Optochin Discs

DD009

Intended use

Recommended for identification and differentiation of *Streptococcus pneumoniae* and viridans streptococci.

Directions

Prepare Soyabean Casein Digest Agar (M290) w/blood or Blood Agar Base (M073) w/blood plates and streak pure culture of organism to be tested across one half of the plate. Streak a known *Pneumococcus* (for eg. *Streptococcus pneumoniae*) culture across the other half of the plate as positive control. Immediately place Optochin discs in the centre of the two halves of the plate and incubate at 35-37°C for 18-24 hours in 5% CO_2. Following incubation observe for zone of inhibition around the discs.

Principle And Interpretation

Alpha haemolytic (viridans) streptococci and *Pneumococcus* (*Streptococcus pneumoniae*) cannot be easily distinguished on Blood Agar plates as pneumococci strain shows partial clearing of blood and greenish discolouration (α-haemolysis). Optochin is inhibitory for pneumococcal growth whereas other streptococci strains show good growth or a very small zone of inhibition. Bowers and Jeffries have shown a correlation between bile solubility and full Optochin susceptibility for the differentiation of *Streptococcus pneumoniae* from other streptococci (1). Hence optochin test is a useful diagnostic aid for identification /differentiation of pneumococci and viridans streptococci. Optochin discs are filter paper discs impregnated with optochin. The test is based on the property of viridans streptococci to grow in the presence of Optochin (ethyl hydrocuprein hydrochloride) which inhibits pneumococci. This test is performed for the diagnosis of penumococcal infections. Specimens of sputum, lung aspirate or urine are first examined by Gram's stain, cultured and the isolates are then subjected to Optochin Sensitivity Test.

Type of specimen

Clinical samples - pure isolate

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 clinical 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. Other biochemical and serological tests must be performed for confirmation.

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

Filter paper discs of 6 mm diameter bearing letters "Op" in continuous printing style

Cultural Response

DD009 : Cultural response observed after an incubation at 35-37°C for 18-24 hours in 5% CO_2 on seeded Soyabean Casein Digest Agar (M290) with added sterile defibrinated blood using Optochin discs.

Organism	Diameter of Zone of Inhibition & Interpretation
Streptococcus pneumoniae ATCC 6303	More than or equal to 15 mm, Sensitive
Streptococcus pyogenes ATCC 19615	No zone of inhibition or a minimal zone, Resistant

Storage and Shelf Life

Store between 2-8°C in a tightly closed container. Use before expiry date on the label. 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. Bowers. E.F. and Jeffries L.R., 1995, J. Clin. Path., 8:58.
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.

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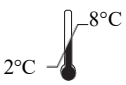
IVD ***In vitro*** **diagnostic medical device**

CE Marking



Storage temperature

Do not use if package is damaged

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Oxidase Discs

DD018

Intended Use

Recommended for detection of oxidase production by microorganisms like *Neisseria, Alcaligenes, Aeromonas, Vibrio, Campylobacter and Pseudomonas* which give positive reactions and for excluding *Enterobacteriaceae*, which give negative reactions.

Directions

Oxidase reaction is carried out by touching and spreading a well isolated colony on the oxidase disc. The reaction is observed within 5-10 seconds at 25-30°C. A change later than 10 seconds or no change at all is considered negative reaction.

Principle And Interpretation

Certain bacteria posses either cytochrome oxidase or indophenol oxidase (an iron-containing haemoprotein), which catalyzes the transport of electrons from donor compounds (NADH) to electron acceptors (usually oxygen). In the oxidase test, a colourless dye such as N, N-dimethy-p-phenylenediamine serves as an artificial electron acceptor for the enzyme oxidase (1). The dye is oxidized to form indophenol blue, a coloured compound. The test is useful in the initial characterization of aerobic gram-negative bacteria of the genera *Aeromonas, Plesiomonas, Pseudomonas, Campylobacter* and *Pasteurella*. Oxidase discs are sterile filter paper discs impregnated with N, N-dimethyl-p-phenylenediamine oxalate, ascorbic acid and a-naphthol. These discs overcome the neccessity of daily preparation of fresh reagent. Gordon and McLeod (2) introduced oxidase test for identifying gonococci based upon the ability of certain bacteria to produce indophenol blue from the oxidation of dimethyl-p-phenylenediamine and a-naphthol. Gaby and Hadley (3) introduced a more sensitive method by using N,N-dimethyl-p-phenylenediamine oxalate where all staphylococci were oxidase negative. In a positive reaction the enzyme cytochrome oxidase combines with N,N-dimethyl-p-phenylenediamine oxalate and a-naphthol to form the dye, indophenol blue.

Type of specimen

Isolated Microorganism

Specimen Collection and Handling

For microbial specimens, 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.Do not use stainless steel or nichrome inoculating wires, as false positive reaction may result from surface oxidation products formed during flame sterilization.
2.Growth from media containing dyes is not suitable for testing.
3.Timing is critical (5-10 sec) for interpretation of results.
4.Perform oxidase test on all gram-negative bacilli.
5.Cytochrome oxidase production may be inhibited by acid production. False negative reactions may be exhibited by *Vibrio, Aeromonas* and *Plesiomonas* species when grown on a medium containing fermentable carbohydrate e.g. MacConkey Agar (M081). Colonies taken from media containing nitrate may give unreliable results. The loss of activity of the oxidase reagent is caused by auto-oxidation which may be avoided by adding 0.1% ascorbic acid (6).

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

Filter paper discs of 10 mm diameter

Cultural response

Typical oxidase reaction given by 18-48 hour culture observed within 5-10 seconds at 25-30°C.

Organism	Reaction Observed
Pseudomonas aeruginosa ATCC 27853 (00025*)	positive : deep purplish blue colouration of disc
Neisseria gonorrhoeae ATCC 19424	positive : deep purplish blue colouration of disc
Escherichia coli ATCC 25922 (00013*)	negative : purplish blue colouration after 10 sec/ no colour change
Staphylococcus aureus subsp. *aureus* ATCC 25923 (00034*)	negative : no colour change

Key : (*) Corresponding WDCM numbers.

Storage and Shelf Life

Store at 2 - 8°C. Use before expiry date on the label. 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. Biochemical tests for Identification of Medical Bacteria, 3rd Edition, Jean F. MacFaddin.
2. Gordon J. and Mcleod J.W., 1928, J. Path. Bact., 31:185
3. Gaby W.L and Hadley C., 1957. J. Bact., 74:356
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.

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CE Marking

2°C 8°C **Storage temperature**

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X Factor

DD020

Intended Use

Recommended for the presumptive identification of *Haemophilus* species on the basis of their requirements for X or V factors or both.

Directions

Inoculate the surface of BHI Agar (M211) plate with the test organisms by either streaking or surface spreading. Aseptically place the X (DD020), V (DD021) and X+V (DD022) factor discs on the plate, in the following positions:
Disc Position on the Agar plate

X factor disc 12 O' clock

V factor disc 4 O' clock

X+V factor disc 8 O' clock

Incubate the plates at 35 - 37°C for 24 - 48 hours. Observe for the growth in the neighborhood of the discs.

Principle And Interpretation

Both X and V factors are growth factors that are essential for certain organisms like *Haemophilus* species and also enhance growth of organisms like *Neisseria* species. X-factor discs are the sterile filter paper discs impregnated with growth factor X which are used for differentiating *Haemophilus* species in conjuction of V factor & X+V factor discs. *Bordetella* and *Haemophilus* species can also be identified on the basis of the requirement of X and V growth factors in the basal medium. The X factor (hemin) and V factor (Coenzyme- Nicotinamide adenine dinucleotide NAD+) are impregnated on the sterile filter paper discs diameter 6mm The test organism requiring X factor alone, grows only in the vicinities of X and X+V factor discs. Those which require V factor alone grow in the vicinities of V and X+V factor discs. If both X and V factors are required, then the organism will grow only in the vicinity of the X+V factor discs. This satellite growth is seen around the disc promoting growth (1).

Type of specimen

Isolated Microorganism

Specimen Collection and Handling

For microbial specimens, 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. Further biochemical or serological testing is recommended to identify the organism accurately.
2. Also some species of *Haemophilus* shows similarities in growth factor requirements.
3. Do not use too heavy suspension of the test organisms as X or V factor carryover medium from the primary growth may take place.

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

Dark Brown to Grayish colour Filter paper discs of 6 mm diameter bearing letters "X" in continuous printing style.

Cultural response

Cultural characteristics observed on BHI Agar (M211) after an incubation at 35-37°C for 24-48 hours.

Organism	Growth with X factor	Growth without growth factor
Haemophilus parainfluenzae ATCC 7901	Negative	Negative
Haemophilus influenzae ATCC 19418	Positive	Negative
Haemophilus influenzae ATCC 49247	Positive	Negative
Haemophilus influenzae ATCC 49766	Positive	Negative
Haemophilus influenzae ATCC 10211	Positive	Negative

Storage and Shelf Life

Store below (-20°C to -10°C). Use before expiry date on the label. 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. Murray PR, Baron EJ, Jorgensen J.H., Pfaller M A, Yolken R.H(Eds.),8th ed, 2003, Manual of Clinical Microbiology, ASM,Washington D.C.

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 : 04/ 2025

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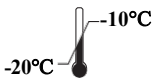

Storage temperature

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CE Marking

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V Factor

DD021

Intended Use

Recommended for the presumptive identification of *Haemophilus* species on the basis of their requirements for X or V factors or both.

Directions

Inoculate the surface of BHI Agar (M211) plate with the test organisms by either streaking or surface spreading. Aseptically place the X (DD020), V (DD021) and X+V (DD022) factor discs on the plate, in the following positions:
Disc Position on the Agar plate:

X factor disc 12 O' clock

V factor disc 4 O' clock

X+V factor disc 8 O' clock

Incubate the plates at 35 - 37°C for 24 - 48 hours. Observe for the growth in the neighbourhood of the discs.

Principle And Interpretation

Both X and V factors are growth factors that are essential for certain organisms like *Haemophilus* species and also enhance growth of organisms like *Neisseria* species. V-factor discs are the sterile filter paper discs impregnated with growth factor V which are used for differentiating *Haemophilus* species in conjuction of X factor & X+V factor discs. *Bordetella* and *Haemophilus* species can also be identified on the basis of the requirement of X and V growth factors in the basal medium. The X factor (hemin) and V factor (Coenzyme- Nicotinamide adenine dinucleotide NAD+) are impregnated on the sterile filter paper discs of diameter 6mm. The test organism requiring X factor alone, grows only in the vicinities of X and X+V factor discs. Those which require V factor alone grow in the vicinities of V and X+V factor discs. If both X and V factors are required, then the organism will grow only in the vicinity of the X+V factor discs. This satellite growth is seen around the disc promoting growth (1).

Type of specimen

Isolated Microorganism

Specimen Collection and Handling

For microbial specimens, 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. Further biochemical or serological testing is recommended to identify the organism accurately.
2. Also some species of *Haemophilus* shows similarities in growth factor requirements.
3. Do not use too heavy suspension of the test organisms as X or V factor carryover from the primary growth medium may take place.

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

Filter paper discs of 6 mm diameter bearing letters "V" in continuous printing style.

Cultural response

Cultural characteristics observed on BHI Agar (M211) after an incubation at 35-37°C for 24-48 hours.

Organism	Growth with V factor	Growth without growth factor
Haemophilus parainfluenzae ATCC 7901	Positive	Negative
Haemophilus influenzae ATCC 19418	Negative	Negative
Haemophilus influenzae ATCC 49247	Negative	Negative
Haemophilus influenzae ATCC 49766	Negative	Negative
Haemophilus influenzae ATCC 10211	Negative	Negative

Storage and Shelf Life

Store below (-20°C to -10°C). Use before expiry date on the label. 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

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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 : 04/ 2025

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-10°C -20°C **Storage temperature**

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X+V Factor

DD022

Intended Use

Recommended for the presumptive identification of *Haemophilus* species on the basis of their requirements for X or V factors or both.

Directions

Inoculate the surface of BHI Agar (M211) plate with the test organisms by either streaking or surface spreading.
Aseptically place the X (DD020), V (DD021) and X+V (DD022) factor discs on the plate, in the following positions:
Disc Position on the Agar plate

X factor disc 12 O' clock

V factor disc 4 O' clock

X+V factor disc 8 O' clock

Incubate the plates at 35 - 37°C for 24 - 48 hours. Observe for the growth in the neighbourhood of the discs.

Principle And Interpretation

Both X and V factors are growth factors that are essential for certain organisms like *Haemophilus* species and also enhance growth of organisms like *Neisseria* species. X+V factor discs are the sterile filter paper discs impregnated with growth factor X and V which are used for differentiating *Haemophilus* species in conjuction of X factor & V factor discs. *Bordetella* and *Haemophilus* species can also be identified on the basis of the requirement of X and V growth factors in the basal medium. The X factor (hemin) and V factor (Coenzyme- Nicotinamide adenine dinucleotide NAD +) are impregnated on the sterile filter paperdiscsofdiameter6mm. The test organism requiring X factor alone, grows only in the vicinities of X and X+V factor discs. Those which require V factor alone grow in the vicinities of V and X+V factor discs. If both X and V factors are required, then the organism will grow only in the vicinity of the X+V factor discs. This satellite growth is seen around the disc promoting growth (1).

Type of specimen

Isolated Microorganism

Specimen Collection and Handling

For microbial specimens, 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. Further biochemical or serological testing is recommended to identify the organism accurately.
2. Also some species of *Haemophilus* shows similarities in growth factor requirements.
3. Do not use too heavy suspension of the test organisms as X or V factor carryover from the primary growth medium may take place.

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

Dark Brown to Grayish colour Filter paper discs of 6 mm diameter bearing letters "X+V" in continuous printing style.

Cultural response

Cultural characteristics observed on BHI Agar (M211) after an incubation at 35-37°C for 24-48 hours.

Organism	Growth with X +V factor	Growth without growth factor
Haemophilus parainfluenzae ATCC 7901	Positive	Negative
Haemophilus influenzae ATCC 19418	Positive	Negative
Haemophilus influenzae ATCC 49247	Positive	Negative
Haemophilus influenzae ATCC 49766	Positive	Negative
Haemophilus influenzae ATCC 10211	Positive	Negative

Storage and Shelf Life

Store below (-20°C to -10°C). Use before expiry date on the label. 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).

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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.

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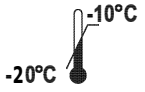

Storage temperature

Do not use if package is damaged

Bile Esculin Discs

DD024

Intended Use

Recommended for detection of esculin hydrolysis in the presence of bile, for differentiating Group D streptococci from other Streptococcal groups.

Directions

Esculin impregnated disc is placed on the seeded Bile Esculin Agar Base (M340) plate and is incubated at 35-37°C for 18-24 hours.

Principle And Interpretation

Group D streptococci hydrolyze esculin to esculetin and dextrose. Esculetin reacts with an iron salt such as ferric citrate to form a blackish brown coloured complex (1). Rochaix found that esculin hydrolysis is an important criteria in the identification of enterococci (2). Meyer and Schonfeld (3) observed that when bile was added to esculin medium, around 60% enterococci were able to grow and split the esculin while other streptococci could not. When a comparative study was performed by Facklam and Moody (4) for presumptive identification of Group D streptococci, they found the bile esculin test as a reliable means of identifying Group D streptococci and differentiating them from other streptococci groups.

Type of specimen

Isolated Microorganism

Specimen Collection and Handling

For microbial specimens, 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. 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. Use known Group D and non-Group D streptococci to determine the accuracy of the discs and inoculum.

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

Plain filter paper discs of 6mm diameter.

Cultural response

Cultural response observed by placing Bile Esculin disc (DD024) on seeded Bile Esculin Agar Base (M340) plate, incubated at 35-37°C for 18-24 hours.

Organism	Growth	Esculin hydrolysis
Enterococcus faecalis ATCC 29212 (00087*)	luxuriant	positive: blackening of media around the disc.
Streptococcus agalactiae ATCC 13813	luxuriant	negative: no blackening

Listeria monocytogenes ATCC 19118	luxuriant	positive: blackening of media around the disc.
Streptococcus pyogenes ATCC 19615	luxuriant	negative: no blackening

Key : (*) Corresponding WDCM numbers.

Storage and Shelf Life

Store at 2 - 8°C. Use before expiry date on the label. 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 (5,6).

Reference

1. MacFaddin J. F., 2000, Biochemical Tests for Identification of Medical Bacteria, 3rd ed., Philadelphia: Lippincott. Williams and Wilkins.
2. Rochaix, 1924, C. R. Soc. Biol., 90:771.
3. Meyer and Schonfeld, 1926, Zentralbl. Bacteriol. Parasitenkd. Infectionskr. Hyg. Abt. I Orig., 99:402.
4. Facklam and Moody, 1970, Appl. Microbiol., 20:245.
5. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
6. 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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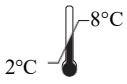

Storage temperature

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

CE Marking

Do not use if package is damaged

Disclaimer :

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.

Spore Strips

DD032

Intended use

Recommended as steam sterilization monitor strips, *Bacillus stearothermophilus* are used for evaluating sterilization process.

Directions

Place indicators in the areas of the pack or load least accessible to steam. Places such as the geometrical center, and the upper and lower regions of both front and rear of the load to be sterilized are considered suitable areas for placement of these indicators. A standard procedure should be established for the routine evaluation of each sterilizer. On completion of the sterilization cycle, remove the indicators from the test loads and deliver them to the laboratory for testing. All sterility tests should be performed in a clean dust free transfer area, preferably under positive air pressure, using rigid aseptic technique throughout the test procedure.

Using sterile scissors, cut open one end of the envelope. Thereafter remove the indicator with sterile tweezers and aseptically transfer it to a tube of sterile Tryptone Soya Broth w/ Yeast Extract and Hemin w/o Dextrose (M207) or Tryptone Soya Broth (M011). Incubate the tubes for seven days at 55 - 60°C. Observe the tubes daily. If turbidity develops, failure of the sterilization process is indicated.

Precautions:

The spore strips or broth cultures of *Bacillus stearothermophilus* must be autoclaved at 121°C for at least 30 minutes prior to discarding. Each spore strip is individually packaged in a steam-permeable envelope.

Principle And Interpretation

Bacillus stearothermophilus is a thermophilic bacteria which can grow at 65°C and above. The spores are highly heat resistant and are used to monitor autoclave performance (1). These indicators which are specified by the U.S. military specification MIL-S- 36586 are GMP requirements of U.S. FDA. Sterilization is the freeing of an article from all living organisms including viable spores (1). Sterilization quality control can only be achieved through the use of calibrated biological indicators (endospores). These indicators consist of *Bacillus stearothermophilus* spores impregnated on chromatography paper strips, individually placed into envelopes. Number of spores present per strip : 106. These organisms are difficult to destroy because they are more resistant to heat than other vegetative bacteria and viruses. Therefore, if they are destroyed during sterilization, it is assumed that all other life forms are also destroyed. This test is considered the most sensitive check of the autoclaves efficiency.

Type of specimen

Isolated micro-organism

Specimen Collection and Handling:

For microbial specimens, follow appropriate techniques for handling specimens as per established guidelines. After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions:

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.Other biochemical and serological tests must be performed to identify the organism accurately.

Quality Control

Appearance

Filter paper strip impregnated with spores of standard culture of *B.stearothermophilus* ATCC 7953

Number of spores

1000000 spores/strip

Cultural response

Sterility checking of the autoclave was carried out using Spore strip. After autoclaving, strip was inoculated in 100ml of sterile Tryptone Soya Broth (M011) and incubated at 55°C upto 7 days. An unexposed spore strip was also inoculated separately in 100ml (M011).

Growth	Unexposed Spore Strip	Exposed Spore Strip	Positive control	Negative control
Growth in M011	Luxuriant	No growth	Luxuriant	No growth

Storage and Shelf Life

Store between 15-27°C. Use before expiry date on the label. 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 ofthis product. Follow established laboratory procedures in disposing of infectious materials and material that into contact comes with sample must be decontaminated and disposed of in accordance with current laboratory techniques (2,3).

Reference

1.Mackie and McCartney, 1996, Practical Medical Microbiology, 14th ed., Vol. 2, Collee J. G., Fraser A. G., Marmion B, P., Simmons A (Eds.), Churchill Livingstone, Edinburgh.

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 : 02 / 2023

DMACA Indole Discs

DD040

Intended Use

Recommended for Indole test to determine the ability of an organism to split indole from the tryptophan molecule, and thus to aid differentiation between *Escherichia coli* from *Klebsiella*.

Directions

Place the DMACA Indole Disc on suspected colony from HiCrome™ UTI Agar (M1353) or HiCrome™ UTI Agar, Modified (M1418) plate. Observe for appearance of blue-purple colour within 10 - 30 seconds.

Principle And Interpretation

In the presence of oxygen, some bacteria are able to split tryptophan into indole and alpha-aminopropionic acid. The presence of indole can be detected by the addition of DMACA (p-Dimethylaminocinnamaldehyde) reagent indicated by formation of bluish-purple colour (1).

Type of specimen

Isolated Microorganism

Specimen Collection and Handling

For microbial specimens, 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.Do not use Mueller- Hinton Agar for test, because tryptophan is destroyed during the acid hydrolysis of casein.

2.Do not used media that contain dyes (e.g., EMB, MAC).

3.Do not use medium with a nitrate disc/strip to perform the indole test, as nitrate can interfere with indole test by showing false positive results.

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

Filter paper discs of 6 mm diameter bearing letters 'Dm' in continuous printing style.

Cultural response

The indole production by organisms was tested after an incubation of 18-24 hours at 35-37°C, using HiCrome™ UTI Agar (M1353) or HiCrome™ UTI Agar, Modified (M1418) plate.

Organism	Indole production
Escherichia coli ATCC 25922 (00013*)	positive reaction, blue purple colour formation
Pseudomonas aeruginosa ATCC 27853 (00025*)	negative reaction.
Klebsiella pneumoniae ATCC 13883 (00097*)	negative reaction.

Key : (*) Corresponding WDCM numbers.

Storage and Shelf Life

Store at 2 - 8°C. Use before expiry date on the label. 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. MacFaddin J. F., 1980, Biochemical Tests for Identification of Medical Bacteria, 2nd ed., Williams and Wilkins, Baltimore.

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 : 02/2023

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

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

CE Marking

2°C 8°C **Storage temperature**

Do not use if package is damaged

Disclaimer :

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.

Colistin Ezy MIC™ Strip (CL) (0.016-256 mcg/ml) EM020

Antimicrobial Susceptibility Testing
For *In Vitro* Diagnostic use

It is a unique MIC determination paper strip which is coated with Colistin on a single paper strip in a concentration gradient manner, capable of showing MICs in the range of 0.016mcg/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 for rapidly growing aerobic organisms as mentioned above.

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 pre-spread 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 Colistin, Amikacin, Vancomycin, Gentamicin, 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: Colistin showing reading of 0.75 mcg/ml should be rounded up to next concentration i.e. 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 strips 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 (mcg/ml)		
		≤ S	I	≥ R
Other non-*Enterobacterales*	35-37°C for 18 hrs.	2	4	8
Enterobacterales , Acinetobacter spp., *P.aeruginosa,*	35-37°C for 18 hrs.	-	≤ 2	4

Quality control:

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

Following are the reference MIC values (mcg/ml) range for Colistin.

Organism	Medium used	Incubation	Std. Quality Control limits (mcg/ml)
E.coli ATCC 25922[a]	Mueller Hinton Agar	35-37°C for 18 hrs.	0.25 - 0.5 – 1.0 - 2.0
P. aeruginosa ATCC 27853	Mueller Hinton Agar	35-37°C for 18 hrs.	0.25 - 0.5 – 1.0 - 2.0
E. coli NCTC 13846	Mueller Hinton Agar	35-37°C for 18 hrs.	2.0 - 4.0 – 8.0

[a] : Quality Control Limit deleted in CLSI 2024.

In-house Quality Control for Resistant Clinical Isolates :

Organism	Medium used	Incubation	MIC values obtained by repeated Microbroth dilution (mcg/ml)	MIC values obtained by Ezy MIC™ Strip (mcg/ml)
Col-Res Clinical Isolate 1	Mueller Hinton Agar	35-37°C for 18 hrs.	**32** (**Range:** 16.0 -32.0-64.0)	32, 32, 24, 32, 24
Col-Res Clinical Isolate 2	Mueller Hinton Agar	35-37°C for 18 hrs.	**16** (**Range:** 8.0 - 16.0 - 32.0)	16, 16, 8, 8, 12
Col-Res Clinical Isolate 3	Mueller Hinton Agar	35-37°C for 18 hrs.	**8** (**Range:** 4.0 - 8.0 - 16.0)	8, 8, 8, 4, 4

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) Colistin 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

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

Do not use if package is damaged

Vancomycin Ezy MIC™ Strip (VAN) (0.016-256 mcg/ml) EM060

Antimicrobial Susceptibility Testing
For *In Vitro* Diagnostic use

It is a unique MIC determination paper strip which is coated with Vancomycin in a concentration gradient manner, capable of showing MICs in the range of 0.016mcg/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.

CLSI RECOMMENDATION FOR VANCOMYCIN SENSITIVITY TEST

High molecular weight antibiotics such as Vancomycin, polymyxin B and colistin do not diffuse in concentration gradient manner while diffusing through the agar medium when the disc susceptibility test is employed. The Antimicrobial Susceptibility Testing using disc diffusion test does not differentiate vancomycin-susceptible isolates of *S.aureus* from Vancomycin intermediate isolates, nor does the test differentiates among Vancomycin–susceptible, intermediate, and resistant isolates of coagulase-negative staphylococci, all of which may give similar size zones of inhibition.

CLSI therefore recommends that MIC test should be performed to determine the susceptibility of all isolates of staphylococci to Vancomycin .[1]

Usefulness of Vancomycin Ezy MIC™ strip

1) Besides obtaining accurate MIC values for Gram- positive cultures, VISA (Vancomycin Intermediate *Staphylococcus aureus*) can be detected when isolated colonies appear within the zone of inhibition of Vancomycin particularly when 1.0 McFarland inoculum is used and MIC is read on full 48 hrs incubation. The sensitivity of the method can be further enhanced for better detection of VISA/ VRSA (Vancomycin Resistant *Staphylococcus aureus* / hVISA (Hetro Vancomycin Intermediate *Staphylococcus aureus*) using BHI agar with higher inoculum and 48 hr incubation.

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 yields 10^5 - 10^6 cells/ml).

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, and 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 supplemented with 5% sterile, defibrinated blood is recommended.
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 Vancomycin, Gentamicin, Amikacin, and members of β-lactams class of drugs, always read the MIC at the point of completion 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: Vancomycin showing reading of 0.75 mcg/ml should be rounded up to next concentration i.e. 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 strips 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 aureus	35-37°C for 18 hrs.	2	4-8	16
Enterococcus spp., *Staphylococcus* spp *other than S. aureus*	35-37°C for 18 hrs.	4	8-16	32
S. pneumoniae, Streptococcus spp. Beta haemolytic group, *Streptococcus* spp. Viridans group	35-37°C for 20-24 hrs at 5% CO_2	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 Vancomycin.

Organism	Medium used	Incubation	Std. Quality Control limits (mcg/ml)
S. aureus ATCC 29213	Mueller Hinton Agar	35-37°C for 18 hrs.	0.5 – 1.0 – 2.0
E. faecalis ATCC 29212	Mueller Hinton Agar	35-37°C for 18 hrs.	1.0 – 2.0 – 4.0
S. pneumoniae ATCC 49619	Mueller Hinton Agar w/ 5% Sheep Blood	35-37°C for 20-24 hrs at 5% CO_2	0.12 – 0.25 – 0.5

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) Vancomycin 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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Wagle Industrial Estate,
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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

Penicillin Ezy MIC™ Strip (PEN) (0.002-32 mcg/ml) EM084

Antimicrobial Susceptibility Testing
For *In Vitro* Diagnostic use

It is a unique MIC determination paper strip which is coated with Penicillin 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 and for *Neisseria gonorrhoeae*, GC Agar Base (M434) with 1% defined growth supplement (FD025) is recommended. For *B. fragilis*, Brucella Agar with Hemin and Vitamin K1, supplemented with 5 % v/v defibrinated sterile sheep blood is recommended.
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 Penicillin and other members of β-lactams class of drugs, Amikacin, Vancomycin, Gentamicin, 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: Penicillin 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.

When testing	Incubation	Interpretive Criteria		
		≤ S	I	≥ R
Staphylococcus spp	35-37°C for 18 hrs.	0.12	-	0.25
Enterococcus spp	35-37°C for 18 hrs.	8	-	16
S.pneumoniae (Non Meningitis) (Parenteral)	35-37°C for 20-24hrs with 5% CO_2	2	4	8
S.pneumoniae (Meningitis) (Parenteral)	35-37°C for 20-24hrs with 5% CO_2	0.06	-	0.12
Streptococcus spp. Beta haemolytic group	35-37°C for 20-24hrs with 5% CO_2	0.12	-	-
Streptococcus spp. Viridans group	35-37°C for 20-24hrs with 5% CO_2	0.12	0.25-2	4
N. gonorrhoeae, S. pneumoniae (Oral)	35-37°C for 20-24hrs with 5% CO_2	0.06	0.12-1	2
N. meningitidis	35-37°C for 20-24hrs with 5% CO_2	0.06	0.12-0.25	0.5
Anaerobes	35-37°C for 24-48hrs under anaerobic condition.	0.5	1	2

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 Penicillin

Organism	Medium used	Incubation	Std. Quality Control limits (mcg/ml)
S. aureus ATCC 29213	Mueller Hinton Agar	35-37°C for 18 hrs.	0.25-0.5-1.0-2.0
E. faecalis ATCC 29212	Mueller Hinton Agar	35-37°C for 18 hrs.	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	0.25 - 0.5 - 1.0
Neisseria gonorrhoeae ATCC 49226	GC Agar Base (M434) with 1% defined growth supplement (FD025)	35-37°C for 20-24hrs at 5% CO_2	0.25 - 0.5 - 1.0
B.fragilis ATCC 25285	Brucella Agar with Hemin and Vitamin K1, supplemented with 5 % v/v defibrinated sterile sheep blood	35-37°C for 24-48 hrs under strict anaerobic condition	8.0 – 16.0 – 32.0

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.

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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 sealed glass vial with a desiccator capsule.

1) Penicillin Ezy MIC™ strip (10/30/60/90/120/150 Strips per pack)
2) Applicator sticks
3) Package Insert

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HIMEDIA®

Technical Data

Egg Yolk Emulsion (50 ml/100 ml per vial)

FD045/FD045L

Sterile stabilized emulsion of egg yolk recommended for use in various culture media.

Composition

Ingredients	Concentration (100 ml per vial)	Concentration (50 ml per vial)
Egg yolk	30ml	15ml
Sterile saline	70ml	35ml

Directions:

Warm up the refrigerated egg yolk emulsion to room temperature. Shake well to attain uniform emulsion. (Since on refrigeration emulsion has a tendency to form layers or small lumps). Aseptically add 50 ml emulsion in 950 ml of sterile, molten, cooled (45-50°C) Baird Parker Agar Base M043/ Baird Parker Agar Base M043S/ Baird Parker HiVeg™ Agar Base MV043/ Baird Parker HiCynth™ Agar MCD043/ Baird Parker Agar (Agar Medium O) ME043/ Baird Parker Agar (Agar Medium O) M043B/ Baird Parker Agar Base, Granulated GM043I/ Baird Parker Agar Base, Granulated GM043/ Baird Parker Agar Medium (In accordance with IP 1996) MM043/ Baird Parker Agar Medium MU043/ Baird Parker Agar Base M043I / Mannitol Salt Agar Base M118/Mannitol Salt Agar Base, Granulated GM118/Mannitol Salt HiCynth™ Agar Base MCD118 / Mannitol Salt HiVeg™ Agar Base MV118/ Baird Parker Agar Base w/Sulpha M1140.

Aseptically add in 475 ml of sterile, molten, cooled (45-50°C) Bacillus Cereus Agar Base M833/ Bacillus Cereus HiVeg™ Agar Base MV833/ Bacillus Cereus HiCynth™ Agar Base MCD833

OR

Aseptically add 100 ml emulsion in 900 ml of sterile, molten, cooled (45-50°C) McClung Toabe Agar Base M387/ McClung Toabe HiVeg™ Agar Base MV387/K.R.A.N.E.P. Agar Base M583/K.R.A.N.E.P. HiVeg™ Agar Base MV583 / MYP Agar Base (Phenol Red Egg Yolk Polymyxin Agar Base) M636/ M636S/ MYP HiVeg™ Agar Base (Phenol Red Egg Yolk Polymyxin HiVeg™ Agar Base MV636/ MYP Agar Base, Granulated (Phenol Red Egg Yolk Polymyxin Agar Base, Granulated) GM636 / MYP HiCynth™Agar Base (Phenol Red Egg Yolk Polymyxin HiCynth™ Agar Base) MCD636/ KG Agar Base M658/KG HiVeg™ Agar Base MV658/ L.D. Egg Yolk Agar Base M744/ Egg Yolk Agar Base M808 / Egg Yolk Agar Base, HiVeg™ MV808/ Egg Yolk Agar Base, Modified M1043 / Modified MYP Agar Base M1139/ Bacillus cereus Selective Agar Base (MYP) ISO 7932 M1139I /Modified MYP HiVeg™ Agar Base MV1139. Aseptically add in 890 ml of sterile, molten, cooled (45-50°C) TPEY Agar Base M402/ TPEY HiVeg™ Agar Base MV402.

Aseptically add 450 ml of sterile, molten, cooled (45-50°C) in C. botulinum Isolation Agar Base M911/ C. botulinum Isolation HiVeg™ Agar Base MV911

OR

Aseptically add 25 ml emulsion in 475 ml of sterile, molten, cooled (45-50°C) Perfringens Agar Base T.S.C./S.F.P.AgarBase) M837/ Perfringens Agar Base, Granulated (Tryptose Sulphite Cycloserine Agar Base, Granulated) (T.S.C./S.F.P. Agar Base, Granulated) GM837/ Perfringens HiCynth™ Agar Base (T.S.C/S.F.P HiCynth™ Agar Base) MCD837/ Perfringens HiVeg™ Agar Base (T.S.C. / S.F.P. HiVeg™ Agar Base) MV837/ S.F.P. Agar Base M1005/ S.F.P. HiVegTM Agar Base MV1005.

OR

Aseptically add 80 ml emulsion in 920 ml of sterile, molten, cooled (45-50°C) Anaerobic Egg Agar Base M902 / Anaerobic Egg HiVeg™ Agar Base MV902.

OR

Aseptically add 20 ml emulsion in 90 ml of sterile, molten, cooled (45-50°C) Polymyxin Pyruvate Egg Yolk Mannitol Bromothymol Blue Agar Base (PEMBA) M1484.

OR

Aseptically add 15 ml emulsion in 420 ml of sterile, molten, cooled (45-50°C) Willis and Hobb's Medium Base M1375.

OR

Aseptically add 7ml of Emulsion in 93ml of sterile, molten, cooled (45-50°C) Lipovitellin Salt Mannitol Agar Base M627.

OR

Aseptically add 2 vials of CC Difficile Supplement (FD010), 40 ml of Egg Yolk Emulsion (FD045) together with 10 ml lysed horse blood in 1000 ml of sterile, molten, cooled (45-50°C) Clostridium Brazier Agar Base M1803

OR

Aseptically add 50ml of concetrated Egg yolk emulsion (FD045) and rehydrated contents of 1 vial of LM Selective Supplement (FD330) in 950 ml of sterile, molten, cooled (45-50°C) L.mono Selective Agar Base (LM Selective Agar Base) M1994.

Mix well and pour into sterile petri plates.

Type of specimen

Clinical samples - faeces, urine etc. ; Food samples

Specimen Collection and Handling

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (1,2).
For Food samples follow appropriate techniques for handling specimens as per established guidelines (3).
After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning & Precautions

In Vitro diagnostic use. 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.
3. Salfinger Y., and Tortorello M.L. Fifth (Ed.), 2015, Compendium of Methods for the Microbiological Examination of Foods, American Public Health Association, Washington, D.C.

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CE Marking

2°C 8°C **Storage temperature**

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Technical Data

Haematin Growth Supplement

FD117

A growth supplement recommended for cultivation of *Haemophilus influenzae*.

Composition

Per vial sufficient for 500 ml medium

Ingredients	Concentration
NAD	7.500mg
Haematin	7.500mg

Directions:

Rehydrate the contents of one vial aseptically with 5.0 ml sterile distilled water. Mix well and aseptically add to 500 ml sterile, molten, cooled (45-50°C) Haemophilus Test Agar Base M1259. Mix well and aseptically pour into sterile petri plate

Type of specimen

Isolated Microorganism from 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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Technical Data

CO Selective Supplement FD119

Recommended for the selective isolation and cultivation of Streptococci.

Composition

Per vial sufficient for 500 ml medium

*Ingredients	Concentration
Colistin sulphate	5mg
Oxolinic acid	2.500mg

Directions:

Rehydrate the contents of one vial aseptically with 1ml of 0.2N NaOH and 4ml of sterile distilled water. Mix well and aseptically add to 500 ml of sterile, molten, cooled (45-50ºC) Columbia Blood Agar Base M144/ Columbia Blood Agar Base, Granulated GM144/ Columbia Blood Agar Base, HiVeg™ MV144/ Columbia Blood HiCynth™ Agar Base MCD144/ Columbia Blood Agar Base w/1% Agar M144A/ Columbia Blood Agar Base w/ 1% Agar, HiVeg™ MV144A/ Columbia Blood HiCynth™ Agar Base w/1% Agar MCD144A along with 5% v/v defibrinated blood. Mix well and pour into sterile petri plates.

Type of specimen

Clinical samples - Respiratory secretions, urine and other clinical material.

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

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

2°C 8°C **Storage temperature**

CE Marking

Do not use if package is damaged

U5 Supplement

FD157

Filter sterilized urea solution recommended for the isolation of *Ureaplasma urealyticum* and *Mycoplasma hominis*.

Composition

Per vial sufficient for 425 ml medium

Ingredients	Concentration
Urea	0.250g
Distilled water	5ml
Final pH (at 25°C)	8.0±0.2

Directions:

Warm up the refrigerated Urea solution to room temperature and aseptically add 1 vial to 425 ml of sterile, molten, cooled (45-50ºC) Mycoplasma Urogenital Broth Base M1374 along with 1 vial of Vitamino Growth Supplement FD025 and 1 vial of PAN Selective Supplement FD175 and 50 ml of Horse Serum RM1239. Mix well and dispense as desired.

Type of specimen

Isolated microorganism from clinical, food and water samples.

Specimen Collection and Handling

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (1,2). For food samples, follow appropriate techniques for sample collection and processing as per guidelines (3). For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards(4). After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning & Precautions

In Vitro diagnostic use. 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).

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3. Salfinger Y., and Tortorello M.L. Fifth (Ed.), 2015, Compendium of Methods for the Microbiological Examination of Foods, 5th Ed., American Public Health Association, Washington, D.C.
4. Baird R.B., Eaton A.D., and Rice E.W., (Eds.), 2015, Standard Methods for the Examination of Water and Wastewater, 23rd ed., APHA, Washington, D.C.

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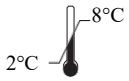
IVD ***In vitro*** **diagnostic medical device**

CE Marking



Storage temperature

Do not use if package is damaged

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AzCe Selective Supplement

FD226

Recommended to differentiate *Enterococcus faecium* from *Enterococcus faecalis*.

Composition

Per vial sufficient for 500 ml medium

*Ingredients	Concentration
Cephalexin	25mg
Aztreonam	37.500mg

Directions:

Rehydrate the contents of 1 vial aseptically with 5 ml sterile distilled water. Mix well and aseptically add to 500 ml of sterile, molten, cooled (45-50°C) Arabinose Agar Base M1576/ HiCrome™ Enterococcus faecium Agar Base M1580 /HiCrome™ Enterococccus Faecium HiVeg™ Agar Base MV1580/ HiCrome™ Enterococcus faecium HiCynth™ Agar Base MCD1580. Mix well and pour into sterile petri plates

Type of specimen

Food samples ; Water samples Clinical samples urine, faeces, etc.

Specimen Collection and Handling

For Food & Water samples follow appropriate techniques for handling specimens as per established guidelines (1,2).
For clinical samples follow appropriate techniques for handling specimens as per established guidelines (3,4).
After use, contaminated materials must be sterili ed by autocla ing before discarding.

Warning & Precautions

In Vitro diagnostic use. 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 (3,4).

Reference

1. Salfinger Y., and Tortorello M.L., 2015, Compendium of Methods for the Microbiological Examination of Foods, 5th Ed., American Public Health Association, Washington, D.C.
2. Baird R.B., Eaton A.D., and Rice E.W., (Eds.), 2015, Standard Methods for the Examination of Water and Wastewater, 23rd ed., APHA, Washington, D.C.
3. Isenberg (Ed.),2004, Clinical Microbiology Procedures Handbook, Vol.3, American Society for Microbiology, Washington. D.C.
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.

*** Not For Medicinal Use**

Revision : 02/2022

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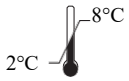

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Technical Data

Coagulase Plasma (0.1gm per vial) FD248

It is recommended for studying coagulase reaction in diagnosis of Staphylococci.

Composition

Per vial sufficient for 6 tests medium

Ingredients	Concentration
Coagulase Plasma	0.100g

Directions:

Rehydrate the contents of one vial aseptically with 3 ml sterile distilled water. Add 0.5 ml of rehydrated FD248 in a tube. To this add approximately 0.05 ml of overnight broth culture of test organisms or 2-3 pure colonies picked from a non-inhibitory agar plate. Mix gently & incubate at 37°C in incubator or water bath for up to 4 hours. Observe for clot formation in the tube at regular intervals. Any degree of clotting within 4 hours is considered as positive results.

Type of specimen

Clinical- skin, throat samples etc; Food samples

Specimen Collection and Handling

For Clinical & Food samples follow appropriate techniques for handling specimens as per established guidelines (1,2,3). After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning & Precautions

In Vitro diagnostic use. 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. For unopened vial, use before the expiry date on the label. The rehydrated solution can be stored for up to 2 weeks at 2-8°C

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.
3. Salfinger Y., and Tortorello M.L., 2015, Compendium of Methods for the Microbiological Examination of Foods, 5th Ed., American Public Health Association, Washington, D.C.

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

2°C 8°C **Storage temperature**

CE **CE Marking**

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Sabouraud Dextrose Broth, Granulated (Sabouraud Liquid Medium, Granulated)

GM033

Intended Use:

For cultivation of yeasts, moulds and aciduric microorganisms from clinical and non-clinical samples.

Composition**

Ingredients	g / L
Dextrose (Glucose)	20.000
Peptone, special	10.000
Final pH (at 25°C)	5.6±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 30.0 grams in 1000 ml purified/ distilled water. Heat if necessary to dissolve the medium completely. Mix well and dispense in tubes or flasks as desired. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

Principle And Interpretation

Sabouraud Dextrose Agar is Carliers modifications (1) of the formulation described by Sabouraud (2) for the cultivation of fungi, particularly those associated with skin infections. The medium is also recommended by APHA (3). Sabouraud Dextrose Broth is also a modification by Sabouraud (4) and serves the same purpose as Sabouraud Dextrose Agar Medium 3.

Sabouraud dextrose media are peptone media supplemented with dextrose to support the growth of fungi. Peptone special provides carbon and nitrogen source, vitamins, minerals, amino acids and growth factors. Dextrose provides an energy source for the growth of microorganisms. The low pH favors fungal growth and inhibits contaminating bacteria from clinical specimens (5). The acid reaction of the final medium is inhibitory to a large number of bacteria making it particularly useful for cultivating fungi and aciduric microorganisms. For isolation of fungi from contaminated specimens, a selective medium should be inoculated simultaneously. Incubate cultures for 4 to 6 weeks before reporting as negative.

Type of specimen

Clinical : skin scrapings; Food and dairy samples.

Specimen Collection and Handling

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (6,7).

For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (3,8). After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions

In Vitro diagnostic use. 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. Since it is a general purpose medium, bacterial cultures will also grow.
2. Further isolation and biochemical tests should be carried out for confirmation.

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 colored granular medium

Colour and Clarity of prepared medium

Light amber coloured clear solution in tubes

Reaction

pH of 3.0% w/v aqueous solution at 25°C. pH : 5.6±0.2

pH

5.40-5.80

Cultural Response

Cultural characteristics was observed after an incubation at 20-25°C for 3-5 days.

Organism	Inoculum (CFU)	Growth
Candida albicans ATCC 10231 (00054*)	50 -100	luxuriant
Candida albicans ATCC 2091 (00055*)	50 -100	luxuriant
Aspergillus brasiliensis ATCC 16404 (00053*)	50 -100	luxuriant
Saccharomyces cerevisiae ATCC 9763 (00058*)	50 -100	luxuriant
Saccharomyces cerevisiae ATCC 2601	50 -100	good-luxuriant
Escherichia coli ATCC 8739 (00012*)	50 -100	Luxuriant (inhibited on media with low pH)
Escherichia coli ATCC 25922 (00013*)	50 -100	good-luxuriant
Escherichia coli NCTC 9002	50 -100	Luxuriant (inhibited on media with low pH)
Lactobacillus casei ATCC 334	50 -100	luxuriant

Key : (*) Corresponding WDCM numbers.

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 15-30°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 (6,7).

Reference

1. Carlier G. I. M., 1984, Brit. J. Derm. Syph., 60:61
2. Sabouraud R., Les Teignes, Paris: Masson et Cie, 1910, p 553
3. Salfinger Y., and Tortorello M.L., 2015, Compendium of Methods for the Microbiological Examination of Foods, 5th Ed., American Public Health Association, Washington, D.C.
4. Sabouraud R., 1892, Ann. Dermatol. Syphil. 3 : 1061.
5. 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.
6. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
7. 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.
8. American Public Health Association, Standard Methods for the Examination of Dairy Products, 1978, 14th Ed., Washington D.C.

Revision :04/2024

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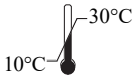

Storage temperature

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Sabouraud Dextrose Agar, Granulated

GM063

Intended Use:

Recommended for the cultivation of yeasts, moulds and aciduric microorganisms from clinical and non-clinical samples.

Composition**

Ingredients	g / L
Dextrose (Glucose)	40.000
Mycological, peptone	10.000
Agar	15.000
Final pH (at 25°C)	5.6±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 65.0 grams 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. Mix well and pour into sterile Petri plates.

Principle And Interpretation

Sabouraud Dextrose Agar is Carlier's modification (1) of the formulation described by is a modification of Sabouraud Dextrose Agar which is described by Sabouraud (2) for the cultivation of fungi (yeasts, moulds), particularly useful for the fungi associated with skin infections. This medium is also employed to determine microbial contamination in food, cosmetics, and clinical specimens (3). Mycological Peptone provides nitrogenous compounds. Dextrose provides an energy source. High dextrose concentration and low pH favors fungal growth and inhibits contaminating bacteria from test samples (4).

Type of specimen

Food and dairy samples ; Clinical samples: skin scrapings

Specimen Collection and Handling

For food and dairy samples follow appropriate techniques for handling specimens as per established guidelines (5,6,7).
For clinical samples, follow appropriate techniques for sample collection and processing as per guidelines (8,9). After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions:

In Vitro diagnostic use. 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. For heavily contaminated samples, the plate must be supplemented with inhibitory agents for inhibiting bacterial growth with lower pH.
2. Some pathogenic fungi may produce infective spores which are easily dispersed in air, so examination should be carried out in safety cabinet
3. Further biochemical tests should be carried out for confirmation.

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 coloured granular media.
Gelling
Firm, comparable with 1.5% Agar gel.
Colour and Clarity of prepared medium
Light yellow to amber coloured clear to slightly opalescent gel forms in Petri plates.

Reaction
Reaction of 6.5% w/v aqueous solution at 25°C (after sterilization). pH : 5.6±0.2
pH
5.40-5.80
Cultural Response
Cultural response was observed after an incubation at 20-25°C for 24-48 hours.

Organism	Inoculum (CFU)	Growth	Recovery
Candida albicans ATCC 10231 (00054*)	50 -100	Luxuriant (white colonies)	>=70 %
#*Aspergillus brasiliensis* ATCC 16404 (00053*)	50 -100	luxuriant	>=70 %
Candida albicans ATCC 2091 (00055*)	50 -100	luxuriant	>=70 %
Saccharomyces cerevisiae ATCC 9763 (00058*)	50 -100	luxuriant	>=70 %
Escherichia coli ATCC 8739 (00012*)	50 -100	luxuriant	>=70 %
Escherichia coli ATCC 25922 (00013*)	50 -100	luxuriant	>=70 %
Escherichia coli NCTC 9002	50 -100	luxuriant	>=70 %
Lactobacillus casei ATCC 334	50 -100	luxuriant	>=70 %
Trichophyton rubrum ATCC 28191		luxuriant	

Key : (*) - Corresponding WDCM numbers. (#) - Formerly known as *Aspergillus niger*

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 20-30°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

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Reference

1.Carlier G. I. M., 1948, Brit. J. Derm. Syph., 60:61.
2.Sabouraud K., 1892, Ann. Dermatol. Syphilol, 3:1061.
3.Bacteriological Analytical Manual, 8th Edition, Revision A, 1998. AOAC, Washington D.C.
4. Murray PR, Baren EJ, Jorgensen JH, Pfaller MA, Yolken RH (editors) 2003, Manual of clinical Microbiology, 8th ed., ASM, Washington, D.C.
5. American Public Health Association, Standard Methods for the Examination of Dairy Products, 1978, 14th Ed., Washington D.C.
6.Salfinger Y., and Tortorello M.L., 2015, Compendium of Methods for the Microbiological Examination of Foods, 5th Ed., American Public Health Association, Washington, D.C.
7. Wehr H. M. and Frank J. H., 2004, Standard Methods for the Microbiological Examination of Dairy Products, 17th Ed., APHA Inc., Washington, D.C.8.
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 : 05/2024

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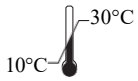

Storage temperature

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Lactobacillus MRS Agar,Granulated(MRS Agar, Granulated)

GM641

Intended use

Recommended for isolation and cultivation of all Lactobacilli from food, dairy and clinical samples.

Composition**

Ingredients	g / L
Proteose peptone	10.000
HM Peptone B #	10.000
Yeast extract	5.000
Dextrose (Glucose)	20.000
Tween 80 (Polysorbate 80)	1.000
Ammonium citrate	2.000
Sodium acetate	5.000
Magnesium sulphate	0.100
Manganese sulphate	0.050
Dipotassium hydrogen phosphate	2.000
Agar	12.000
Final pH (at 25°C)	6.5±0.2

**Formula adjusted, standardized to suit performance parameters
Equivalent to Beef extract

Directions

Suspend 67.15 grams 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. Mix well and pour into sterile Petri plates.

Principle And Interpretation

Lactobacilli MRS media are based on the formulation of deMan, Rogosa and Sharpe (1) with slight modification. It supports luxuriant growth of all Lactobacilli from oral cavity (2), dairy products (3), foods (2), faeces (4,5) and other sources (6).

Proteose peptone and HM peptone B supply nitrogenous and carbonaceous compounds. Yeast extract provides vitamin B complex and dextrose is the fermentable carbohydrate and energy source. Polysorbate 80 supplies fatty acids required for the metabolism of Lactobacilli. Sodium acetate and ammonium citrate inhibit Streptococci, moulds and many other microorganisms. Magnesium sulphate and manganese sulphate provide essential ions for multiplication of lactobacilli. Phosphates provide good buffering action in the media.

Lactobacilli are microaerophilic and generally require layer plates for aerobic cultivation on solid media. When the medium is set, another layer of un-inoculated MRS Agar is poured over the surface to produce a layer plate. Lactobacilli isolated on MRS Agar should be further confirmed biochemically.

Type of specimen

Clinical samples - urine, faeces, etc.; Food and dairy samples

Specimen Collection and Handling:

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (4,5).

For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (2,7,8).

After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions :

In Vitro diagnostic Use. 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. Individual organisms differ in their growth requirement and may show variable growth patterns on the medium.
2. Further Biochemical and serological testing is required for complete identification.

Performance and Evaluation

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

Quality Control

Appearance

Cream to light yellow coloured granular medium

Gelling

Firm, comparable with 1.2% Agar gel.

Colour and Clarity of prepared medium

Medium to dark amber coloured, clear to slightly opalescent gel forms in Petri plates

Reaction

Reaction of 6.71% w/v aqueous solution at 25°C. pH : 6.5±0.2

pH

6.30-6.70

Cultural Response

Cultural characteristics observed after an incubation at 35-37°C for 18-24 hours or longer.(with 5% CO2)

Organism	Inoculum (CFU)	Growth	Recovery
Lactobacillus rhamnosus ATCC 9595	50-100	luxuriant	>=50%
Lactobacillus fermentum ATCC 9338	50-100	luxuriant	>=50%
^*Lactobacillus delbrueckii* subsp. *lactis ATCC 7830*	50-100	luxuriant	>=50%
#*Lactiplantibacillus plantarum ATCC 8014*	50-100	luxuriant	>=50%
Lactobacillus saki ATCC 15521(00015)*	50-100	luxuriant	>=50%
Lactobacillus lactis ATCC 19435(00016)*	50-100	luxuriant	>=50%
Pediococcus pentosaceas ATCC 33316 (00158)*	50-100	luxuriant	>=50%
Escherichia coli ATCC 25922 (00013)*	>=10^4	inhibited	0%
Bacillus cereus ATCC 11778 (00001*)	>=10^4	inhibited	0%

Key: (*) Corresponding WDCM numbers.

#Formerly known as *Lactobacillus plantarum* ^ Formerly known as *Lactobacillus leichmannii*

Storage and Shelf Life

Store dehydrated powder 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).

References

1. deMan J., Rogosa M. and Sharpe M., 1960, J. Appl. Bacteriol., 23:130.
2. American Public Health Association, Standard Methods for the Examination of Dairy Products, 1978, 14th Ed., Washington D.C.
3. Marshall R.T. (Ed.), 1992, Standard Methods for the Examination of Dairy Products, 16th ed., APHA, Washington,D.C.
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.
6. MacFaddin J., 1985, Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria, Vol.1, Williams and Wilkins, Baltimore.
7. Salfinger Y., and Tortorello M.L., 2015, Compendium of Methods for the Microbiological Examination of Foods, 5th Ed., American Public Health Association, Washington, D.C.
8. Wehr H. M. and Frank J. H., 2004, Standard Methods for the Microbiological Examination of Dairy Products, 17th Ed., APHA Inc., Washington, D.C.

Revision : 06/2024

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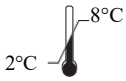

Storage temperature

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Technical Data

HiCombi™ Dual Performance Medium

LQ012

Intended use

Recommended for rapid growth of *Enterobacteria, Pseudomonas*, Staphylococci and *Candida*. Combination of solid (20ml) and liquid (40ml) media in single bottle.

Composition**

Ingredients	g / L
Solid	20.000 ml
HM infusion powder #	12.500
BHI powder	5.000
Proteose peptone	10.000
Dextrose (Glucose)	2.000
Sodium chloride	5.000
Disodium hydrogen phosphate	2.500
Agar	15.000
Liquid	40.000 ml

Same as solid media without Agar

**Formula adjusted, standardized to suit performance parameters
Equivalent to Calf brain infusion from

Directions

Label the ready to use blood culture bottle. Do not unscrew the cap. Remove the top seal of the cap. Disinfect the part of the rubber stopper which is now exposed. Draw patient's blood with the sterile or disposable needle and syringe. Transfer the blood sample immediately into the culture bottle by puncturing the rubber stopper with the needle and injecting the blood. Incubate the bottle for 4-6 hours at 30 -35°C. For adsorption on solid surface. DO NOT SHAKE OR HOLD MORE THAN 15 SECONDS. Revert into an upright position and incubate for 18-24 hours at 30-35°C or longer if necessary. Venting: Use sterile venting needle (LA038). Keep the bottle in an upright position preferably in a biological safety cabinet, place an alcohol swab over the rubber stopper and insert the venting needle with filter through it. Insertion and withdrawal of the needle should be done in a straight line. discard the needle and mix the contents by gently inverting the bottle 2-3 times. Do Not vent the bottle for anaerobic cultures. Incubate at 30-35°C for 18-24 hours and further for seven days. Recommended volume of blood to be tested in LQ012: 8-10 ml (For Adult use).

Principle And Interpretation

BHI Medium is useful for cultivating a wide variety of microorganisms since it is a highly nutritive medium. It is also used to prepare the inocula for antimicrobial susceptibility testing. BHI Broth is a modification of the original formulation of Rosenow, where he added pieces of brain tissues to dextrose broth (1). BHI Broth is also the preferred medium for anaerobic bacteria, yeasts and moulds (2,3). This medium is nutritious and well buffered to support the growth of wide variety of organisms (1,4,5). With the addition of 10% defibrinated sheep blood, it is useful for isolation and cultivation of *Histoplasma capsulatum* (6) and other fungi. For selective isolation of fungi, addition of gentamicin and/or chloramphenicol is recommended (7).

Proteose peptone, HM infusion powder and BHI powder serve as sources of carbon, nitrogen, essential growth factors, amino acids and vitamins. Dextrose serves as a source of energy. Disodium phosphate helps in maintaining the buffering action of the medium whereas sodium chloride maintains the osmotic equilibrium of the medium.

Type of specimen

Clinical samples : Blood

Specimen Collection and Handling

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (8,9).
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. Further biochemical and serological testing is required for complete identification.
2. Individual organisms differ in their growth requirement and may show variable growth patterns on the medium.
3. Each lot of the medium has been tested for the organisms specified on the COA. It is recommended to users to validate the medium for any specific microorganism other than mentioned in the COA based on the user's unique requirement.

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

In a sterile glass bottle combination of broth and one agar coated surface.

Colour of Agar medium
Yellow coloured media

Colour of liquid medium
Amber coloured solution

Quantity of medium

20ml of medium in glass bottle 40ml of medium in glass bottle

pH of Agar medium
7.20- 7.60

pH of liquid medium
7.20- 7.60

Sterility Check

Passes release criteria

Cultural response

Cultural characteristics was observed after incubation at 35-37°C for 18-48 hours.

Organism	Inoculum (CFU)	Growth on agar medium	Growth on liquid medium
Candida albicans ATCC 10231 (00054*)	50-100	Luxuriant	Luxuriant
Haemophilus influenzae ATCC 19418	50-100	Luxuriant	Luxuriant
Pseudomonas aeruginosa ATCC 27853 (00025*)	50-100	Luxuriant	Luxuriant
Streptococcus pyogenes ATCC 19615	50-100	Luxuriant	Luxuriant
Staphylococcus aureus subsp. *aureus* ATCC 25923 (00034*)	50-100	Luxuriant	Luxuriant
Neisseria meningitidis ATCC 13090	50-100	Luxuriant	Luxuriant
Streptococcus pneumoniae ATCC 6303	50-100	Luxuriant	Luxuriant

Key : (*) Corresponding WDCM numbers.

Storage and Shelf Life

On receipt store between 15-30°C. Use before expiry date on the label. 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.Rosenow, 1919, J. Dental Research, 1:205.
2.MacFaddin J. F., 1985, Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria, Vol. I, Williams and Wilkins, Baltimore.
3.Atlas R. M., 1993, Handbook of Microbiological Media, 147-153, CRC Press, Boca Raton, FL.
4.Roseburg T. et al, 1944, J. Inf. Dis., 74:
5.Conant N. F., 1950, Diagnostic Procedures and Reagents, 3rd Ed., APHA Inc., New York
6.Howard B., Keiser J. F., Weissfeld A. et al, 1994, Clinical and Pathogenic Microbiology, 2nd Ed., Mosby Co.
7.Murray P. R., Baron J. H., Pfaller M. A., Jorgensen J. H. and Yolken R. H., (Eds.), 2003, Manual of Clinical Microbiology,8th Ed., American Society for Microbiology, Washington, D.C.
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.

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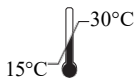
IVD ***In vitro*** **diagnostic medical device**



Storage temperature

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

CE Marking

Do not use if package is damaged

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Nutrient Agar

M001

Intended use

Nutrient Agar is used as a general purpose medium for the cultivation of less fastidious microorganisms, can be enriched with blood or other biological fluids.

Composition**

Ingredients	g / L
Peptone	5.000
Sodium chloride	5.000
HM peptone B#	1.500
Yeast extract	1.500
Agar	15.000
Final pH (at 25°C)	7.4±0.2

**Formula adjusted, standardized to suit performance parameters
- Equivalent to Beef extract

Directions

Suspend 28.0 grams 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. If desired ,the medium can be enriched with 5-10% blood or other biological fluids. Mix well and pour into sterile Petri plates.

Principle And Interpretation

Nutrient media are basic culture media used for maintaining microorganisms, cultivating fastidious organisms by enriching with serum or blood and are also used for purity checking prior to biochemical or serological testing (1,2). Nutrient Agar is ideal for demonstration and teaching purposes where a more prolonged survival of cultures at ambient temperature is often required without risk of overgrowth that can occur with more nutritious substrate. This relatively simple formula has been retained and is still widely used in the microbiological examination of variety of materials and is also recommended by standard methods. It is one of the several non-selective media useful in routine cultivation of microorganisms (3,4). It can be used for the cultivation and enumeration of bacteria which are not particularly fastidious. Addition of different biological fluids such as horse or sheep blood, serum, egg yolk etc. makes it suitable for the cultivation of related fastidious organisms. Peptone, HM peptone B and yeast extract provide the necessary nitrogen compounds, carbon, vitamins and also some trace ingredients necessary for the growth of bacteria. Sodium chloride maintains the osmotic equilibrium of the medium.

Type of specimen

Clinical samples - faeces, urine ; Food and dairy samples; Water samples

Specimen Collection and Handling:

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (5,6).
For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (3,4,7).
For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards (8).
After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions :

In Vitro diagnostic use. 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. Individual organisms differ in their growth requirement and may show variable growth patterns on the medium.
2. Each lot of the medium has been tested for the organisms specified on the COA. It is recommended to users to validate the medium for any specific microorganism other than mentioned in the COA based on the user's unique requirement.