

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<sub>2</sub>. 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 ( $\alpha$ -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 $^{\circ}$ C for 18-24 hours in 5% CO<sub>2</sub> 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

No zone of inhibition or a minimal zone, Resistant

ATCC 19615

## 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.

Revision: 02/2022



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In vitro diagnostic medical device



Storage temperature



**CE Marking** 



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## Disclaimer:

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## **Xylose-Lysine Deoxycholate Agar (XLD Agar)**

**M031** 

#### Intended use

Recommended for the isolation and enumeration of Salmonella Typhi and other Salmonella species from clinical and non-clinical samples.

## Composition\*\*

Ingredients	g/L
Yeast extract	3.000
L-Lysine	5.000
Lactose	7.500
Sucrose	7.500
Xylose	3.500
Sodium chloride	5.000
Sodium deoxycholate	2.500
Sodium thiosulphate	6.800
Ferric ammonium citrate	0.800
Phenol red	0.080
Agar	15.000
Final pH ( at 25°C)	$7.4 \pm 0.2$

<sup>\*\*</sup>Formula adjusted, standardized to suit performance parameters

#### **Directions**

Suspend 56.68 grams in 1000 ml purified / distilled water. Heat with frequent agitation until the medium boils. **DO NOT AUTOCLAVE OR OVERHEAT**. Transfer immediately to a water bath at 50°C. After cooling, pour into sterile Petri plates. It is advisable not to prepare large volumes that will require prolonged heating, thereby producing precipitate. **Note**: Slight precipitation in the medium may occur, which is inheritant property of the medium, and does not affect the performance of the medium.

## **Principle And Interpretation**

XLD Agar has been recommended for the identification of *Enterobacteriaceae* (1) and for the microbiological testing. XLD Agar was formulated by Taylor (2-6) for the isolation and differentiation of enteric pathogens including *Salmonella* Typhi from other *Salmonella* species of foods, water and dairy products (7-11). XLD Agar exhibits increased selectivity and sensitivity as compared to other plating media e.g. SS Agar (M108), EMB Agar (M022) and Bismuth Sulphite Agar (M027) (3,5,7,12-15). The media formulation does not allow the overgrowth of other organisms over *Salmonella* and *Shigella* (16). Samples suspected of containing enteric pathogens, along with other mixed flora, are initially enriched in Modified Semisolid RV Medium Base (M1482) (17). It is also recommended by FDA (18).

The medium contains yeast extract, which provides nitrogen and vitamins required for growth. Though the sugars xylose, lactose and sucrose provide sources of fermentable carbohydrates, xylose is mainly incorporated into the medium since it is not fermented by Shigellae but practically by all enterics. This helps in the differentiation of *Shigella* species. Sodium chloride maintains the osmotic balance of the medium. Lysine is included to differentiate the *Salmonella* group from the non-pathogens. Salmonellae rapidly ferment xylose and exhaust the supply. Subsequently lysine is decarboxylate by the enzyme lysine decarboxylase to form amines with reversion to an alkaline pH that mimics the *Shigella* reaction. However, to prevent this reaction by lysine-positive coliforms, lactose and sucrose are added to produce acid in excess. Degradation of xylose, lactose and sucrose to acid causes phenol red indicator to change its colour to yellow. Bacteria that decarboxylate lysine to cadaverine can be recognized by the appearance of a red colouration around the colonies due to an increase in pH. These reactions can proceed simultaneously or successively, and this may cause the pH indicator to exhibit various shades of colour or it may change its colour from yellow to red on prolonged incubation. To add to the differentiating ability of the formulation, an H<sub>2</sub>S indicator system, consisting of sodium thiosulphate and ferric ammonium citrate, is included for the visualization of hydrogen sulphide produced, resulting in the formation of colonies with black centers. The non-pathogenic H<sub>2</sub>S producers do not decarboxylase lysine; therefore, the acid reaction produced by them prevents the blackening of the colonies (2).

XLD Agar is both selective and differential medium. It utilizes sodium deoxycholate as the selective agent and therefore it is inhibitory to gram-positive microorganisms.

## Type of specimen

Clinical samples - Faeces; Food and dairy samples; Water samples.

## **Specimen Collection and Handling:**

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (19,20). For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (9,10). 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. Slight precipitation in the medium may occur, which is inheritant property of the medium, and does not affect the performance of the medium.
- 2. This medium is general purpose medium and may not support the growth of fastidious organisms.
- 3. Some *Proteus* strains may give red to yellow colouration with most colonies developing black centers, giving rise to false positive reactions.
- 4. Non-enterics like Pseudomonas and Providencia may exhibit red colonies.
- 5. S. Paratyphi A, S.Choleraesuis, S. Pullorum and S. Gallinarum may form red colonies without H<sub>2</sub>S, thus resembling Shigella species.

## **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

Light yellow to pink homogeneous free flowing powder

#### Gelling

Firm, comparable with 1.5% Agar gel

## Colour and Clarity of prepared medium

Red coloured clear to slightly opalescent gel forms in Petri plates

#### Reaction

Reaction of 5.67% w/v aqueous solution at 25°C . pH :  $7.4\pm0.2$ 

#### рH

7.20-7.60

### **Cultural Response**

Cultural response was observed after an incubation at 35-37°C for specified time. Recovery rate is considered as 100% for bacteria growth on Soyabean Casein Digest Agar.

Organism	Inoculum (CFU)	Growth	Observed Lot value (CFU)	t Recovery	Colour of Colony	Incubation period
Salmonella Typhimurium ATCC 14028 (00031*)	50 -100	luxuriant	25 -100	>=50 %	red with black centres	18 -72 hrs
Salmonella Abony NCTC 6017 (00029*)	50 -100	good-luxuriant	25 -100	>=50 %	red with black centres	18 -72 hrs
Escherichia coli ATCC 8739 (00012*)	50 -100	fair	10 -30	20 -30 %	yellow	18 -72 hrs
Escherichia coli ATCC 25922 (00013*)	50 -100	fair	10 -30	20 -30 %	yellow	18 -72 hrs

Please refer disclaimer Overleaf.

\$ Proteus hauseri ATCC 13315	50 -100	good-luxuriant	25 -100	>=50 %	grey with black centres	x 18 -72 hrs
Salmonella Paratyphi A ATCC 9150	50 -100	good-luxuriant	25 -100	>=50 %	red	18 -72 hrs
Salmonella Paratyphi B ATCC 8759	50 -100	good-luxuriant	25 -100	>=50 %	red with black centres	18 -72 hrs
Salmonella Enteritidis ATCC 13076 (00030*)	50 -100	good-luxuriant	25 -100	>=50 %	red with black	18 -72 hrs
Salmonella Typhi ATCC 6539	50 -100	good-luxuriant	25 -100	>=50 %	red with black	18 -72 hrs
Shigella dysenteriae ATCC 13313	50 -100	good-luxuriant	25 -100	>=50 %	red	18 -72 hrs
Shigella flexneri ATCC 12022 (00126*)	50 -100	fair-good	15 -40	30 -40 %	red	18 -72 hrs
Shigella sonnei ATCC 25931	50 -100	fair-good	15 -40	30 -40 %	red	18 -72 hrs
# Klebsiella aerogenes ATCC 13048 (00175*)	50 -100	fair	10 -30	20 -30 %	yellow	18 -72 hrs
Enterobacter cloacae ATCC 13047 (00083*)	50 -100	fair	10 -30	20 -30 %	yellow	18 -72 hrs
Staphylococcus aureus subsp. aureus ATCC 25923 (00034*)	>=104	inhibited	0	0%		>=72 hrs
Staphylococcus aureus subsp. aureus ATCC 6538 (00032*)	>=104	inhibited	0	0%		>=72 hrs
Enterococcus faecalis ATCC 29212 (00087*)	>=104	inhibited	0	0%		>=72 hrs

 $Key: *Corresponding \ WDCM \ numbers.$ 

(#) Formerly known as Enterobacter aerogenes \$ Formerly known as Proteus vulgaris

## 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

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 (19,20).

## Reference

- 1. Chadwick P., Delisle G. H and Byer M., 1974, Can. J. Microbiol., 20, 1653-1664.
- 2. Taylor W. L., 1965, Am. J. Clin. Pathol., 44:471-475.
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9. 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.

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- 16. Taylor W. L. and Schelhart B., 1969, Appl. Micro. 18, 1387-1392.
- 17. Isenberg H. D., Kominos S., and Sigeal M., 1969, Appl Microbiol., 18, 656-659.
- 18. FDA Bacteriological Analytical Manual, 2005, 18th Ed., AOAC, Washington, D.C.
- 19. Isenberg, H.D. Clinical Microbiology Procedures Handbook. 2nd Edition.
- 20. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.

Revision: 06/2024



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## Kligler Iron Agar M078

## **Intended Use:**

Recommended for differential identification of gram-negative enteric bacilli from clinical and non-clinical samples on the basis of the fermentation of glucose (dextrose), lactose and hydrogen sulphide production.

## Composition\*\*

Ingredients	g/L
Peptone	15.000
HM Peptone B #	3.000
Yeast extract	3.000
Proteose peptone	5.000
Lactose	10.000
Dextrose	1.000
Ferrous sulphate	0.200
Sodium chloride	5.000
Sodium thiosulphate	0.300
Phenol red	0.024
Agar	15.000
Final pH ( at 25°C)	$7.4\pm0.2$

<sup>\*\*</sup>Formula adjusted, standardized to suit performance parameters

## **Directions**

Suspend 57.52 grams in 1000 ml purified/distilled water. Heat to boiling to dissolve the medium completely. Mix well and distribute into tubes. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Allow the tubes to cool in slanted position to form slopes with about 1 inch butts. Best reactions are obtained on freshly prepared medium. Do not use screw capped tubes or bottles.

Note: Avoid overheating otherwise it may produce precipitate in the medium.

## **Principle And Interpretation**

Kligler Iron Agar is a combination of the lead acetate medium described by Kligler (1,2) and Russels Double Sugar Agar (3) and is used as a differentiation medium for typhoid, dysentery and allied bacilli (4). Bailey and Lacey substituted phenol red for andrade indicator previously used as pH indicator (4). Kligler Iron Agar differentiates lactose fermenters from the non-fermenters. It differentiates Salmonella Typhi from other Salmonellae and also Salmonella Paratyphi A from Salmonella Scottmuelleri and Salmonella Enteritidis (5). Fermentation of dextrose results in production of acid, which turns the indicator from red to yellow. Since there is little sugar i.e. dextrose, acid production is very limited and therefore a reoxidation of the indicator is produced on the surface of the medium, and the indicator remains red. However, when lactose is fermented, the large amount of acid produced, avoids reoxidation and therefore the entire medium turns yellow. Kligler Iron Agar, in addition to Peptone, HM peptone B and yeast extract, contains lactose and glucose (dextrose), which enables the differentiation of species of enteric bacilli. Phenol red is the pH indicator, which exhibits a colour change in response to acid produced during the fermentation of sugars. The combination of ferrous sulphate and sodium thiosulphate enables the detection of hydrogen sulfide production, which is evidenced by a black color either throughout the butt, or in a ring formation near the top of the butt. Lactose non-fermenters (e.g., Salmonella and Shigella) initially produce a yellow slant due to acid produced by the fermentation of the small amount of glucose (dextrose). When glucose (dextrose) supply is exhausted in the aerobic environment of the slant, the reaction reverts to alkaline (red slant) due to oxidation of the acids produced. The reversion does not occur in the anaerobic environment of the butt, which therefore remains acidic (yellow butt). Lactose fermenters produce yellow slants and butts because of lactose fermentation. The high amount of acids thus produced helps to maintain an acidic pH under aerobic conditions. Tubes showing original colour of the medium indicates the fermentation of neither glucose (dextrose) nor lactose. Gas production (aerogenic reaction) is detected as individual bubbles or by splitting or displacement of the agar by the formation of cracks in the butt of the medium.

Pure cultures of suspected organisms from plating media such as MacConkey Agar (M081), Bismuth Sulphite Agar (M027) or Deoxycholate Citrate Agar (M065), SS Agar (M108) etc. are inoculated on Kligler Iron Agar for identification.

## Type of specimen

Isolated microorganism from clinical, food, dairy and water samples.

<sup># -</sup> Equivalent to Beef extract

## **Specimen Collection and Handling**

For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards (6). For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (7,8,9). For clinical samples follow appropriate techniques for handling specimens as per established guidelines (10,11). 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. Results should be noted after 18-24 hours to avoid erroneous results.
- 2. Straight wire loop should be used for inoculation.
- 3. Pure isolates should be used to avoid erroneous results.
- 4. Other biochemical and serological tests must be performed for complete identification

#### **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

Light yellow to pink homogeneous free flowing powder

#### Gelling

Firm, comparable with 1.5% Agar gel

#### Colour and Clarity of prepared medium

Red coloured, clear to slightly opalescent gel forms in tubes as slants

#### Reaction

Reaction of 5.75% w/v aqueous solution at 25°C. pH: 7.4±0.2

#### pН

7.20-7.60

## **Cultural Response**

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

Organism	Growth	Gas	H2S	Slant	Butt
Escherichia coli ATCC 25922 (00013*)	luxuriant	positive reaction	negative reaction, no blackening of medium	acidic reaction, yellowing of the medium	acidic reaction, yellowing of the medium
#Klebsiella aerogenes ATCC 13048 (00175*)	luxuriant	positive reaction	negative reaction, no blackening of medium	acidic reaction, yellowing of the medium	acidic reaction, yellowing of the medium
Citrobacter freundii ATCC 8090	luxuriant	positive reaction	positive reaction, blackening of medium	acidic reaction, yellowing of the medium	acidic reaction, yellowing of the medium
## Proteus hauseri ATCC 13315	luxuriant	negative reaction	positive reaction, blackening of medium	alkaline reaction, red colour of the medium	acidic reaction, yellowing of the medium
Klebsiella pneumoniae ATCC 13883 (00087*)	luxuriant	positive reaction	negative reaction,no blackening of medium	acidic reaction, yellowing of the medium	acidic reaction, yellowing of the medium
Salmonella Paratyphi A ATCC 9150	luxuriant	positive reaction	negative reaction,no blackening of medium	alkaline reaction, red colour of the medium	acidic reaction, yellowing of the medium

Salmonella Schottmuelleri ATCC 10719	luxuriant	positive reaction	positive reaction, blackening of medium	alkaline reaction, red colour of the medium	acidic reaction, yellowing of the medium
Salmonella Typhi ATCC 6539	luxuriant	negative reaction	positive reaction, blackening of medium	alkaline reaction, red colour of the medium	acidic reaction, yellowing of the medium
Salmonella Enteritidis ATCC 13076 (00030*)	luxuriant	positive reaction	positive reaction, blackening of medium	alkaline reaction, red colour of the medium	acidic reaction, yellowing of the medium
Shigella flexneri ATCC 12022 (00126*)	luxuriant	negative reaction	negative reaction,no blackening of medium	alkaline reaction, red colour of the medium	acidic reaction, yellowing of the medium
Pseudomonas aeruginosa ATCC 27853 (00025*)	luxuriant	negative reaction	negative reaction, blackening of medium	alkaline reaction, red colour of the medium	alkaline reaction,red colour of the medium
Yersinia enterocolitica ATCC 27729	luxuriant	variable reaction	negative reaction,no blackening of medium	alkaline reaction,red colour of the medium	acidic reaction, yellowing of the medium
Enterobacter cloacae ATCC 13047 (00083*)	luxuriant	positive reaction	negative reaction,no blackening of medium	acidic reaction, yellowing of the medium	acidic reaction, yellowing of the medium

Key: \* Corresponding WDCM numbers

(#) Formerly known as *Enterobacter aerogenes* 

## Formerly known as Proteus vulgaris

## **Storage and Shelf Life**

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

#### **Disposal**

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (10,11).

## Reference

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Revision: 05/2024



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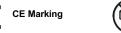


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Storage temperature



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# Columbia Blood Agar Base Intended Use:

M144

For preparation of blood agar, chocolate agar and for preparation of various selective and identification media and isolation of organisms from clinical and non clinical samples.

## Composition\*\*

Ingredients	g/L
Peptone, special	23.000
Corn starch	1.000
Sodium chloride	5.000
Agar	15.000
Final pH ( at 25°C)	7.3±0.2

<sup>\*\*</sup>Formula adjusted, standardized to suit performance parameters

### **Directions**

Suspend 44.0 grams of 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 before adding heat sensitive compounds. For Blood Agar: Add 5% v/v sterile defibrinated sheep blood to sterile cool base.

For Chocolate Agar: Add 10% v/v sterile defibrinated sheep blood to sterile cool base. Heat to 80°C for 10 minutes with constant agitation.

The medium can be made selective by adding different antimicrobials to sterile base.

For Brucella species: Add rehydrated contents of 1 vial of NPBCVN Selective Supplement (FD005) to 500 ml sterile molten base.

For *Campylobacter* species: Add rehydrated contents of 1 vial of Blaser-Wang Selective Supplement (FD006) or Butzler Selective Supplement (FD007) or Skirrow Selective Supplement (FD008) or VTCA Selective Supplement (FD090) or Butzler VI Selective Supplement (FD106) to 500 ml sterile molten base along with rehydrated contents of 1 vial of Minerals Growth Supplement (FD009) and 5-7% v/v horse or sheep blood.

For Gardnerella species: Add rehydrated contents of 1 vial of GNA Selective Supplement (FD056) to 500 ml sterile molten base.

For Cocci: Add rehydrated contents of 1 vial of NC Selective Supplement (FD030) or NNP Selective Supplement (FD031) or CO Selective Supplement (FD119) to 500 ml sterile molten base.

## **Principle And Interpretation**

Columbia Blood Agar Base was devised by Ellner et al (1). This medium contains special peptone which supports rapid and luxuriant growth of fastidious and non-fastidious organisms. Also, this medium promotes typical colonial morphology; better pigment production and more sharply defined haemolytic reactions. Fildes found that Nutrient Agar supplemented with a digest of sheep blood supplied both of these factors and the medium would support the growth of *H. influenzae* (2,3). The inclusion of bacitracin makes the enriched Columbia Agar Medium selective for the isolation of *Haemophilus* species from clinical specimens, especially from upper respiratory tract (4). Columbia Agar Base is used as the base for the media containing blood and for selective media formulations in which different combinations of antimicrobial agents are used as additives.

Corn starch serves as an energy source and also neutralizes toxic metabolites. Sheep blood permits the detection of haemolysis and also provides heme (X factor) which is required for the growth of many bacteria. However it is devoid of V factor (Nicotinamide adenine dinucleotide) and hence *Haemophilus influenzae* which needs both the X and V factors, will not grow on this medium.

Columbia Agar Base with added sterile serum provides an efficient medium for *Corynebacterium diphtheriae* virulence test medium. After following the established technique for *C. diphtheriae*, lines of toxin-antitoxin precipitation are clearly visible in 48 hours. Many pathogens require carbon dioxide; therefore, plates may be incubated in an atmosphere containing approximately 3-10% CO<sub>2</sub>.

Precaution: Brucella cultures are highly infective and must be handled carefully; incubate in 5-10% CO<sub>2</sub>. Campylobacter species are best grown at  $42^{\circ}$ C in a micro aerophillic atmosphere. Plates with Gardenerella supplements plates should be incubated at  $35^{\circ}$ C for 48 hours containing 7% CO<sub>2</sub> (2).

#### Type of specimen

Clinical samples: throat swabs, pus.

## **Specimen Collection and Handling**

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (5,6).

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

## **Warning and Precautions**

In Vitro diagnostic use only. 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. Certain fastidious organisms like *Haemophilus influenzae* may not grow on the medium, blood supplementation may be required.
- 2. As this medium have a relatively high carbohydrate content, beta-hemolytic *Streptococci* may exhibit a greenish hemolytic reaction which may be mistaken for the alpha haemolysis.
- 3. Biochemical characterization is required on colonies of pure culture for complete identification.

#### **Performance and Evaluation**

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

## **Quality Control**

#### **Appearance**

Cream to yellow homogeneous free flowing powder.

#### Gelling

Firm, comparable with 1.5% Agar gel.

#### Colour and Clarity of prepared medium

Basal medium: Light amber coloured clear to slightly opalescent gel.

After addition of 5%w/v sterile defibrinated blood: Cherry red coloured opaque gel forms in Petri plates.

#### Reaction

Reaction of 4.4% w/v aqueous solution at 25°C. pH: 7.3±0.2

## pН

7.10-7.50

#### **Cultural Response**

Cultural characteristics observed with added 5% w/v sterile defibrinatedblood, after an incubation at 35-37°C for 24-48 hours.

Organism	Inoculum (CFU)	Growth	Recovery	Haemolysis
Neisseria meningitidis ATCC 13090	50-100	luxuriant	>=70%	none
Staphylococcus aureus subsp. aureus ATCC 25923 (00034*)	50-100	luxuriant	>=70%	beta / gamma
Staphylococcus epidermidis ATCC 12228 (00036*)	50-100	luxuriant	>=70%	gamma
Staphylococcus aureus subsp. aureus ATCC 6538 (00032*)	50-100	luxuriant	>=70%	beta / gamma
Streptococcus pneumoniae ATCC 6303	50-100	luxuriant	>=70%	alpha
Streptococcus pyogenes ATCC 19615	50-100	luxuriant	>=70%	beta
Clostridium sporogenes ATCC 19404 (00008*)	50-100	luxuriant	>=50 %	
Clostridium sporogenes ATCC 11437	50-100	luxuriant	>=50 %	
Clostridium perfringens ATCC 13124 (00007*)	50-100	luxuriant	>=50 %	
Clostridium perfringens ATCC 12934	50-100	luxuriant	>=50 %	
aureus ATCC 6538 (00032*)  Streptococcus pneumoniae ATCC 6303  Streptococcus pyogenes ATCC 19615  Clostridium sporogenes ATCC 19404 (00008*)  Clostridium sporogenes ATCC 11437  Clostridium perfringens ATCC 13124 (00007*)  Clostridium perfringens	50-100 50-100 50-100 50-100	luxuriant luxuriant luxuriant luxuriant	>=70% >=70% >=50 % >=50 % >=50 %	alpha

Key: (\*) Corresponding WDCM numbers.

## Storage and Shelf Life

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

## **Disposal**

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (5,6).

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Revision: 05/2024



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In vitro diagnostic medical device



Storage temperature



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**CE Marking** 



Do not use if package is damaged

#### Disclaimer:

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## **Mueller Hinton Agar**

**M173** 

#### **Intended Use:**

Recommended for determination of susceptibility of microorganisms to antimicrobial agents isolated from clinical samples.

## Composition\*\*

Ingredients	g/L
HM infusion solids B # (from 300g)	2.000
Acicase ##	17.500
Starch	1.500
Agar	17.000
Final pH (at 25°C)	$7.3 \pm 0.1$

<sup>\*\*</sup>Formula adjusted, standardized to suit performance parameters

#### **Directions**

Suspend 38.0 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. Note: The performance of this batch has been tested and standardised as per the current CLSI (formerly, NCCLS) document M6-protocols for Evaluating Dehydrated Mueller Hinton Agar.

## **Principle And Interpretation**

The Mueller Hinton formulation was originally developed as a simple, transparent agar medium for the cultivation of pathogenic *Neisseria* species (1). Other media were subsequently developed that replaced the use of Mueller Hinton Agar for the cultivation of pathogenic *Neisseria* species, but it became widely used in the determination of sulfonamide resistance of gonococci and other organisms. Mueller Hinton Agar is now used as a test medium for antimicrobial susceptibility testing (2). Mueller Hinton Agar is recommended for the diffusion of antimicrobial agents impregnated on paper disc through an agar gel as described in CLSI Approved Standard (3). Mueller Hinton Agar has been selected by the CLSI for several reasons:

- i. It demonstrates good batch-to-batch reproducibility for susceptible testing.
- ii. It is low in sulfonamide, trimethoprim and tetracycline inhibitors.
- iii. It supports the growth of most non-fastidious bacterial pathogens and
- iv. Many data and much experience regarding its performance have been recorded (4).

Kirby-Bauer et al recommended this medium for performing antibiotic susceptibility tests using a single disc of high concentration (5). WHO Committee on Standardization of Susceptibility Testing has accepted Mueller Hinton Agar for determining the susceptibility of microorganisms because of its reproducibility (6). Mueller Hinton Agar with 5% sheep blood and Mueller Hinton Agar with Hemoglobin have been recommended for antimicrobial susceptibility testing of *Streptococcus pneumoniae* and *Haemophilus influenzae*.

HM infusion B from and acicase provide nitrogenous compounds, carbon, sulphur and other essential nutrients. Starch acts as a protective colloid against toxic substances present in the medium. Starch hydrolysis yields dextrose, which serves as a source of energy. These ingredients are selected for low thymine and thymidine content as determined by MIC values for *Enterococcus faecalis* with sulfamethoxazole trimethoprim (SXT).

The Kirby-Bauer procedure is based on agar diffusion of antimicrobial substances impregnated on paper discs. This method employs disc with a single concentration of antimicrobial agent and the zone diameters observed are correlated with minimum inhibitory concentration (MIC) values (7,1,2). A standardized suspension of the organism is swabbed over the entire surface of the medium.

Paper discs impregnated with specific amounts of antimicrobial agents are then placed on the surface of the medium, incubated and zones of inhibition around each disc are measured. The susceptibility is determined by comparing with CLSI standards (4). The various factors, which influence disc diffusion susceptibility tests, are agar depth, disc potency, inoculum concentration, pH of the medium and beta-lactamase production by test organisms (4,8).

Mueller Hinton Agar is not appropriate for assay by disc diffusion method with slow growing organisms, anaerobes and

<sup># -</sup> Equivalent to Beef heart infusion

<sup>## -</sup> Equivalent to Casein acid hydrolysate

capnophiles. With slow growing organisms, increased incubation may cause deterioration of diffusing antibiotic and produce unprecise readings (9). Mueller Hinton Agar is recommended for the diffusion of antimicrobial agents impregnated on paper disc through an agar gel as described in NCCLS (National Committee for Clinical Laboratory Standards), now CLSI (Clinical and Laboratory Standards Institute) Approved Standard (10).

## Type of specimen

Clinical samples: Pure cultures isolated from urine, stool, blood etc.

## **Specimen Collection and Handling**

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (2,10-13).

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. This medium is recommended for susceptibility testing of pure cultures only.
- 2. Inoculum density may affect the zone size. Heavy inoculum may result in smaller zones or too less inoculum may result in bigger zones.
- 3. Fastidious organisms may not grow on this medium and may require supplementation of blood.
- 4. Fastidious anaerobes may not grow on this medium.
- 5. As antimicrobial susceptibility is carried with antibiotic disc, proper storage of the disc is desired which may affect the potency of the disc.
- 6. Under certain circumstances, the in vitro results of antibiotic susceptibility may not show the same in vivo.

#### **Performance and Evaluation**

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

## **Quality Control**

## Appearance

Cream to yellow homogeneous free flowing powder

## Gelling

Firm, comparable with 1.7% agar gel.

## Colour and Clarity of prepared medium

Light amber coloured clear to slightly opalescent gel froms in Petri plates

#### Reaction

Reaction of 3.8% w/v aqueous solution at 25°C. pH: 7.3±0.1

#### pН

7.20-7.40

## **Cultural Response**

Antibiotic susceptibility tests are performed in accordance with, and meet the acceptance limits of the current ISO/TS 16782 (15). Performance of the medium is checked in accordance with the CLSI/EUCAST guidelines.

For testing S. pneumoniae: The medium was supplemented with 5% Horse blood and 20 mg/l NAD, incubated at 34-36°C for 18-20 hours in 5% CO<sub>2</sub>.

For testing *H. influenzae*: The medium was supplemented with 5% Horse blood and 20 mg/l -NAD, incubated at 34-36°C for 18-20 hours in 5% CO2.

#### **Antibiotic Sensitivity test**

Various discs were tested for standard ATCC strains and zone of inhibition were measured after an incubation 30-35°C for 18 hours. (As per the latest CLSI Protocol M6 & Standards as per the current CLSI M100).

#### **Thymine/Thymidine Content**

# The zones for these discs are indicative of the Thymine/Thymidine content of the medium.

## **Divalent Cation Content**

\$ The zones for these discs are indicative of the Divalent Cation content of the medium

Organism	Growth	Standard Zone	Incubation temperature	Incubation period
Escherichia coli ATCC 25922 (00013*) Cephalothin CEP 30mcg Ampicillin AMP 10mcg Chloramphenicol C 30 mcg Gentamicin GEN 10mcg Co-Trimoxazole (Sulpha/ Trimethoprim) (COT) 25 mcg Sulphafurazole SF 300 mcg Cefotaxime CTX 5 mcg Tigecycline TGC 15mcg Tetracycline TE 30 mcg Amoxicillin- clavulanate AMC 30 mcg Ciprofloxacin CIP 5mcg	luxuriant	15-21 mm 15-22 mm 21-27 mm 19-26 mm 23-29 mm 15-23 mm 25-31 mm 20-27 mm 18-25 mm 18-24 mm	34-36°C	16-20 hours
Escherichia coli ATCC 35218 Amoxicillin- clavulanate AMC 30 mcg Piperacillin/Tazobactam PIT 100/10 mcg	luxuriant	17-22 mm 24-30 mm	34-36°C	16-20 hours
Ticarcillin TI 75 mcg Ticarcillin/Clavulanic acid TCC 75/10mcg Ampicillin AMP 10 mcg Ampicillin/Sulbactam A/S 10/10 mcg		6 mm 21-25mm 6 mm 13-19 mm		
Staphylococcus aureus subsp. aureus ATCC 25923 (00034*)  Erythromycin E 15 mcg Linezolid LZ 30 mcg Tetracycline TE 30 mcg Ciprofloxacin CIP 5mcg Amoxyclav(Amoxicillin/ Clavulanic acid) AMC 30 mcg Co-Trimoxazole COT 25 mcg Cefoxitin CX 30 mcg Oxacillin OX 1mcg Pristinomycin RP 15 mcg Gentamicin GEN 10 mcg Penicillin-G 10 units Ampicillin/Sulbactam A/S 10/10 mcg	luxuriant	22-30 mm 24-30 mm 24-30 mm 22-30 mm 28-36 mm 24-32 mm 23-29 mm 18-24 mm 21-28 mm 19-27 mm 26-37 mm 29-37 mm	34-36°C	16-20 hours
Staphylococcus aureus subsp. aureus ATCC 29213 (00131*) Penicillin-G P 1 unit Cefoxitin CX 30 mcg Erythromycin E 15 mcg Linezolid LZ 10 mcg Gentamicin GEN 10 mcg Tetracycline TE 30 mcg \$ Ciprofloxacin CIP 5mcg	luxuriant	12-18 mm 24-30 mm 23-29 mm 21-27 mm 19-25 mm 23-31 mm 21-27 mm	34-36°C	16-20 hours

Staphylococcus aureus subsp. aureusATCC 43300 (MRSA) (00211*)	luxuriant		34-36°C	24 hours
Oxacillin OX 1 mcg		Very Hazy to No Zone		
Cefoxitin CX 30 mcg		<=21 mm		
Pseudomonas aeruginosa ATCC 27853 (00025*)	luxuriant		34-36°C	16-20 hours
Ceftazidime CAZ 30 mcg		22-29 mm		
Ciprofloxacin CIP 5mcg		25-33 mm		
Tobramycin TOB 10 mcg \$ Amikacin AK 30 mcg \$		20-26 mm 20-26 mm		
Aztreonam AT 3mcg		23-29 mm		
Cephotaxime CTX 30 mcg		18-22 mm		
Gentamicin GEN 10 mcg \$		17-23 mm		
Imipenem IPM 10 mcg		20-28 mm		
Piperacillin PI 100 mcg		25-33 mm		
Piperacillin Tazobactum PIT 30/6 mcg		23-29 mm		
Enterococcus faecalis	luxuriant		34-36°C	16-20 hours
ATCC 29212 (00087*) Trimethoprim TR 5 mcg #		24-32 mm		
Ampicillin AMP 2 mcg		15-21 mm		
Imipenem IPM 10 mcg		24-30 mm		
Linezolid LZ 10 mcg		19-25 mm		
Nitrofurantoin NIT 100 mcg		18-24 mm		
Co-Trimoxazole (Sulpha/ Trimethoprim) (COT) 25 mcg		26-34 mm		
Vancomycin VA 5 mcg		10-16 mm		
Enterococcus faecalis ATCC33186 (00210*)	luxuriant		34-36°C	16-20 hours
Co-Trimoxazole (Sulpha/ Trimethoprim) (COT) 25 mcg		<=20 mm		
Streptococcus pneumoniae ATCC 49619	luxuriant		34-36°C	18-20 hours
Vancomycin VA 5 mcg		17-23 mm		
Haemophilus influenzae ATCC 49247	luxuriant		34-36°C	18-20 hours
Ampicillin AMP 2 mcg		6-12 mm		
Haemophilus influenzae ATCC 49766	luxuriant		34-36°C	18-20 hours
Cefixime CFM 5 mcg		29-35 mm		
** ** ****				

Key: \*Corresponding WDCM numbers.

## **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**

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 (3,5).

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- 12. European Committee on Antimicrobial Susceptibility Testing Breakpoint tables for interpretation of MICs and zone diameters Version 14.0, valid from 2024-01-01.
- 13. European Committee on Antimicrobial Susceptibility Testing Routine and extended internal quality control as recommended by EUCAST Version 14.0, valid from 2024-01-01.

Revision: 06/2024



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In vitro diagnostic medical device





Storage temperature



Do not use if package is damaged

#### Disclaimer:

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## HiCrome<sup>TM</sup> UTI Agar

M1353

#### Intended use

Recommended for presumptive identification and confirmation of microorganisms mainly causing urinary tract infections, can also be used for testing water, food, environmental and other clinical samples.

## Composition\*\*

Ingredients	<b>g</b> / <b>L</b>
Peptone, special	15.000
Chromogenic mixture	2.450
Agar	15.000
Final pH ( at 25°C)	$6.8 \pm 0.2$

<sup>\*\*</sup>Formula adjusted, standardized to suit performance parameters

#### **Directions**

Suspend 32.45 gram in 1000 ml purified /distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C. Mix well and pour into sterile Petri plates.

## **Principle And Interpretation**

Urinary tract infections are bacterial infections affecting parts of urinary tract. The common symptoms of urinary tract infection are urgency and frequency of micturition, with associated discomfort or pain. The common condition is cystitis, due to infection of the bladder with a uropathogenic bacterium, which most frequently is *Escherichia coli*, but sometimes *Staphylococcus saprophyticus* or especially in hospital-acquired infections, *Klebsiella* species, *Proteus mirabilis*, other coliforms, *Pseudomonas aeruginosa* or *Enterococcus faecalis* (1). HiCrome<sup>TM</sup> UTI Agar is formulated on basis of work carried out by Pezzlo (2) Wilkie et al (3), Friedman et al (4), Murray et al (5), Soriano and Ponte (6) and Merlino et al (7). These media are recommended for the detection of urinary tract pathogens where HiCrome<sup>TM</sup> UTI Agar has broader application as a general nutrient agar for isolation of various microorganisms. It facilitates and expedites the identification of some gram-negative bacteria and some gram-positive bacteria on the basis of different contrasted colony colours produced by reactions of genus or species specific enzymes with two chromogenic substrates. The chromogenic substrates are specifically cleaved by enzymes produced by *Enterococcus* species, *E.coli* and coliforms. Presence of amino acids like phenylalanine and tryptophan from peptones helps for detection of tryptophan deaminase activity, indicating the presence of *Proteus* species, *Morganella* species and *Providencia* species.

One of the chromogenic substrate is cleaved by ß-glucosidase possessed by Enterococci resulting in formation of blue colonies. *E.coli* produce pink colonies due to the enzyme ß-D-galactosidase that cleaves the other chromogenic substrate. Further confirmation of *E.coli* can be done by performing the indole test. Coliforms produce purple coloured colonies due to cleavage of both the chromogenic substrate. Colonies of *Proteus, Morganella* and *Providencia* species appear brown because of tryptophan deaminase activity. Peptone special provides nitrogenous, carbonaceous compounds, long chain amino acids, vitamins and other essential growth nutrients. This medium can be made selective by supplementation with antibiotics for detecting microorganisms associated with hospital borne infections.

## Type of specimen

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

## **Specimen Collection and Handling**

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (8,9).

For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (10,11). For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards (12). 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 an enzyme-substrate based reaction, the intensity of colour may vary with isolates.

#### **Performance and Evaluation**

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

## **Quality Control**

#### **Appearance**

Cream to yellow homogeneous free flowing powder

#### Gelling

Firm, comparable with 1.5% Agar gel

#### Colour and Clarity of prepared medium

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

#### Reaction

Reaction of 3.24% w/v aqueous solution at 25°C. pH: 6.8±0.2

pН

6.60-7.20

#### **Cultural Response**

Cultural characteristics observed after an incubation at 35-37°C for 16-24 hours.

Organism	Inoculum (CFU)	Growth	Recovery	Colour of Colony
Escherichia coli ATCC 25922 (00013*)	50-100	luxuriant	>=70%	Purple to magenta
Enterococcus faecalis ATCC 29212 (00087*)	50-100	luxuriant	>=70%	blue-green (small)
Klebsiella pneumoniae ATCC 13883 (00097*)	50-100	luxuriant	>=70%	blue to purple, mucoid
Proteus mirabilis ATCC 12453	50-100	luxuriant	>=70%	light brown
Pseudomonas aeruginosa ATCC 27853 (00025*)	50-100	luxuriant	>=70%	colourless (greenish pigment may be observed)
Staphylococcus aureus subsp. aureus ATCC 25923 (00034*)	50-100	luxuriant	>=70%	golden yellow

Key: \*Corresponding WDCM numbers.

## **Storage and Shelf Life**

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

### **Disposal**

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

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Revision: 07/2024



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## Cefoxitin (Cephoxitin) CX 30 mcg

**SD041** 

Cefoxitin (Cephoxitin) CX 30 mcg discs are used for antimicrobial susceptibility testing of bacterial cultures as per Kirby-Bauer Method

## **Composition**

## \*Ingredients

Cefoxitin Concentration (Cephoxitin) 30 mcg/disc

## **Susceptibility Test Procedure:**

- 1. Prepare plates with Mueller Hinton Agar (M173/M1084) for rapidly growing aerobic organisms as per Kirby- Bauer Method. The medium in the plates should be sterile and should have a depth of about 4 mm.
- 2. Inoculate 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 (prepared by mixing 0.5 ml of 1.175% barium chloride and 99.5 ml of 0.36N sulfuric acid). Dilute the inoculum or incubate further as necessary to attain comparative turbidity. Alternatively, the inoculum can be standardized by other appropriate optical method (0.08 0.13 OD turbid suspension at 625 nm)
- 3. 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. Allow the inoculum to dry for 5 15 minutes with lid in place.
- 4. Apply the discs using aseptic technique. When using cartridges, the discs can be applied using the specially designed applicator. When the vials are used, apply the discs using sterile forceps.
- 5. Deposit the discs with centers at least 24 mm apart. For fastidious organisms and for Penicillins and Cephalosporins, the discs should preferably be deposited with centers 30 mm apart.
- 6. Incubate immediately at  $35 \pm 2^{\circ}$ C and examine after 16-18 hours or longer, if necessary. For fastidious organisms incubate at appropriate temperature and time.
- 7. Measure the zones showing complete inhibition and record the diameters of the zones to the nearest millimeter using a calibrated instrument like zone scales (PW096/PW297)

## **Principle:**

Antimicrobial susceptibility testing (AST) of bacterial and fungal isolates is a common and important technique in most clinical laboratories. The results of these tests are used for selection of the most appropriate antimicrobial agent(s) for treatment against the infectious organisms. Till the 1950s, laboratories were lacking in the methodologies and equipments for the accurate determination of in vitro responses of organisms to antimicrobial agents. Bauer et al (1) began the development of standardized methods for antimicrobial susceptibility testing, using disc diffusion system. However the susceptibility results may not always correlate with the patient's response to therapy. The response of an infected patient to antimicrobial agent(s) is a complex interrelationship of host responses, drug dynamics and microbial activity. Antimicrobial susceptibility tests are either quantitative or qualitative. Disc diffusion test is a qualitative test method. The National Committee for Clinical Laboratory Standards (NCCLS), now known as Clinical Laboratory Standards Institute (CLSI) has published comprehensive documents regarding the disc diffusion systems. The agar disc diffusion test is the most convenient and widely used method for routine antimicrobial susceptibility testing. In subsequent and current practice, antimicrobial impregnated paper discs are applied onto the agar surface. Based on the Kirby- Bauer Method, standardized reference procedures for the disc systems were published by WHO and FDA and are periodically updated by the CLSI (formerly NCCLS)(2). For any antimicrobial testing, Quality control or clinical testing, the method to be followed is the same as mentioned above.

However few precautions are to be maintained while handling of the Sensitivity discs,

- On receipt the discs are to be immediately stored at the recommended temperature.
- Medium preparation, Inoculum preparation and incubation to be done as specified.

## **Interpretation:**

Use following interpretive criteria for susceptibility categorization\*

		Sensitive	Intermediate	Resistant
Antimicrobial agent	Interpretative criteria for	mm or more	mm	mm or less
	Enterobacterales	16	13-15	14
Cefoxitin	For S.aureus & S.lugdunensis	22	-	21
(Cephoxitin) 30 mcg	For Coagulase- negative Staphylococci except S.lugdunensis & S.pseudintermedius	25	-	24
	Neisseria gonorhoeae	28	24-27	23

## **Quality Control:**

**Appearance:** Filter paper discs of 6mm diameter with printed "CX 30" on centre of each side of the disc.

**Cultural response**: Average diameter of zone of inhibition observed on Mueller Hinton Agar (M173) after 18 hours incubation at 35-37°C for standard cultures.

Organisms (ATCC)	Std. zone of diameter (mm)*
E.coli (25922)	23-29
S.aureus (25923)	23-29

<sup>\* =</sup> Interpretive criteria & QC ranges as per CLSI & EUCAST standards.

## **Storage and Shelf-life:**

On receipt discs should always be stored at  $-20^{\circ}$ C under dry conditions, along with the dessicator provided in individual pack. Use before expiry date on the label.

#### **References:**

- 1. Bauer, Kirby, Sherris and Turck, 1966, Am. J. Clin. Path., 45: 493
- 2. Performance standards of Antimicrobial Disc Susceptibility Tests, M100S, 32nd Ed., CLSI Vol. 42 No.2, Feb-2022.
- 3. EUCAST, Breakpoint tables for interpretation of MIC's & zone diameters, version 12.0, valid from 01.01.2022.

#### Note:

Use following media to carry out susceptibility test

For rapidly growing aerobic organisms : Mueller Hinton Agar (M173/M1084)

For *Haemophilus* spps: Haemophilus Test Agar (M1259 + FD117)

For *S.pneumoniae*: Muller Hinton Agar supplemented with 5% Sheep Blood For Neisseria spps: G.C.Agar +1% defined growth supplement (M434 + FD025)

#### \* Not for Medicinal Use

IVD

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Ceftazidime CAZ 30 mcg

**SD062** 

Ceftazidime CAZ 30 mcg discs are used for antimicrobial susceptibility testing of bacterial cultures as per Bauer-Kirby Method

Composition

\*Ingredients Concentration
Ceftazidime 30 mcg/disc

## **Susceptibility Test Procedure:**

- 1. Prepare plates with Mueller Hinton Agar (M173/M1084) for rapidly growing aerobic organisms as per Bauer-Kirby Method. The medium in the plates should be sterile and should have a depth of about 4 mm.
- 2. Inoculate 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 (prepared by mixing 0.5 ml of 1.175% barium chloride and 99.5 ml of 0.36N sulfuric acid). Dilute the inoculum or incubate further as necessary to attain comparative turbidity. Alternatively, the inoculum can be standardized by other appropriate optical method (0.08 0.13 OD turbid suspension at 625 nm)
- 3. 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. Allow the inoculum to dry for 5 15 minutes with lid in place.
- 4. Apply the discs using aseptic technique. When using cartridges, the discs can be applied using the specially designed applicator. When the vials are used, apply the discs using sterile forceps.
- 5. Deposit the discs with centers at least 24 mm apart. For fastidious organisms and for Penicillins and Cephalosporins, the discs should preferably be deposited with centers 30 mm apart.
- 6. Incubate immediately at  $35 \pm 2^{\circ}$ C and examine after 16-18 hours or longer, if necessary. For fastidious organisms incubate at appropriate temperature and time.
- 7. Measure the zones showing complete inhibition and record the diameters of the zones to the nearest millimeter using a calibrated instrument like zone scales (PW096/PW297)

#### **Principle:**

Antimicrobial susceptibility testing (AST) of bacterial and fungal isolates is a common and important technique in most clinical laboratories. The results of these tests are used for selection of the most appropriate antimicrobial agent(s) for treatment against the infectious organisms. Till the 1950s, laboratories were lacking in the methodologies and equipments for the accurate determination of in vitro responses of organisms to antimicrobial agents. Bauer et al (1) began the development of standardized methods for antimicrobial susceptibility testing, using disc diffusion system. However the susceptibility results may not always correlate with the patient's response to therapy. The response of an infected patient to antimicrobial agent(s) is a complex interrelationship of host responses, drug dynamics and microbial activity. Antimicrobial susceptibility tests are either quantitative or qualitative. Disc diffusion test is a qualitative test method. The National Committee for Clinical Laboratory Standards (NCCLS), now known as Clinical Laboratory Standards Institute (CLSI) has published comprehensive documents regarding the disc diffusion systems. The agar disc diffusion test is the most convenient and widely used method for routine antimicrobial susceptibility testing. In subsequent and current practice, antimicrobial impregnated paper discs are applied onto the agar surface. Based on the Bauer-Kirby Method, standardized reference procedures for the disc systems were published by WHO and FDA and are periodically updated by the CLSI (formerly NCCLS)(2). For any antimicrobial testing, Quality control or clinical testing, the method to be followed is the same as mentioned above.

However few precautions are to be maintained while handling of the Sensitivity discs,

- On receipt the discs are to be immediately stored at the recommended temperature.
- Medium preparation, Inoculum preparation and incubation to be done as specified.

## **Interpretation:**

Use following interpretive criteria for susceptibility categorization\*

		Sensitive	Intermediate	Resistant
Antimicrobial agent	Interpretative criteria for	mm or more	mm	mm or less
	Enterobacteriaceae, B.cepacia	21	18-20	17
Ceftazidime	P.aeruginosa, Acientobacter & Staphylococcus	18	15-17	14
30 mcg	Haemophilus influenzae & Haemophilus parainfluenzae	26	-	-
	Neisseria gonorhoeae	31	-	-

## **Quality Control:**

**Appearance:** Filter paper discs of 6mm diameter with printed "CAZ 30" on centre of each side of the disc.

**Cultural response**: Average diameter of zone of inhibition observed on Mueller Hinton Agar (M173) after 18 hours incubation at 35-37°C for standard cultures.

Organisms (ATCC)	Std. zone of diameter (mm)*
E.coli (25922)	25-32
S.aureus (25923)	16-20
P.aeruginosa (27853)	22-29
K.pneumonaie (700603)	10-18

<sup>\* =</sup> Interpretive criteria & QC ranges as per CLSI standards.

## **Storage and Shelf-life:**

On receipt discs should always be stored at -20°C under dry conditions, along with the dessicator provided in individual pack. Use before expiry date on the label.

#### **References:**

- 1. Bauer, Kirby, Sherris and Turck, 1966, Am. J. Clin. Path., 45: 493
- 2. Performance standards of Antimicrobial Disc Susceptibility Tests, M100S, 32nd Ed., CLSI Vol. 42 No.2, Feb-2022.
- 3. EUCAST, Breakpoint tables for interpretation of MIC's & zone diameters, version 12.0, valid from 01.01.2022.

#### Note:

Use following media to carry out susceptibility test

For rapidly growing aerobic organisms: Mueller Hinton Agar (M173/M1084)

For Haemophilus spps: Haemophilus Test Agar (M1259 + FD117)

For *S.pneumoniae*: Muller Hinton Agar supplemented with 5% Sheep Blood For Neisseria spps: G.C.Agar +1% defined growth supplement (M434 + FD025)

<sup>\*</sup> Not for Medicinal Use





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Storage temperature





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## Amoxyclav AMC 30 mcg (Amoxycillin/Clavulanic acid) (20/10mcg)

**SD063** 

Amoxyclav (Amoxycillin/Clavulanic acid) AMC 30mcg (20/10mcg) discs are used for antimicrobial susceptibility testing of bacterial cultures as per Bauer-Kirby Method

## **Composition**

\*Ingredients Concentration
Amoxyclav 30mcg/disc
(Amoxycillin/Clavulanic acid)

## **Susceptibility Test Procedure:**

- 1. Prepare plates with Mueller Hinton Agar (M173/M1084) for rapidly growing aerobic organisms as per Bauer-Kirby Method. The medium in the plates should be sterile and should have a depth of about 4 mm.
- 2. Inoculate 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 (prepared by mixing 0.5 ml of 1.175% barium chloride and 99.5 ml of 0.36N sulfuric acid). Dilute the inoculum or incubate further as necessary to attain comparative turbidity. Alternatively, the inoculum can be standardized by other appropriate optical method (0.08 0.13 OD turbid suspension at 625 nm)
- 3. 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. Allow the inoculum to dry for 5 15 minutes with lid in place.
- 4. Apply the discs using aseptic technique. When using cartridges, the discs can be applied using the specially designed applicator. When the vials are used, apply the discs using sterile forceps.
- 5. Deposit the discs with centers at least 24 mm apart. For fastidious organisms and for Penicillins and Cephalosporins, the discs should preferably be deposited with centers 30 mm apart.
- 6. Incubate immediately at  $35 \pm 2^{\circ}$ C and examine after 16-18 hours or longer, if necessary. For fastidious organisms incubate at appropriate temperature and time.
- 7. Measure the zones showing complete inhibition and record the diameters of the zones to the nearest millimeter using a calibrated instrument like zone scales (PW096/PW297)

#### **Principle:**

Antimicrobial susceptibility testing (AST) of bacterial and fungal isolates is a common and important technique in most clinical laboratories. The results of these tests are used for selection of the most appropriate antimicrobial agent(s) for treatment against the infectious organisms. Till the 1950s, laboratories were lacking in the methodologies and equipments for the accurate determination of in vitro responses of organisms to antimicrobial agents. Bauer et al (1) began the development of standardized methods for antimicrobial susceptibility testing, using disc diffusion system. However the susceptibility results may not always correlate with the patient's response to therapy. The response of an infected patient to antimicrobial agent(s) is a complex interrelationship of host responses, drug dynamics and microbial activity. Antimicrobial susceptibility tests are either quantitative or qualitative. Disc diffusion test is a qualitative test method. The National Committee for Clinical Laboratory Standards (NCCLS), now known as Clinical Laboratory Standards Institute (CLSI) has published comprehensive documents regarding the disc diffusion systems. The agar disc diffusion test is the most convenient and widely used method for routine antimicrobial susceptibility testing. In subsequent and current practice, antimicrobial impregnated paper discs are applied onto the agar surface. Based on the Bauer-Kirby Method, standardized reference procedures for the disc systems were published by WHO and FDA and are periodically updated by the CLSI (formerly NCCLS)(2). For any antimicrobial testing, Quality control or clinical testing, the method to be followed is the same as mentioned above.

However few precautions are to be maintained while handling of the Sensitivity discs,

- On receipt the discs are to be immediately stored at the recommended temperature.
- Medium preparation, Inoculum preparation and incubation to be done as specified.

## **Interpretation:**

Use following interpretive criteria for susceptibility categorization\*

Antimicrobial agent	Interpretative criteria for	Sensitive mm or more	Intermediate mm	Resistant mm or less
	Enterobacteriaceae	18	14-17	13
Amoxyclav (Amoxycillin/Clavulanic acid) 30mcg (20/10mcg)	Haemophilus influenzae & Haemophilus parainfluenzae	20	-	19

## **Quality Control:**

**Appearance:** Filter paper discs of 6mm diameter with printed "AMC 30" on centre of each side of the disc.

**Cultural response**: Average diameter of zone of inhibition observed on Mueller Hinton Agar (M173) after 18 hours incubation at 35-37°C for standard cultures.

Organisms (ATCC)	Std. zone of diameter (mm)*
E. coli (25922)	18-24
S.aureus (25923)	28-36
E.coli (35218)	17-22

<sup>\* =</sup> Interpretive criteria & QC ranges as per CLSI standards.

## **Storage and Shelf-life:**

On receipt discs should always be stored at -20°C under dry conditions, along with the dessicator provided in individual pack. Use before expiry date on the label.

#### **References:**

- 1. Bauer, Kirby, Sherris and Turck, 1966, Am. J. Clin. Path., 45: 493
- Performance standards of Antimicrobial Disc Susceptibility Tests, M100S, 32nd Ed., CLSI Vol. 42 No.2, Feb-2022.
- 3. EUCAST, Breakpoint tables for interpretation of MIC's & zone diameters, version 12.0, valid from 01.01.2022.

#### Note:

Use following media to carry out susceptibility test

For rapidly growing aerobic organisms: Mueller Hinton Agar (M173/M1084)

For *Haemophilus* spps: Haemophilus Test Agar (M1259 + FD117)

For *S.pneumoniae*: Muller Hinton Agar supplemented with 5% Sheep Blood For Neisseria spps: G.C.Agar +1% defined growth supplement (M434 + FD025)

#### \* Not for Medicinal Use



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Ceftriaxone CTR 30 mcg

**SD065** 

Ceftriaxone CTR 30 mcg discs are used for antimicrobial susceptibility testing of bacterial cultures as per Bauer-Kirby Method

**Composition** 

\*Ingredients Concentration Ceftriaxone 30 mcg/disc

## **Susceptibility Test Procedure:**

- 1. Prepare plates with Mueller Hinton Agar (M173/M1084) for rapidly growing aerobic organisms as per Bauer-Kirby Method. The medium in the plates should be sterile and should have a depth of about 4 mm.
- 2. Inoculate 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 (prepared by mixing 0.5 ml of 1.175% barium chloride and 99.5 ml of 0.36N sulfuric acid). Dilute the inoculum or incubate further as necessary to attain comparative turbidity. Alternatively, the inoculum can be standardized by other appropriate optical method (0.08 0.13 OD turbid suspension at 625 nm)
- 3. 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. Allow the inoculum to dry for 5 15 minutes with lid in place.
- 4. Apply the discs using aseptic technique. When using cartridges, the discs can be applied using the specially designed applicator. When the vials are used, apply the discs using sterile forceps.
- 5. Deposit the discs with centers at least 24 mm apart. For fastidious organisms and for Penicillins and Cephalosporins, the discs should preferably be deposited with centers 30 mm apart.
- 6. Incubate immediately at  $35 \pm 2^{\circ}$ C and examine after 16-18 hours or longer, if necessary. For fastidious organisms incubate at appropriate temperature and time.
- 7. Measure the zones showing complete inhibition and record the diameters of the zones to the nearest millimeter using a calibrated instrument like zone scales (PW096/PW297)

#### **Principle:**

Antimicrobial susceptibility testing (AST) of bacterial and fungal isolates is a common and important technique in most clinical laboratories. The results of these tests are used for selection of the most appropriate antimicrobial agent(s) for treatment against the infectious organisms. Till the 1950s, laboratories were lacking in the methodologies and equipments for the accurate determination of in vitro responses of organisms to antimicrobial agents. Bauer et al (1) began the development of standardized methods for antimicrobial susceptibility testing, using disc diffusion system. However the susceptibility results may not always correlate with the patient's response to therapy. The response of an infected patient to antimicrobial agent(s) is a complex interrelationship of host responses, drug dynamics and microbial activity. Antimicrobial susceptibility tests are either quantitative or qualitative. Disc diffusion test is a qualitative test method. The National Committee for Clinical Laboratory Standards (NCCLS), now known as Clinical Laboratory Standards Institute (CLSI) has published comprehensive documents regarding the disc diffusion systems. The agar disc diffusion test is the most convenient and widely used method for routine antimicrobial susceptibility testing. In subsequent and current practice, antimicrobial impregnated paper discs are applied onto the agar surface. Based on the Bauer-Kirby Method, standardized reference procedures for the disc systems were published by WHO and FDA and are periodically updated by the CLSI (formerly NCCLS)(2). For any antimicrobial testing, Quality control or clinical testing, the method to be followed is the same as mentioned above.

However few precautions are to be maintained while handling of the Sensitivity discs,

- On receipt the discs are to be immediately stored at the recommended temperature.
- Medium preparation, Inoculum preparation and incubation to be done as specified.

## **Interpretation:**

Use following interpretive criteria for susceptibility categorization\*

		Sensitive	Intermediate	Resistant
Antimicrobial agent	Interpretative criteria for	mm or more	mm	mm or less
	Enterobacteriaceae	23	20-22	19
	P.aeruginosa, Acientobacter & Staphylococcus	21	14-20	13
	Haemophilus influenzae & Haemophilus parainfluenzae	26	-	-
Ceftriaxone 30 mcg	Neisseria meningitidis	34	-	-
	Neisseria gonorhoeae	35	-	-
	Streptococcus spp. Viridians group	27	25-26	24
	Streptococcus spp. beta haemolytic gruop	24	-	-

## **Quality Control:**

Appearance: Filter paper discs of 6mm diameter with printed "CTR 30" on centre of each side of the disc.

**Cultural response**: Average diameter of zone of inhibition observed on Mueller Hinton Agar (M173) after 18 hours incubation at 35-37°C for standard cultures.

Organisms (ATCC)	Std. zone of diameter (mm)*
E.coli (25922)	29-35
S.aureus (25923)	22-28
P.aeruginosa (27853)	17-23
K.pneumoniae (700603)	16-24

<sup>\* =</sup> Interpretive criteria & QC ranges as per CLSI standards.

## **Storage and Shelf-life:**

On receipt discs should always be stored at -20°Cunder dry conditions, along with the dessicator provided in individual pack. Use before expiry date on the label.

## References:

- 1. Bauer, Kirby, Sherris and Turck, 1966, Am. J. Clin. Path., 45: 493
- 2. Performance standards of Antimicrobial Disc Susceptibility Tests, M100S, 32nd Ed., CLSI Vol. 42 No.2, Feb-2022.
- 3. EUCAST, Breakpoint tables for interpretation of MIC's & zone diameters, version 12.0, valid from 01.01.2022.

## Note:

Use following media to carry out susceptibility test

For rapidly growing aerobic organisms: Mueller Hinton Agar (M173/M1084)

For Haemophilus spps: Haemophilus Test Agar (M1259 + FD117)

For S.pneumoniae: Muller Hinton Agar supplemented with 5% Sheep Blood For Neisseria spps: G.C.Agar +1% defined growth supplement (M434 + FD025)

<sup>\*</sup> Not for Medicinal Use

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Pefloxacin PF 5 mcg

**SD070** 

Pefloxacin PF 5 mcg discs are used for antimicrobial susceptibility testing of bacterial cultures as per Bauer-Kirby Method

Composition

\*Ingredients Concentration
Pefloxacin 5 mcg/disc

## **Susceptibility Test Procedure:**

- 1. Prepare plates with Mueller Hinton Agar (M173/M1084) for rapidly growing aerobic organisms as per Bauer-Kirby Method. The medium in the plates should be sterile and should have a depth of about 4 mm.
- 2. Inoculate 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 (prepared by mixing 0.5 ml of 1.175% barium chloride and 99.5 ml of 0.36N sulfuric acid). Dilute the inoculum or incubate further as necessary to attain comparative turbidity. Alternatively, the inoculum can be standardized by other appropriate optical method (0.08 0.13 OD turbid suspension at 625 nm)
- 3. 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. Allow the inoculum to dry for 5 15 minutes with lid in place.
- 4. Apply the discs using aseptic technique. When using cartridges, the discs can be applied using the specially designed applicator. When the vials are used, apply the discs using sterile forceps.
- 5. Deposit the discs with centers at least 24 mm apart. For fastidious organisms and for Penicillins and Cephalosporins, the discs should preferably be deposited with centers 30 mm apart.
- 6. Incubate immediately at  $35 \pm 2^{\circ}$ C and examine after 16-18 hours or longer, if necessary. For fastidious organisms incubate at appropriate temperature and time.
- 7. Measure the zones showing complete inhibition and record the diameters of the zones to the nearest millimeter using a calibrated instrument like zone scales (PW096/PW297)

#### **Principle:**

Antimicrobial susceptibility testing (AST) of bacterial and fungal isolates is a common and important technique in most clinical laboratories. The results of these tests are used for selection of the most appropriate antimicrobial agent(s) for treatment against the infectious organisms. Till the 1950s, laboratories were lacking in the methodologies and equipments for the accurate determination of in vitro responses of organisms to antimicrobial agents. Bauer et al (1) began the development of standardized methods for antimicrobial susceptibility testing, using disc diffusion system. However the susceptibility results may not always correlate with the patient's response to therapy. The response of an infected patient to antimicrobial agent(s) is a complex interrelationship of host responses, drug dynamics and microbial activity. Antimicrobial susceptibility tests are either quantitative or qualitative. Disc diffusion test is a qualitative test method. The National Committee for Clinical Laboratory Standards (NCCLS), now known as Clinical Laboratory Standards Institute (CLSI) has published comprehensive documents regarding the disc diffusion systems. The agar disc diffusion test is the most convenient and widely used method for routine antimicrobial susceptibility testing. In subsequent and current practice, antimicrobial impregnated paper discs are applied onto the agar surface. Based on the Bauer-Kirby Method, standardized reference procedures for the disc systems were published by WHO and FDA and are periodically updated by the CLSI (formerly NCCLS)(2). For any antimicrobial testing, Quality control or clinical testing, the method to be followed is the same as mentioned above.

However few precautions are to be maintained while handling of the Sensitivity discs,

- On receipt the discs are to be immediately stored at the recommended temperature.
- Medium preparation, Inoculum preparation and incubation to be done as specified.

## **Quality Control:**

**Appearance:** Filter paper discs of 6mm diameter with printed "PF 5" on centre of each side of the disc.

**Cultural response**: Average diameter of zone of inhibition observed on Mueller Hinton Agar (M173) after 18 hours incubation at 35-37°C for standard cultures.

Organisms (ATCC)	Std. zone of diameter (mm)
E. coli (25922)	29-33
S.aureus (25923)	24-28
P.aeruginosa (27853)	17-21

### **Storage and Shelf-life:**

Discs should always be stored at -20°C to +8°C under dry conditions, along with the dessicator provided in individual pack. Use before expiry date on the label.

#### **References:**

- 1. Bauer, Kirby, Sherris and Turck, 1966, Am. J. Clin. Path., 45: 493
- 2. Performance standards of Antimicrobial Disc Susceptibility Tests, M100S, 32nd Ed., CLSI Vol. 42 No.2, Feb-2022.
- 3. EUCAST, Breakpoint tables for interpretation of MIC's & zone diameters, version 12.0, valid from 01.01.2022.

#### Note:

Use following media to carry out susceptibility test

For rapidly growing aerobic organisms: Mueller Hinton Agar (M173/M1084)

For Haemophilus spps: Haemophilus Test Agar (M1259 + FD117)

For *S.pneumoniae*: Muller Hinton Agar supplemented with 5% Sheep Blood For Neisseria spps: G.C.Agar +1% defined growth supplement (M434 + FD025)

## \* Not for Medicinal Use



In vitro diagnostic medical



CE Marking



Storage temperature



Do not use if package is damaged



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Revision: 03 / 2022

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