www.cepartner4u.eu

CE Marking

Do not use if package is damaged

Single sterile barrier system

Disclaimer :

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AC3F Selective Supplement

FD278

Recommended for the detection of Extended Spectrum Beta lactamase producing organisms.

Composition

Per vial sufficient for 500 ml medium

*Ingredients	Concentration
Ceftazidime	1.50mg
Cefotaxime	1.50mg
Ceftriazone	1.00mg
Aztreonam	1.00mg
Fluconazole	5.00mg

Directions:

Rehydrate the contents of 1 vial aseptically with 5 ml sterile distilled water. Mix well and aseptically add to 500ml of sterile, molten, cooled (45-50°C) HiCrome™ ESBL Agar M1829. Mix well and pour into sterile Petri plates.

Type of specimen

Clinical samples - rectal screening swabs, faecal samples, etc. or from isolated colony

Specimen Collection and Handling

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (1,2). After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning & Precautions

In Vitro diagnostic use only. For professional use only. Read the label before opening the container. Wear protective gloves/ protective clothing/eye protection/face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling clinical specimens. Safety guidelines may be referred in individual safety data sheets.

Storage and Shelf Life

Store at 2 - 8°C. Use before expiry date on the label.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (1,2).

Reference

1. Isenberg (Ed.),2004, Clinical Microbiology Procedures Handbook, Vol.3, American Society for Microbiology, Washington. D.C.
2. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology,11th Edition. Vol. 1.

*** Not For Medicinal Use**

Revision : 02/2022

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IVD ***In vitro* diagnostic medical device**

CE **CE Marking**

2°C 8°C **Storage temperature**

Do not use if package is damaged

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Technical Data

Carba Selective Supplement FD357

Recommended for isolation of Carbapenem resistant *Enterobacteriaceae* from clinical samples.

Composition

Per vial sufficient for 1000 ml medium

*Ingredients	Concentration
Selective mix	50mg

Directions:

Rehydrate the contents of one vial aseptically with 2 ml of 0.2N NaOH and 8ml of sterile distilled water. Mix well and aseptically add to 1000 ml of sterile, molten, cooled (45-50°C) HiCrome™ CarbaResist Agar Base M2099. Mix well and pour into sterile Petri plates.

Type of specimen

Clinical samples

Specimen Collection and Handling

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (1,2). After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning & Precautions

In Vitro diagnostic use only. For professional use only. Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling clinical specimens. Safety guidelines may be referred in individual safety data sheets.

Storage and Shelf Life

Store at 2 - 8°C. Use before expiry date on the label.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (1,2).

Reference

1. Isenberg (Ed.),2004, Clinical Microbiology Procedures Handbook, Vol.3, American Society for Microbiology, Washington. D.C.
2. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology,11th Edition. Vol. 1.

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IVD ***In vitro* diagnostic medical device**

CE Marking

2°C 8°C **Storage temperature**

Do not use if package is damaged

Charcoal Agar Base

M344

Intended Use:

Recommended for cultivation of *Bordetella pertussis*, for vaccine production and also for stock culture maintenance.

Composition**

Ingredients	g / L
HM infusion B from 500g #	12.000
Peptone	10.000
Yeast extract	3.500
Starch, soluble	10.000
Charcoal	4.000
Sodium chloride	5.000
Agar	18.000
Final pH (at 25°C)	7.3±0.2

**Formula adjusted, standardized to suit performance parameters

#Equivalent to beef heart, infusion from

Directions

Suspend 31.25 grams in 450 ml purified/distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50° and aseptically add sterile 10% of defibrinated blood and rehydrated contents of one vial of Bos Selective Supplement (FD004).Mix well and pour into sterile Petri plates. For *Haemophilus* species, the medium can be converted to Chocolate Agar.

Principle And Interpretation

The genus *Bordetella* contains four species: *Bordetella pertussis, Bordetella parapertussis, Bordetella bronchiseptica* and Bordetella avium (1). Genetic studies have shown that these organisms are very closely related to each other. Humans are the only host of *B.pertussis* and *B.parapertussis,* while *B.bronchoseptica* is found in a wide variety of animals and occasionally found in humans (2). *B.avium* is found in birds. *Bordetella* species are obligately aerobic and metabolically not very active. They are non-motile except *B.bronchoseptica. B.pertussis* is the major cause of whooping cough or pertussis.

B.parapertussis is associated with a milder form of the disease (3). Primary isolation of *B.pertussis* in particular, requires the addition of charcoal, 15-20% blood to neutralize the growth-inhibiting effects. Isolation of this organism requires enrichment medium.Charcoal Agar is prepared according to the method of Mishulow, Sharpe and Cohen (2). This medium can be used as a replacement for Bordet-Gengou Agar for isolation of *B. pertussis* and for the production of *B.pertussis* vaccines. Charcoal Agar supplemented with horse blood can also be used for the cultivation and isolation of *Haemophilus influenzae* (4). The difficulty in the isolation of *Bordetella pertussis* from nasopharyngeal secretions is the repression of unwanted flora during the long incubation period on nutritious media. Penicillin can be added to the medium as an antimicrobial agent for restricting the other contaminants. However Penicillin resistant floras still cause contamination, which as observed by Lacey (4). Methicillin was found to be superior than Penicillin in suppressing unwanted nasopharyngeal flora as observed by Broome et al (5). Sutcliffe and Abbott found that Cephalexin was still better than Methicillin (6).

The ingredients like HM infusion B from, Peptone, yeast extract provide essential nutrients to the organisms. Sodium chloride maintains osmotic balance. Starch soluble and charcoal neutralizes substances toxic to *Bordetella* species such as fatty acids. Charcoal has the tendency to settle at the bottom of the flask. Therefore, before dispensing, swirl the flasks gently to obtain a uniform charcoal suspension (7).

Examine plates after 40 hours incubation and twice daily thereafter. Small shiny grayish white, round corner, colonies of *Bordetella* species are observed on plates. Confirm the findings with DFA i.e. Direct Fluorescent Antibody testing. To make earlier diagnosis, perform direct fluorescent antibody testing on the secretion.

Type of specimen

Nasal swabs

Specimen Collection and Handling:

Collect the nasal swabs in early stage of the illness and place in tubes of half strength Charcoal Agar Base supplemented with 10% v/v lysed defibrinated horse blood and Bos Selective Supplement (FD004). Generously inoculate the swabs on to thick layer of Charcoal Agar Base containing 10% v/v blood and Bos Selective Supplement (FD004). Non-selective medium (without FD004) may be used in addition. Replace the swab in the original transport medium and hold at room temperature. Incubate the plates at 35°C in a moist atmosphere (60-70% humidity) upto 6 days. After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions :

In Vitro diagnostic use only. For professional use only. Read the label before opening the container. Wear protective gloves/ protective clothing/eye protection/ face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling clinical specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations :

1. Swirl the flask gently when dispensing to obtain a uniform charcoal suspension.
2. Confirm the findings with DFA i.e. Direct Fluorescent Antibody testing.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

Quality Control

Appearance

Grey to greyish black homogeneous free flowing powder

Gelling

Firm, comparable with 1.8% Agar gel

Colour and Clarity of prepared medium

Black coloured, opaque gel with undissolved black particles forms in Petri plates

Reaction

Reaction of 6.25% w/v aqueous solution at 25°C. pH : 7.3±0.2

pH

7.10-7.50

Cultural Response

Cultural characteristics observed with added sterile defibrinated blood and Bos Selective Supplement (FD004), after an incubation at 35 - 37°C for 24 - 48 hours

Organism	Inoculum (CFU)	Growth	Recovery
Bordetella bronchiseptica ATCC 4617	50-100	good-luxuriant	>=50%
Bordetella parapertussis ATCC 15311	50-100	good-luxuriant	>=50%
Bordetella pertussis ATCC 8467	50-100	good-luxuriant	>=50%
Staphylococcus aureus subsp. aureus ATCC 25923 (00034*)	>=10^4	inhibited	0%
Klebsiella pneumoniae ATCC 13883 (00097*)	>=10^4	inhibited	0%

Key : *Corresponding WDCM numbers.

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 2-8°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition. Seal the container tightly after use. Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (8,9).

Reference

1. Murray P. R., Baron J. H., Pfaller M. A., Jorgensen J. H. and Yolken R. H., (Ed.), 2003, Manual of Clinical Microbiology, 8th Ed., American Society for Microbiology, Washington, D.C.
2. Mishulow, Sharpe and Cohen, 1953, Am. J. Public Health, 43:1466.
3. Ensminger P. W., Gulberston C. G. and Powell H. M., 1953. J. Infect. Dis., 93(3):266.
4. Lacey B. W., 1954, J. Hyg., 59:273
5. Broome C. V., Fraser D. W. and English J. W., 1979, Internat. Symp. on Pertussis DHEW J., Washington D.C., pp 19-29.
6. Sutcliffe E. M. and Abbott J. D., 1979, B.M.J. II: 732-733.
7. MacFaddin J. F., 1985, Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria, Vol. I, Williams and Wilkins, Baltimore.
8. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
9. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.

Revision : 04 / 2024

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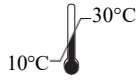

Storage temperature

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HiCrome™ ESBL Agar Base

M1829

Intended Use:

Recommended for selective isolation Extended-Spectrum ß-lactamase-Producing *Enterobacteriaeceae.*

Composition**

Ingredients	g/ L
Peptone mix	12.000
Chromogenic mixture	4.000
Sodium chloride	5.000
Buffer mix	4.000
Agar	15.000
Final pH (at 25°C)	6.8±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 40.0 gram in 1000 ml purified / distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C and add rehydrated contents of two vials of AC3F Selective Supplement (FD278). Mix well and pour into sterile Petri plates.

Principle And Interpretation

Extended-spectrum ß-lactamase (ESBL)-producing organisms are an increasing challenge for healthcare practitioners fighting healthcare-associated infections (HAIs). *Escherichia coli, Klebsiella pneumoniae,* and *Klebsiella oxytoca* are the most common ESBL-producing pathogens (1). ESBL-producing organisms are generally resistant to many classes of antibiotics, including aminoglycosides and fluoroquinolones; ESBL-producing organisms are able to attack newer cephems and monobactams as well as narrow-spectrum cephalosporins and antigram-negative penicillins (1). They are associated with increased mortality and are difficult to detect and treat. The widespread use of extended-spectrum, third-generation cephalosporins, introduced in the 1980's to treat antibiotic-resistant bacteria, is believed to be a major contributor to the emergence of ESBL-producing organisms.

HiCrome™ ESBL Agar Base is chromogenic screening medium for the selective isolation of ESBL producing organisms. It contains peptone mix which serves as the carbon and nitrogen sources, long chain amino acids, vitamins and other growth nutrients. Chromogenic mixture is used to differentiate the ESBL producing organisms on the basis of colour. AC3F Selective Supplement (FD278) helps in inhibition of other contaminating organisms. ESBL producing *E.coli* grow as either pink or purple colonies.

ESBL producing members of the KESC group produce bluish green colonies; *Proteus, Morganella* and *Providencia* do not utilize any chromogen resulting in colourless to light brown colonies. This medium can be inoculated with liquid suspension equivalent to 0.5 McFarland turbidity, prepared from rectal screening swabs, faecal samples or from isolated colony. Isolated colonies should not be directly plated on to this medium, because the high level inoculum may cause false positive results. Further confirmation using biochemical identification tests is recommended.

Type of specimen

Clinical samples - rectal screening swabs, urine, faecal samples, etc. or from isolated colony.

Specimen Collection and Handling

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (2,3).
After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions

In Vitro diagnostic Use only. For professional use only. Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/ face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling clinical specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations

1. Some species may show poor growth due to nutritional variations.
2. Slight colour variation may be observed depending upon strains.
3. Isolated colonies should not be directly plated on to this medium, because the high level inoculum may cause false positive results.
4. Further confirmation using biochemical identification tests is recommended.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

Quality Control

Appearance

Cream to yellow homogeneous free flowing powder

Gelling

Firm, comparable with 1.5% Agar gel

Colour and Clarity of prepared medium

Yellow coloured opalescent gel forms in Petri plates

Reaction

Reaction of 4.0% w/v aqueous solution at 25°C. pH : 6.8±0.2

pH

6.60-7.00

Cultural Response

Cultural characteristics observed after an incubation at 35-37°C for 24 hours with added AC3F Selective Supplement (FD278).

Organism	Inoculum (CFU)	Growth	Recovery	Colour of Colony
Escherichia coli NCTC 13351	50-100	luxuriant	>=50%	pink to purple
Klebsiella pneumoniae ATCC 700603	50-100	luxuriant	>=50%	bluish green
Enterobacter cloacae ATCC 23355	>=10^4	inhibited	0%	-
Citrobacter freundii ATCC 8090	>=10^4	inhibited	0%	
Candida albicans ATCC 10231 (00054*)	>=10^4	inhibited	0%	

Key : (*) Corresponding WDCM numbers.

Storage and Shelf Life

Store between 15-25°C in a tightly closed container and the prepared medium at 2-8°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition Seal the container tightly after use. Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (2, 3).

Reference

1. Journal of Clinical Microbiology, February 2007, Page 501-505, Vol. 45, No. 2
2. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition
3. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015), Manual of Clinical Microbiology, 11th Edition. Vol. 1.

Revision : 06/2024

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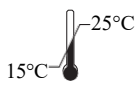
IVD ***In vitro* diagnostic medical device**



Storage temperature

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CE Marking

Do not use if package is damaged

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HiCrome™ VRE Agar Base

M1830

Intended Use

Recommended as a selective media for isolation of Vancomycin Resistant Enterococci (VRE) from clinical specimens.

Composition**

Ingredients	g / L
Peptone special	25.000
Chromogenic mixture	0.450
Sodium chloride	5.000
Buffering agent	1.250
Salt mixture	4.250
Agar	15.000
Final pH (at 25°C)	6.5±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 50.95 gram in 1000 ml purified / distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C)for 15 minutes. Cool to 45-50°C and aseptically add the rehydrated contents of two vials of VF Selective Supplement (FD277). Mix well and pour into sterile Petri plates.

Principle And Interpretation

Enterococci are the common habitants of the normal flora residing in the intestines of mammals (1). Vancomycin Resistant Enterococci are the group of Enterococci that have developed resistance towards many antibiotics particularly vancomycin. Enterococcal infections that result in human disease can be fatal, particularly those caused by strains of vancomycin-resistant enterococci (VRE) (2). Early detection of VRE is important to prevent the emergence of vancomycin resistant in *Enterococcus faecalis*. VRE can be transmitted from person to person, especially in a hospital or chronic-care facility. Microscopic amounts of fecal material from an infected or colonized patient can contaminate the hospital environment and be a reason for the spread of infection. There are many traditional media for the detection of VRE which includes Vancomycin Resistant Enterococci Broth Base/Agar or Bile Esculin Agar supplemented with vancomycin. Peptone special in the medium supplies nitrogenous and carbonaceous compounds, long chain amino acids, vitamins and other necessary nutrients required for the growth of microorganisms. Sodium chloride maintains the osmotic balance. Buffering agents provides buffering to the medium. *Enterococcus faecalis* cleaves the chromogenic substrate in the medium to produce blue coloured colonies, which are clearly visible against the opaque background. The supplement added to the medium allows the selective isolation of Vancomycin Resistant Enterococci. This medium can be inoculated directly from screening swab, isolated colony prepared as a liquid suspension approximately equivalent to 0.5 McFarland turbidity.

Type of specimen

Clinical samples - faeces, urine, etc.

Specimen Collection and Handling

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (3,4).
After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions

In Vitro diagnostic Use only. For professional use only. Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/ face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations

1. Some intermediate species may show poor growth due to nutritional variations and tolerance to vancomycin.
2. Slight colour variation may be observed depending upon the utilization of the substrate by the organism.
3. Interspecies differentiation between *Enterococcus faecalis* and *Enterococcus faecium* cannot be confirmed.
4. Further confirmation has to be carried using sensitivity testing.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

Quality Control

Appearance

Cream to yellow homogeneous free flowing powder

Gelling

Firm, comparable with 1.5% Agar gel.

Colour and Clarity of prepared medium

Off white coloured opaque gel forms in Petri plates.

Reaction

Reaction of 5.1% w/v aqueous solution at 25°C. pH : 6.5±0.2

pH

6.30-6.70

Cultural Response

Cultural characteristics observed with added VF Selective Supplement (FD277), after an incubationat 35-37°C for 24-48 hours.

Organism	Inoculum (CFU)	Growth	Recovery	Colour of colony
Enterococcus faecalis (VRE) ATCC 51299 (00085*)	50-100	luxuriant	>=50%	bluish green
Enterococcus faecalis ATCC 29212 (00087*)	>=10^4	inhibited	0%	
Staphylococcus aureus subsp. *aureus* ATCC 25923 (00034*)	>=10^4	inhibited	0%	

Key :* Corresponding WDCM numbers.

Storage and Shelf Life

Store between 15-25°C in a tightly closed container and the prepared medium at 2-8°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition Seal the container tightly after use. Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with sample must be decontaminated and disposed of in accordance with current laboratory techniques (3,4).

Reference

1.Mara D., Horan NJ: The Handbook of water, wastewater and microbiology, Amsterdam, The Netherlands, Academic Press; 2003.

2.Mascini EM, Bonten MJ: Vancomycin- resistant enterococci: consequences for therapy and infection control. Clin Microbiol Infect.2005,11 (Suppl.4) :43-56

3.Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.

4.Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.

Revision :07/2024

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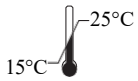
IVD ***In vitro*** **diagnostic medical device**



Storage temperature

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CE Marking

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HiCrome™ MRSA Agar Base, Modified

M1953

Intended Use:

Recommended for the differentiation and identification of MRSA and MRSE *Staphylococcus* species from clinical samples.

Composition**

Ingredients	g / L
Peptone	23.000
Sodium chloride	10.000
Sodium puruvate	5.000
Chromogenic substrate	0.770
Inhibitor mixture	7.000
Agar	15.000
Final pH (at 25°C)	7.2±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 30.38 gram in 500 ml distilled water. Heat to boiling to dissolve the medium completely. **DO NOT AUTOCLAVE.** Cool to 45-50°C. Aseptically add sterile rehydrated contents of 1 vial of MeRS Selective Supplement (FD229) or CF Selective Supplement II (FD259) or both in combination for more selectivity as desired. Mix well and pour into sterile Petri plates.

Principle And Interpretation

MRSA is a resistant variation of the common bacterium *Staphylococcus aureus* and MRSE is a resistant variation of the common bacterium *Staphylococcus epidermidis*. *Staphylococcus aureus* is an invasive pathogen that can cause disease in almost any tissue or organ in the human body, primarily in compromised individuals (1,2). Staphylococcal infections were earlier treated using Penicillin. But over the years resistance to this drug developed. Methicillin was the next drug of choice. While methicillin is very effective in treating most Staphylococcus infections, some strains have developed resistance to methicillin and can no longer be killed by this antibiotic. These resistant bacteria are called Methicillin Resistant *Staphylococcus aureus* (MRSA) (3).

Patients with breaks in their skin due to wounds, indwelling catheters or burns are those with certain risk of developing MRSA infection. Spread of MRSA infections can be controlled to a great extent by maintaining personal hygiene after interaction with an MRSA infected person (3).

Peptone provide the essential nutrients along with carbonaceous, nitrogenous and Vitamin B complex nutrients. The chromogenic mixture incorporated in the medium is specifically cleaved by *Staphylococcus aureus* to give green coloured colonies. Sodium pyruvate enhances the growth of *Staphylococcus* species. Sodium chloride in the medium helps to maintain the osmotic equilibrium of the medium. High concentration of sodium chloride also helps in inhibiting the accompanying microflora. Cefoxitin is recommended to use for selective isolation of MRSA. The medium is made selective for MRSA by the addition of MeRS Selective Supplement (FD229) or CF Selective Supplement II (FD259) or both in combination.

Type of specimen

Clinical samples - Mouth, skin lesions, intestine, upper respiratory tract of humans, urine, wound samples, etc.

Specimen Collection and Handling

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (4,5).
After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions

In Vitro diagnostic use only. For professional use only. Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling clinical specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations

1. Some intermediate strains may show poor growth due to nutritional variations and resistance to methicillin/cefoxitin.
2. Slight colour variation may be observed depending upon the utilization of the substrate by the organism.
3. Further confirmation must be carried out by sensitivity testing.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

Quality Control

Appearance

Cream to beige homogeneous free flowing powder

Gelling

Firm, comparable with 1.5% Agar gel

Colour and Clarity of prepared medium

Light purple, clear to slightly opalescent gel forms in Petri plates

Reaction

Reaction of 6.08% w/v aqueous solution 25°C. pH : 7.2±0.2

pH

7.00-7.40

Cultural Response

Cultural characteristics observed with added MeRS Selective Supplement (FD229) or CF Selective Supplement II (FD259) or both after an incubation at 35-37°C for 18-48 hours.

Organism	Inoculum (CFU)	Growth	Recovery	Growth w/ FD229 or FD259 or both	Recovery w/ FD229 or FD259 or both	Colour of Colony
Escherichia coli ATCC 25922 (00013*)	50-100	poor-good	30-40%	inhibited	0%	purple
Staphylococcus aureus, MRSA ATCC 43300	50-100	luxuriant	>=50%	luxuriant	>=50%	green
Staphylococcus epidermidis, MRSE	50-100	luxuriant	>=50%	luxuriant	>=50%	blue
Staphylococcus xylosus ATCC 29971	50-100	luxuriant	>=50%	inhibited	0%	blue
Enterococcus faecalis ATCC 29212 (00087*)	50-100	none-poor	<=10%	inhibited	0%	

Key : (*) Corresponding WDCM numbers

Storage and Shelf Life

Store between 15-25°C in a tightly closed container and the prepared medium at 2-8°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition Seal the container tightly after use. Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (4,5).

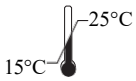
Reference

1.DWorkin M et. al 2006. The Prokaryotes (a Handbook on the Biology of Bacteria) 3rd ed, Vol. 2, page 345.
2.Dr. Alan Johnson, methicillin resistant staphylococcus aureus (MRSA) infection. The Support group for MSRA sufferers and Dependents, Aug 1st , 2005.
3.Methicillin Resistant Staphylococcus aureus Copyright a 1997-2005 Canadian Centre for Occupational Health and Safety, Sept 19th, 2005.
4.Isenberg, H.D. Clinical Microbiology Procedures Handbook. 2nd Edition.
5.Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.

Revision : 04/ 2024

HiMedia Laboratories Pvt. Limited,
Plot No.C-40, Road No.21Y,
MIDC, Wagle Industrial Area,
Thane (W) -400604, MS, India

IVD ***In vitro*** **diagnostic medical device**



Storage temperature

EC REP CEpartner4U, Esdoornlaan 13,
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www.cepartner4u.eu

CE Marking

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HiCrome™ CarbaResist Agar Base

M2099

Intended Use:

Recommended for isolation and differentiation of Carbapenem resistant *Enterobactericeae* from clinical samples.

Composition**

Ingredients	g / L
Acicase#	24.000
Chromogenic mixture	1.500
Agar	17.000
Final pH (at 25°C)	7.2±0.2

**Formula adjusted, standardized to suit performance parameters

Casein Acid Hydrolysate

Directions

Suspend 42.50 gram in 1000 ml purified / distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15lbs pressure (121°C) for 15 minutes. Cool to 45-50°C. Aseptically add rehydrated content of 1 vial of Carba Selective Supplement (FD357). Mix well and pour into sterile Petri plates.

Principle And Interpretation

HiCrome™ CarbaResist Agar Base is a chromogenic medium designed for the detection and differentiation of Carbapenemase producing *Enterobacteriaceae* species. Carbapenems are the last line of defense against invasive or serious infections and are used to treat these life threatening infections that are caused by gram negative, drug resistant pathogens (1). Production of carbapenemase enzyme results in resistance to penicillins, cephalosporins (i.e. cefepime, ceftriaxone), carbapenems (i.e. meropenem, ertapenem) and aztreonam there by making these pathogens multi drug resistant. Most carbapenemase producing bacteria are included in the family *Enterobacteriaceae*, and are thus termed as carbapenem resistant *Enterobacteriaceae* (CRE). Besides the *Enterobacteriaceae* family, rare strains of *Pseudomonas aeruginosa* and *Acinetobacter baumannii* have also been found to produce catrbapenemase (1,2,3).

Acicase provide nitrogenous and carbonaceous compounds, long chain amino acids, sulphur and other essential nutrients. Chromogenic mixture incorporated helps in colour differentiation. The chromogenic substrates are specifically cleaved by enzyme ß-D-galactosidase produced by colistin resistant *E.coli* resulting in pink to purple coloured colonies. Whereas colistin resistant *K. pneumoniae* cleaves the other chromogenic substrate producing metallic blue coloured colonies. *Pseudomonas* species produce colourless colonies or may produce with light greenish pigment. The medium is intended to be used as a screening medium. Isolates should be tested further for Carbapenem susceptibility following CLSI guidelines.

Type of specimen

Clinical Samples

Specimen Collection and Handling

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (5,6).
After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions

In Vitro diagnostic use only. Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling clinical specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations

1. Final identification must be carried out by biochemical tests or antibiotic susceptibility as per CLSI.
2. Some intermediate strains of carbapenem may show poor growth.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

Quality Control

Appearance

Cream to yellow homogeneous free flowing powder

Gelling

Firm, comparable with 1.7% agar gel.

Colour and Clarity of prepared medium

Light amber coloured clear to slight opalscent gel froms in Petri plates.

Reaction

Reaction of 4.25% w/v aqueous solution at 25°C. pH : 7.2±0.2

pH

7.00-7.40

Cultural Response

Cultural characteristics observed after incubation at 35-37°C for 18 -24 hours.

Organism	Inoculum (CFU)	Growth	Recovery	Colour of Colony
Carba Resistant *Klebsiella pneumoniae* ATCC BAA 1705	50 -100	luxuriant	>=50 %	metallic blue
Carba Resistant *Klebsiella pneumoniae* NCTC 13438	50 -100	luxuriant	>=50 %	metallic blue
Carba Resistant *Escherichia coli*	50 -100	luxuriant	>=50 %	pink-purple
Carba Sensitive *Enterobactericeae*	>=10^4	inhibited	0 %	-
Enterococcus faecalis 29212 (00087*)	>=10^4	inhibited	0 %	-

Key: (*) Corresponding WDCM numbers

Storage and Shelf Life

Store between 15-25°C and prepared medium on receipt at 2-8°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition Seal the container tightly after use. Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (4,5).

Reference

1. Samra, Z., 2008, J. Clin. Microbiol; Vol. 146, P.3110-3111.
2. Hindiyeth, M., et. al. 2008, J. Clin. Microbiol.; Vol. 46, p.2879 -2883.
3. Pillai D.R. et.al. 2009. Emerg. Infect. Dis; Vol. 15, P.827-829.
4. Isenberg, H.D. Clinical Microbiology Procedures Handbook. 2nd Edition.
5. Jorgensen, J.H., Pfaller , M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W.(2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.

Revision : 01 / 2024

HiMedia Laboratories Pvt. Limited,
Plot No.C-40, Road No.21Y,
MIDC, Wagle Industrial Area,
Thane (W) -400604, MS, India

IVD ***In vitro*** **diagnostic medical device**

25°C
15°C **Storage temperature**

EC REP CEpartner4U, Esdoornlaan 13,
3951DB Maarn, NL
www.cepartner4u.eu

CE Marking

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Mast Group Ltd.
Mast House, Derby Road, Bootle
Liverpool, Merseyside, L20 1EA
United Kingdom
Tel: + 44 (0) 151 472 1444
Fax: + 44 (0) 151 944 1332
email: sales@mast-group.com
Web: www.mast-group.com

Mast Diagnostica GmbH
Feldstrasse 20
DE-23858 Reinfeld
Germany
Tel: + 49 (0) 4533 2007 0
Fax: + 49 (0) 4533 2007 68
email: mast@mast-diagnostica.de
Web: www.mast-group.com

Mast Diagnostic
12 rue Jean-Jacques Mention
CS91106, 80011 Amiens, CEDEX 1
France
Tél: + 33 (0) 3 22 80 80 67
Fax: + 33 (0) 3 22 80 99 22
email: info@mast-diagnostic.fr
Web: www.mast-group.com

MAST® CARBA PAcE

Intended Use

PACE-ID. For the rapid detection of carbapenemase producing Enterobacterales, *Pseudomonas*, OXA 48 and 23-like enzyme production in *Acinetobacter.*

FOR IN VITRO DIAGNOSTIC USE ONLY

Contents

- **Vial PEL.** Freeze dried pellet* - 4 vials containing inhibitors and lysis components, each designed for 12 tests.
- **Vial RB.** Reconstitution buffer* - 4 vials containing chromogenic indicator resuspension buffer, each sufficient for 12 tests.
- Plastic 0.5 ml tubes, sufficient for 48 tests.

Storage and shelf life

Store at 2 to 8°C in the containers provided until the expiry date shown on the pack label. Allow to equilibrate to room temperature before opening. Once reconstituted, test solution stored at 2 to 8°C, must be used within 4 weeks.

Precautions

For *in vitro* diagnostic use only. Observe approved biohazard and aseptic techniques. To be used by only trained and qualified laboratory personnel. Sterilise all biohazard waste before disposal. Refer to product safety data sheets.

Materials required but not provided

Standard microbiological supplies and equipment such as loops, MAST Group Ltd. culture media, table top vortexes, pipettes, incinerators and incubators, etc.

Procedure

1. Reconstitute the pellet by tipping the entire contents of vial RB into vial PEL.
2. Allow the pellet to fully dissolve at room temperature for 1 minute and mix contents by gently vortexing for 10 seconds. Reconstituted solution should be yellow, if the solution is any other colour do not use.
3. Dispense 250µl of reconstituted solution into the tubes provided. One tube per test.
4. Using a pure, fresh culture of the test organism, take an approximate 1 to 5µl loopful of organism, and add to the tube containing test solution. Mix well by vortexing for 20 seconds.
   Note: to obtain distinct results, ensure that the bacterial resuspension is similar to the turbidity of a 3.0 to 3.5 McFarland standard; Approx. 10^9 CFU/ml.
5. Incubate at 35±1°C for 10 minutes.
6. Record the colour of the test solution immediately or up to 20 minutes after incubation.

Please refer to corresponding steps on the image page.

Interpretation of results

If a colour change is recorded; from yellow to orange/red, record the organism as demonstrating carbapenemase activity.
If no colour change is recorded; solution remains yellow, record the organism as negative for carbapenemase activity.

Quality control

Check for signs of deterioration. Quality control must be performed with at least one organism to demonstrate a positive reaction and another to demonstrate a negative reaction. Do not use the product if the reactions with the control organisms are incorrect. The list below illustrates a range of performance control strains which the end user can easily obtain.

Test Organism	Result
Acinetobacter baumanii NCTC 13301	Orange/Red Carbapenemase positive
Pseudomonas aeruginosa NCTC 13437	Orange/Red Carbapenemase positive
Acinetobacter lwoffi ATCC® 15309	Remains Yellow Carbapenemase negative
Pseudomonas aeruginosa ATCC® 25668	Remains Yellow Carbapenemase negative
Klebsiella pneumoniae NCTC 13438	Orange/Red Carbapenemase positive

Limitations

1. Colonies isolated from indicator media are not recommended.
2. This product only detects the presence of a carbapenemase, differentiation can be carried out by using a suitable genotypic or phenotypic test (for example **MAST**DISCS® *Combi Carba Plus*; D73C).
3. Some GES-type carbapenemases might be difficult to detect.
4. To avoid potentially erroneous results, ensure that equipment used for testing is free of contamination.
5. Test results must be recorded within 20 minutes following the initial 10 minute incubation.
6. Results obtained with this kit must be considered alongside other clinically relevant data when diagnosing an infection.

References

Bibliography available on request.

Acknowledgement

HMRZ compound used in this product was developed by Dr. Hideaki Hanaki of Kitasato, Institute, Japan.

IFU159 GB 05/19 V2

*Formulation may change to meet performance criteria

Mast Group Ltd.
Mast House, Derby Road, Bootle
Liverpool, Merseyside, L20 1EA
United Kingdom
Tel: + 44 (0) 151 472 1444
Fax: + 44 (0) 151 944 1332
email: sales@mast-group.com
Web: www.mast-group.com

Mast Diagnostica GmbH
Feldstrasse 20
DE-23858 Reinfeld
Germany
Tel: + 49 (0) 4533 2007 0
Fax: + 49 (0) 4533 2007 68
email: mast@mast-diagnostica.de
Web: www.mast-group.com

Mast Diagnostic
12 rue Jean-Jacques Mention
CS91106, 80011 Amiens, CEDEX 1
France
Tél: + 33 (0) 3 22 80 80 67
Fax: + 33 (0) 3 22 80 99 22
email: info@mast-diagnostic.fr
Web: www.mast-group.com

Mast Group

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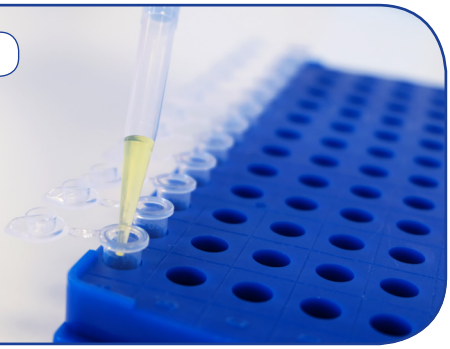

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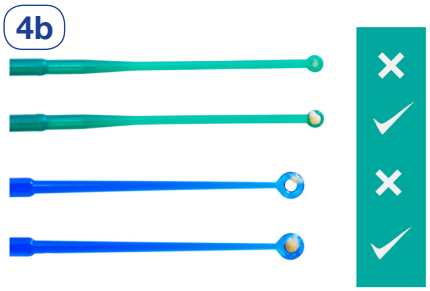

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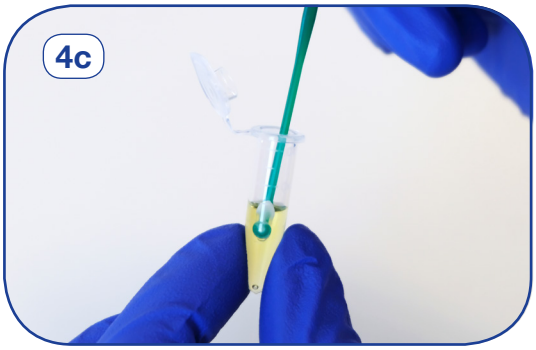

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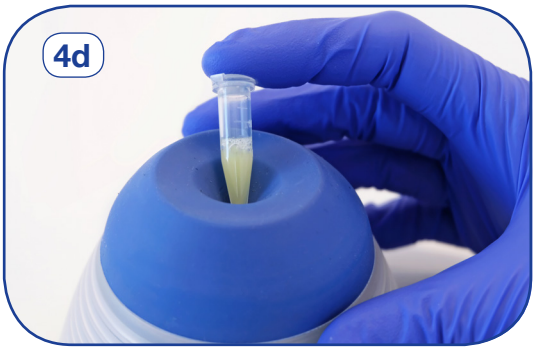

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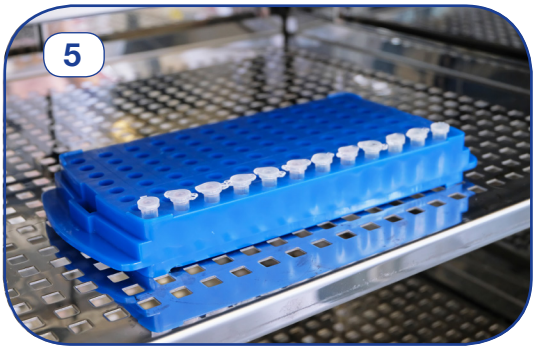

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