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
pH
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).

Reference

1. Collee J. G., Fraser A. G., Marmion B. P., Simmons A., (Eds.), Mackie and McCartney, Practical Medical Microbiology, 1996, 14th Edition, Churchill Livingstone.
2. Pezzlo M., 1998, Clin. Microbiol. Rev., 1:268-280.
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12. Lipps WC, Braun-Howland EB, Baxter TE,eds. Standard methods for the Examination of Water and Wastewater, 24th ed. Washington DC:APHA Press; 2023.

Revision : 07/2024

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

CE Marking

15°C – 25°C **Storage temperature**

Do not use if package is damaged

Disclaimer :

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Bifidobacterium Broth

M1395

Intended Use:

Recommended for cultivation of *Bifidobacterium infantis.*

Composition**

Ingredients	g / L
Tryptone	20.000
Peptone	10.000
Yeast extract	10.000
Tomato juice, solids	16.650
Dextrose (Glucose)	20.000
Polysorbate 80 (Tween 80)	2.000
Final pH (at 25°C)	6.8±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 78.65 grams in 1000 ml purified / distilled water . Heat if necessary to dissolve the medium completely. Distribute in tubes or flasks as desired. Sterilize by autoclaving at 15lbs pressure for 15 minutes.

Principle And Interpretation

The genus *Bifidobacterium* is the third most numerous bacterial population found in the human intestine after Bacteroides and *Eubacterium*. It is an anaerobic bacteria that makes up the gut microbial flora, it resides in the colon and have health benefits for their hosts. Bifidobacteria are also associated with lower incidences of allergies (1,2). Bifidobacterium Broth is used for the cultivation and maintenance of *Bifidobacterium* species. The medium is used exclusively for the cultivation of *Bifidobacterium infantis* (3).

Tryptone, Peptone and yeast extract provides essential growth nutrients. Glucose is the energy source and sodium chloride maintains isotonic conditions. Tomato juice helps in maintaining acidic pH while polysorbate 80 provides fatty acids required for metabolic activity of *Bifidobacterium*.

Type of specimen

Clinical samples- faeces; Dairy samples.

Specimen Collection and Handling:

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (4,5). For dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (6,7). 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. Further biochemical and serological tests must be carried out for complete identification.
2. *Bifidobacterium* species are strict anaerobes, hence condition must be appropriately maintained.

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
Colour and Clarity of prepared medium
Amber coloured clear solution in tubes

Reaction
Reaction of 7.86% w/v solution at 25°C. pH : 6.8±0.2
pH
6.60-7.00
Cultural Response
Cultural characteristics observed after an incubation at 35-37°C for 24-48 hours.

Organism	Inoculum (CFU)	Growth
Bifidobacterium infantis ATCC 25962	50-100	good-luxuriant
Bifidobacterium bifidum ATCC 15696	50-100	good-luxuriant
Bifidobacterium breve ATCC 15698	50-100	good-luxuriant

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 15-25°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition. Seal the container tightly after use. Product performance is best if used within stated expiry period.

Disposal

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

Reference

1.Bjorksten B., Sepp E., Julge K., Voor T., and Mikelsaar M., 2001, J. Allergy Clin. Microbiol., Volume 108, Issue 4, 516-520.
2.Guarner F., and Malagelada J. R., 2003, The Lancet, Vol. 361, Issue 9356, 8 February 2003, 512-519
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6.American Public Health Association, Standard Methods for the Examination of Dairy Products, 1978, 14th Ed., WashingtonD.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.

Revision : 04/2024


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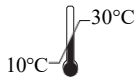
IVD *In vitro* diagnostic medical device



Storage temperature

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CE CE Marking


Do not use if package is damaged

HiCrome™ Enterococcus faecium Agar Base M1580

Intended use

Recommended for the chromogenic identification of *Enterococcus faecium* from faeces, sewage and water supplies.

Composition**

Ingredients	g / L
Peptone, special	23.000
Corn starch	1.000
Sodium chloride	5.000
Arabinose	10.000
Phenol red	0.100
Chromogenic substrate	0.100
Agar	15.000
Final pH (at 25°C)	7.8±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 27.1 grams in 500 ml purified / distilled water. Heat to boiling to dissolve the medium completely. DO NOT AUTOCLAVE. Cool to 45-50°C and aseptically add sterile rehydrated contents of 1 vial of AC Selective Supplement (FD226). Mix well and pour into sterile Petri plates.

Principle And Interpretation

HiCrome™ Enterococcus faecium Agar Base is recommended for the chromogenic detection of *Enterococcus faecium* from urine, faeces, soil, food, water, plants and animals. *E.faecium* is commonly found in the gastrointestinal tracts of humans (1). The resistance exhibited by *Enterococcus* species to various antimicrobials has led them to being a major cause of human infections including nosocomial infections (2). *E.faecalis* causes 80-90% of infection while *E.faecium* causes the majority of the remainder (3). The use of selective media for the isolation of Enterococci has been previously reviewed, including those containing chromogenic substrates (4) and media containing cephalexin-aztreonam supplements. *Enterococcus* species possess the enzyme ß-glucosidase, which specifically cleaves the chromogenic substrate to produce blue coloured colonies. *E.faecium* ferment arabinose; and cleaves the chromogenic substrate present in the media to produce green coloured colonies along with yellow colouration to the medium. *E.faecalis* does not ferment arabinose and therefore retains the blue colour. Peptone special serves as a source of carbon, nitrogen and essential growth nutrients. Corn starch neutralizes the toxic metabolites while sodium chloride maintains the osmotic equilibrium. Phenol red serves as a pH indicator with arabinose being the fermentable carbohydrate.

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 (5,6).
For food samples, follow appropriate techniques for sample collection and processing as per guidelines (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 Precaution

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 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.
3. Slight colour variations may be observed depending on the utilization of the substrate by the organism.

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 pinkish beige 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 5.42% w/v aqueous solution at 25°C. pH : 7.8±0.2

pH

7.60-8.00

Cultural Response

Cultural characteristics observed with added AC Selective Supplement (FD226) after an incubation at 35-37°C for 24-48 hours.

Organism	Inoculum (CFU)	Growth	Recovery	Colour of Colony
Escherichia coli ATCC 25922 (00013*)	>=10^4	inhibited	0%	
Enterococcus faecalis ATCC 29212 (00087*)	50-100	luxuriant	>=50%	blue
Enterococcus faecium ATCC 19434 (00010*)	50-100	luxuriant	>=50%	green
Enterococcus hirae ATCC 10541 (00011*)	50-100	luxuriant	>=50%	blue
Pseudomonas aeruginosa ATCC 27853 (00026*)	>=10^4	inhibited	0%	
Staphylococcus aureus subsp.*aureus* ATCC 25923 (00034*)	>=10^4	inhibited	0%	

Key : (*) Corresponding WDCM numbers.

Storage and Shelf Life

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

Disposal

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

Reference

1. Skinner F. A. and Quesnel L. B., (Ed.), 1978, Streptococci. Academic Press, Inc. (London) Ltd., London, United Kingdom, p. 245-261.
2. Chenoweth C., Schaberg D., The Epidemiology of Enterococci, Eur. J.Clin. Micorbiol. Infect. Dis., 9:80-89, 1990.
3. Moellering R. C., 1992, Clin. Infect. Dis. 14:1173.
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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.
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. Lipps WC, Braun-Howland EB, Baxter TE,eds. Standard methods for the Examination of Water and Wastewater, 24th ed. Washington DC:APHA Press; 2023.

Revision : 06/2024

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

15°C – 25°C **Storage temperature**

CE Marking

Do not use if package is damaged

Urea Indole Medium

M1784

Intended Use

Recommended for differentiation of micro-organisms especially *Enterobacteriaceae* on the basis of their ability to hydrolyze urea and indole production.

Composition**

Ingredients	g / L
L- Tryptophan	3.000
Sodium chloride	5.000
Potassium dihydrogen phosphate	1.000
Dipotassium hydrogen phosphate	1.000
Urea	20.000
Phenol red	0.012
Final pH (at 25°C)	6.8±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 30.01 grams in 1000 ml purified/distilled water. Dissolve the medium completely and sterilize by filtration. **DO NOT AUTOCLAVE**. Aseptically, dispense into sterile tubes or flasks as desired.

Principle And Interpretation

Strains of *Enterobacteria* are associated with abscesses, pneumonia, meningitis, septicemia and infections of wounds, the urinary tract and the intestine. They are a major component of the normal intestinal flora of humans but are relatively uncommon at other body sites. Of clinically significant isolates, *Enterobacteriaceae* may account for 80% of gram-negative bacilli and 50% of all clinically significant isolates in clinical microbiology laboratories (1).

Urea Indole Medium is used for the identification of *Enterobacteria* on the basis of Urease and indole production and the transdeamination of tryptophan. This medium is very useful in the identification of *Proteus* species from *Salmonella* and *Shigella* species. The results for urease production should be noted prior to indole reaction, as addition of Kovac's reagent, decolourizes the medium, due to drop in pH.

L-Tryptophan is an essential amino acid and is converted to skatole and indole, which is detected by the addition of Kovac's Reagent (R008). Sodium chloride maintains the osmotic balance. The phosphates helps in the buffering of the medium. Microorganisms that possess the enzyme urease hydrolyse urea, releasing ammonia, which is detected by the pH indicator phenol red. The alkalinity so developed imparts pink colour to the medium (2).

Type of specimen

Isolated Microorganisms from clinical samples

Specimen Collection and Handling

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (3,4).

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

Warning and Precautions

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

Limitations

1. All urea test media rely on the alkalinity formation and so they are not specific for determining the absolute rate of urease activity.
2. Further biochemical and serological tests must be carried out 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 light pink homogeneous free flowing powder

Colour and Clarity of prepared medium

Yellow to light orange coloured clear solution

Reaction

Reaction of 3.00% w/v aqueous solution at 25°C. pH : 6.8±0.2

pH

6.60-7.00

Cultural Response

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

Organism	Growth	Urease
Escherichia coli ATCC 25922 (00013*)	luxuriant	Negative reaction,no change
Proteus mirabilis ATCC 12453	luxuriant	Positive reaction, Pink colour
## *Proteus hauseri* ATCC 13315	luxuriant	Positive reaction, Pink colour
Salmonella Typhimurium ATCC 14028 (00031*)	luxuriant	Negative reaction,no change

Key : (*) Corresponding WDCM numbers. ## Formerly known as *Proteus vulgaris*

Storage and Shelf Life

Store between dehydrated 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 (3,4).

Reference

1. Patrick R. Murray et al, Manual of Clinical Microbiology, Sixth Edition, 444 - 445.
2. Roland F. Bourbon D, Sztrum S. Ann. Inst. Pasteur, 73. 914-916.
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4. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.

Revision :02/2024

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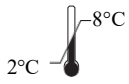

Storage temperature

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Mueller Hinton Agar 2% Glucose w/ Methylene blue

M1825

Intended Use:

Recommended for performing antifungal disc diffusion susceptibility of yeasts.

Composition**

Ingredients	g / L
HM infusion B #	2.000
Acicase™	17.500
Starch	1.500
Dextrose (Glucose)	20.000
Methylene blue	0.0005
Agar	17.000
Final pH (at 25°C)	7.3±0.1

**Formula adjusted, standardized to suit performance parameters

Equivalent to Beef Infusion from

Directions

Suspend 58.0 grams in 1000 ml purified/distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15lbs pressure (121°C) for 15 minutes. Cool to 45-50°C. Mix well and pour into sterile Petri plates.

The performance of this batch has been tested and standardized as per the current CLSI (formerly NCCLS) document M44-A2 in Method for Antifungal Disk Diffusion susceptibility testing of yeasts.

Principle And Interpretation

The Mueller Hinton formulation was originally developed as a simple, transparent agar medium for the cultivation of pathogenic species (1). Mueller Hinton Agar, Modified (as per CLSI for antifungal) is recommended for the diffusion of antifungal agents impregnated on paper disc through an agar gel as described in CLSI Approved Standard (2). When supplemented with glucose to a final concentration of 2%, it provides for suitable fungal growth. The addition of methylene blue to a final concentration of 5µg/ml enhances zone edge definition.

Kirby-Bauer et al recommended Mueller Hinton Agar for performing antibiotic susceptibility tests using a single disc of high concentration (3). WHO Committee on Standardization of Susceptibility Testing has accepted Mueller Hinton Agar for determining the susceptibility of microorganisms because of its reproducibility (4). Mueller Hinton Agar with 5% sheep blood and Mueller Hinton Agar with Haemoglobin have been recommended for antimicrobial susceptibility testing of *Streptococcus pneumoniae* and *Haemophilus influenzae.* Similarly Mueller Hinton Agar, Modified (as per CLSI for antifungal) is recommended for antifungal susceptibity testing of discs.

HM infusion B 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. Dextrose (Glucose) serves as an energy source for fungal cultures while Methylene blue enhances zone edge definition.

Technique:

Preparation of Inoculum:

1.Inoculum is prepared by picking five distinct colonies of approximately 1mm from 24 hours old culture grown on Sabouraud Dextrose Agar (M063) and incubated at 35 ± 2°C. Colonies are suspended in 5ml of sterile 0.85% Saline.

2.Vortex the resulting suspension and adjust the turbidity to yield 1 x 106 - 5 x 106 cells /ml (i.e. 0.5 McFarland standard).

Test Procedure:

1.Prepare plates with Mueller Hinton Agar, Modified (as per CLSI for antifungal) for carrying out susceptibility of antifungal discs. The medium in the plates should be sterile and have a depth of about 4 mm.

2.Dip a sterile non-toxic cotton swab on a wooden applicator into the standardized inoculum (turbidity so adjusted, as to obtain semi confluent growth on the Petri plate) 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.
3.Apply the discs using aseptic technique. Deposit the discs with centers at least 24 mm apart. (Not more than 12 discs should be placed on a 150-mm plate or not more than 5 discs on a 100-mm plate.

4.Invert the plates and place in an incubator set to 35 ± 2°C within 15 minutes after the discs are applied.

5.Examine each plate after 20 - 24 hours of incubation. If plate was satisfactorily streaked the resulting zones of inhibition will be uniformly circular and there will be a semi-confluent lawn of growth. Read at 48 hours only when insufficient growth is observed after 24 hours incubation.

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 (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. 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 bigger zones.
3. Fastidious organisms may not grow on this medium due to nutritional variations.
4. Antifungal disc are used to carry out the susceptibility, proper storage of the disc is desired of the disc.
5. Under certain circumstances, the in vitro results of antifungal 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 at recommended temperature.

Quality Control

Appearance

Light yellow to yellow may have slight blue tinge homogeneous free flowing powder

Gelling

Firm, comparable with 1.7% agar gel.

Colour and Clarity of prepared medium

amber coloured clear to slightly opalescent gel forms in Petri plates

Reaction

Reaction of 5.8% w/v aqueous solution at 25°C. pH : 7.3±0.1

pH

7.20-7.40

Cultural response

A luxuriant growth of test organisms was observed on Mueller Hinton Agar, Modified (as per CLSI for antifungal) in 24-48 hours at 33-37°C along with inhibition zones with respective antibiotic concentrations

Organism	Inoculum (CFU)	Growth	Recovery	Amphotericin-B AP(100units)	Amphotericin-B AP(20 mcg)	Amphotericin-B AP(50 mcg)
Candida albicans ATCC 90028	50-100	luxuriant	>=70%	10 -17 mm	10 -15 mm	31- 42 mm
Candida parapsilosis ATCC 22019	50-100	luxuriant	>=70%	11 -20 mm	10 -17 mm	28 -37 mm
Candida tropicalis ATCC 750	50-100	luxuriant	>=70%	8 -12 mm	8 -10 mm	13 -17 mm

Candida krusei ATCC 6258	50-100	luxuriant	>=70%	9 -14 mm	8 -12 mm	16 -25 mm
Candida albicans ATCC 10231 (00054*)	50-100	luxuriant	>=70%	10 -18 mm	10 -16 mm	30 -40 mm
Saccharomyces cerevisiae ATCC 9763 (00058*)	50-100	luxuriant	>=70%	11 -18 mm	8 -12 mm	29 -38 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 (5,6).

Reference

1. Mueller J. H. and Hinton J., 1941, Proc. Soc. Exp. Biol. Med., 48:330.
2. Method for Antifungal Disk Diffusion Susceptibilty Testing of yeasts; Approved Guideline Second Edition M44-A2 Vol.24 No.17.
3. Bauer A. W., Kirby W. M., Sherris J. L. and Turck M., 1966, Am. J. Clin. Pathol., 45:493.
4. Present Status and Future Work, WHO Sponsored collaborative study, Chicago, Oct. 1967.
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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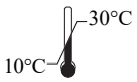
IVD ***In vitro* diagnostic medical device**



Storage temperature

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MacConkey Agar

MH081

Intended Use

Recommended for selective isolation and differentiation of *E.coli* and other enteric bacteria from pharmaceutical products in accordance with the microbial limit testing by harmonized methodology of USP/EP/BP/JP.

Composition**

Ingredients	g / L
Gelatin peptone #	17.000
HMC peptone ##	3.000
Lactose monohydrate	10.000
Sodium chloride	5.000
Bile salts	1.500
Neutral red	0.030
Crystal violet	0.001
Agar	13.500
pH after sterilization (at 25°C)	7.1±0.2

**Formula adjusted, standardized to suit performance parameters

Equivalent to Pancreatic digest of gelatin ## Equivalent to Peptones (meat and casein)

Directions

Suspend 49.53 grams (the equivalent weight of dehydrated medium per litre) in 1000 ml purified/distilled water. Boil for 1 minute with constant stirring. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes or as per validated cycle. Avoid overheating. Cool to 45-50°C. Mix well before pouring into sterile Petri plates. The surface of the medium should be dry when inoculated.

Principle And Interpretation

MacConkey Agar is the earliest selective and differential medium for cultivation of coliform organisms (1,2). Subsequently MacConkey Agar and Broth have been recommended for use in microbiological examination of foodstuffs (3) and for direct plating / inoculation of water samples for coliform counts (4). This medium is also accepted by the Standard Methods for the Examination of Milk and Dairy Products (5). It is recommended in pharmaceutical preparations and is in accordance with the harmonized method of USP/EP/BP/JP (6-9).

Gelatin peptone and HMC peptone provide the essential nutrients, vitamins and nitrogenous factors required for growth of microorganisms. Lactose monohydrate is the fermentable source of carbohydrate. The selective action of this medium is attributed to crystal violet and bile salts, which are inhibitory to most species of gram-positive bacteria. Sodium chloride maintains the osmotic balance in the medium.

After enrichment of *Escherichia coli* in MacConkey Broth (MH083), it is then subcultured on MacConkey Agar. Gram-negative bacteria usually grow well on the medium and are differentiated by their ability to ferment lactose. Lactose fermenting strains grow as red or pink and may be surrounded by a zone of acid precipitated bile. The red colour is due to production of acid from lactose, absorption of neutral red and a subsequent colour change of the dye when the pH of medium falls below 6.8. Lactose non-fermenting strains, such as *Shigella* and *Salmonella* are colourless and transparent and typically do not alter appearance of the medium. *Yersinia enterocolitica* may appear as small, non-lactose fermenting colonies after incubation at room temperature.

Type of specimen

Pharmaceutical samples, Food and dairy samples; Water samples.

Specimen Collection and Handling

For pharmaceutical samples, follow appropriate techniques for sample collection, processing as per guidelines (6-9).

For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (3,5). For water samples, follow appropriate techniques for sample collection and processing as per guidelines (4).

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 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.
3. Though the medium is recommended for selective isolation, further biochemical and serological testing must be carried out for further confirmation.
4. The surface of the medium should be dry when inoculated.

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 1.35% Agar gel.

Colour and Clarity of prepared medium

Red with purplish tinge coloured clear to slightly opalescent gel forms in Petri plates.

pH

6.90-7.30

Cultural Response

Growth Promotion is carried out in accordance with the harmonized method of ICH (USP/EP/BP/JP). Cultural response was observed after an incubation at 30-35°C for 18-72 hours. Recovery rate is considered as 100% for bacteria growth on Soybean Casein Digest Agar.

Growth promoting properties

Growth of microorganism comparable to that previously obtained with previously tested and approved lot of medium occurs at the specified temperature for not more than the shortest period of time specified inoculating 100 cfu (at 30-35°C for <=18 hours).

Indicative properties

Colonies are comparable in appearance and indication reaction to those previously obtained with previously tested and approved lot of medium occurs for the specified temperature for a period of time within the range specified inoculating <=100 cfu (at 30-35°C for 18-72 hours).

Organism	Inoculum (CFU)	Growth	Observed Lot value (CFU)	Recovery	Colour of colony	Incubation period
Growth Promoting + Indicative						
Escherichia coli ATCC 8739 (00012*)	50 -100	luxuriant	25 -100	>=50 %	pink-red with bile precipitate	18 -72 hrs
Additional Microbiological testing						
Escherichia coli ATCC 25922 (00013*)	50 -100	luxuriant	25 -100	>=50 %	pink to red with bile precipitate	18 -24 hrs
# *Klebsiella aerogenes* ATCC 13048 (00175*)	50 -100	luxuriant	25 -100	>=50 %	pink to red	18 -24 hrs
Enterococcus faecalis ATCC 29212 (00087*)	50 -100	fair-good	0 - 10	<=10 %	colourless to pale pink	18 -24 hrs
Salmonella Typhimurium ATCC 14028 (00031*)	50 -100	luxuriant	25 -100	>=50 %	colourless	18 -24 hrs

Staphylococcus aureus subsp. *aureus* ATCC 6538 (00032*)	>=10³	inhibited	0	0 %		>=24 hrs
Staphylococcus aureus subsp. *aureus* ATCC 25923 (00034*)	>=10³	inhibited	0	0 %		>=24 hrs
Salmonella Enteritidis ATCC 13076 (00030*)	50 -100	luxuriant	25 -100	>=50 %	colourless	18 -24 hrs
Salmonella Paratyphi A ATCC 9150	50 -100	luxuriant	25 -100	>=50 %	colourless	18 -24 hrs
Salmonella Paratyphi B ATCC 8759	50 -100	luxuriant	25 -100	>=50 %	colourless	18 -24 hrs
Salmonella Typhi ATCC 6539	50 -100	luxuriant	25 -100	>=50 %	colourless	18 -24 hrs
Salmonella Abony NCTC 6017 (00029*)	50 -100	luxuriant	25 -100	>=50 %	colourless	18 -24 hrs
## *Proteus hauseri* ATCC 13315	50 -100	luxuriant	25 -100	>=50 %	colourless	18 -24 hrs
Shigella flexneri ATCC 12022 (00126*)	50 -100	fair to good	15 -40	30 -40 %	colourless	18 -24 hrs
Staphylococcus epidermidis ATCC 12228 (00036*)	>=10³	inhibited	0	0 %		>=24 hrs
Corynebacterium diphtheriae type *gravis*	>=10³	inhibited	0	0 %		>=24 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. For better performance ot is advised to store the plates 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

1. MacConkey, 1900, The Lancet, ii:20.
2. MacConkey, 1905, J. Hyg., 5:333.
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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5. Wehr H. M. and Frank J. H., 2004, Standard Methods for the Microbiological Examination of Dairy Products, 17th Ed., APHA Inc., Washington, D.C.
6. British Pharmacopoeia, 2022, The Stationery office British Pharmacopoeia.
7. European Pharmacopoeia, 2022 European Dept. for the quality of Medicines.
8. Japanese Pharmacopoeia, 2016.
9. The United States Pharmacopoeia, 2022, The United States Pharmacopeial Convention. Rockville, MD.
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11. 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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McFarland Standard set **R092**

McFarland standards are used to perform spectrophotometric comparisions of bacterial densities in water, saline or liquid growth medium. It provides laboratory guidance for the standardization of numbers of bacteria for susceptibility testing or other procedure requiring a standardization of the inoculum like growth promotion test (GPT).

Set Contains:

R092A (Standard 0.5)- 1 tube

R092B (Standard 1)-1 tube

R092C (Standard 2)- 1 tube

R092D (Standard 3)- 1 tube

R092E (Standard 4)- 1 tube

Directions

Prepare the inoculum of culture required for testing by using sterile saline. Match the density of the resultant suspension with the density of the desired standard. The standards must be thoroughly mixed on a vortex mixture at the time of use to obtain a uniform suspension. Adjust the density of cell suspension by adding saline if it is more turbid as compared to the desired standard or by adding culture if it is dilute. Check the density of the turbidity by determining the absorbance of 0.5 McFarland standard using a spectrophotometer with a 1 cm light path. The absorbance at 625 nm should be 0.08 to 0.10. The standards should be checked regularly to ensure the density accuracy.

Interpretation

McFarland standards are a set of tubes with increasing concentration of Barium Sulphate suspension. The turbidity of Barium Sulphate's white precipitation is used as a point of comparision of bacterial suspensions to known bacterial turbidity.

McFarland Standard	0.5	1	2	3	4
Approximate Corresponding suspension x 10^8 CFU/ml	1.5	3	6	9	12

Limitation of procedure

1. Coloured media may interfere with result interpretation and give incorrect results.

2. Bacterial suspensions of older cultures may not be comparable with expected bacterial counts.

Storage

Store the standards at 2-8°C, away from light after each use.

Reference

1. McFarland, J.1907. Nephelometer: JAMA 14:1176-1178

2. Murry,PR; Baron,EJ; Jorgensen,JH;Landry,ML;Pfaller,MA; Manual of Clinical Microbiology 9th edition ASM press, Washington DC.

Gram's Iodine

S013

Intended use

Grams's Iodine is used as mordant in Gram's staining method.

Composition**

Ingredients

Iodine	1.0 g
Potassium iodide	2.0 g
Distilled water	300.0 ml

**Formula adjusted, standardized to suit performance parameters

Directions

1. Prepare a thin smear on clear, dry glass slide.
2. Allow it to air dry and fix by gentle heat.
3. Flood with Gram's Crystal Violet (S012) for 1 minute. (If over staining results in improper decolourization of known gram-negative organisms, use less crystal violet).
4. Wash with tap water.
5. Flood the smear with Gram’s Iodine (S013). Allow it to remain for 1 minute.
6. Decolourize with Gram's Decolourizer (S032) until the blue dye no longer flows from the smear. (Acetone may be used as a decolourizing agent with caution, since this solvent very rapidly decolourized the smear).
7. Wash with tap water.
8. Counter stain with 0.5% w/v Safranin (S027) for 20 seconds and rinses off with water.
9. Wash with tap water.
10. Allow the slide to air dry or blot dry between sheets of clean bibulous paper and examine under oil immersion objective.

Principle And Interpretation

The Gram stain is a differential staining technique most widely applied in all microbiology disciplines laboratories. It is one of the most important criteria in any identification scheme for all types of bacterial isolates. Different mechanisms have been proposed to explain the gram reaction. There are many physiological differences between gram-positive and gram negative cell walls Ever since Christian Gram has discovered Gram staining, this process has been extensively investigated and redefined In practice, a thin smear of bacterial cells is stained with crystal violet, then treated with an iodine containing mordant to increase the binding of primary stain A decolourizing solution of alcohol or acetone is used to remove the crystal violet from cells which bind it weakly and then the counterstain (like safranin) is used to provide a colour contrast in those cells that are decolourized. Gram-positive bacteria have a thick mesh-like cell wall made of peptidoglycan (50–90% of cell envelope), and as a result are stained purple by crystal violet, whereas gram-negative bacteria have a thinner layer (10% of cell envelope), so do not retain the purple stain and are counter-stained pink by safranin. In a properly stained smear by gram staining procedure, the gram-positive bacteria appear blue to purple and gram negative cells appear pink to red.

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Type of specimen

Any isolated colony on primary or subculture plates can be isolated from following specimens. Clinical specimen: Blood, urine, CSF, pus, wounds, lesions, body tissues, sputum etc. From environment: Air, water, soil, sludge, waste water, food, dairy samples etc.

Specimen Collection and Handling

For clinical samples follow appropriate techniques for handling specimens as per established guidelines
For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines
For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards.
Generally, the smear is made in laboratory; however, when there is a concern that transport will be delayed or that the preservation for culture will alter the specimen, prepare smear and submit slides to the laboratory.

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 results of Gram stains in conjunction with other clinical and laboratory findings. Use additional procedures (e.g., special stains, inclusion of selective media, etc.) to confirm findings suggested by gram-stained smears.
2. Proper smear preparation is key to obtaining good gram staining results. Avoid excessive material or thick smears which may interfere with the passage of light and lead to distortion of images.
3. Overheating slides during heat fixation can distort the appearance of the organisms.
4. Only fresh cultures and specimens should be gram stained since cell wall integrity of older cells may give improper gram staining characteristics. Gram positive organisms that have lost cell wall integrity because of old age or antibiotic treatment may appear pink.
5. The decolorization step is the most important step in the gram-staining process. Over decolorization results in an abundance of bacteria that appear gram negative, while under decolorization results in too many bacteria that appear to be gram-positive.
6. The procedure given is based on an ideal thin smear of cells. Staining and decolorization times may vary depending on the sample and its thickness.
7. False Gram stain results may be related to inadequately collected specimens or delay in transit.

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 :** Yellow to dark brown coloured solution.
- **Clarity :** Clear without any particles.

- **Microscopic Examination :** Gram staining is carried out where Gram's Iodine is used as one of the stains and staining characteristics of organisms are observed under microscope by using oil immersion lens.
- **Results :** Gram-positive microorganisms : violet
  Gram-negative microorganims : pinkish red

Storage and Shelf Life

Store between 10 - 30 °C in tightly closed container and away from bright light. Use before expiry date on label. On opening, product should be properly stored in dry ventilated area protected from extremes of temperature and sources of ignition. Seal the container tightly after use.

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 .

Reference

1. Downes F. P. and Ito K. (Ed.), 2001, Compendium of Methods for the Microbiological Examination of Foods, 4th ed., APHA, Washington, D.C.
2. Rice E.W., Baird, R.B., Eaton A. D., Clesceri L. S. (Eds.), 2012, Standard Methods for the Examination of Water and Wastewater, 22nd ed., APHA, Washington, D.C.
3. Wehr H. M. and Frank J. H., 2004, Standard Methods for the Microbiological Examination of Dairy Products, 17th Ed., APHA Inc., 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.
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9. Washington, J.A.1986.Rapid diagnosis by microscopy. Clin.Microbiol. Newsl.8:135-137
10. Lamanna and Mallette, 1965, Basic BActeriology,3rd ed.,Williams and Wilkins Co.,Baltimore.
11. Salton,1964,The Bacterial cell Wall, Elsevier, Amsterdam.

Storage temperature

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Ampicillin AMP 10mcg SD002

Ampicillin AMP 10mcg discs are used for antimicrobial susceptibility testing of of bacterial cultures as per Kirby-Bauer Method

Composition

*Ingredients	Concentration
Ampicillin	10mcg/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 ± 2°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*

Antimicrobial agent	Interpretative criteria for	Sensitive mm or more	Intermediate mm	Resistant mm or less
Ampicillin AMP 10mcg	*Enterobacteriaceae*	17	14-16	13
	Staphylococcus	29	-	28
	Enterococcus spp.	17	-	16
	Haemophilus influenzae & Haemophilus parainfluenzae	22	19-21	18
	Streptococcus spp. beta haemolytic gruop	24	-	-

Quality Control:

Appearance: Filter paper discs of 6mm diameter with printed "AMP 10" 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)	15-22
S.aureus (25923)	27-35
E.coli (35218)	6
S.pneumoniae (49619)	30-36

* = 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)

*** Not for Medicinal Use**

IVD — In vitro diagnostic medical device

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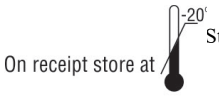

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Ampicillin AMP 2 mcg SD002A

Ampicillin AMP 2mcg discs are used for antimicrobial susceptibility testing of bacterial cultures as per Kirby-Bauer Method.

Composition

*Ingredients	Concentration
Ampicillin	2 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 ± 2°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.

Quality Control:

Appearance: Filter paper discs of 6mm diameter with printed "AMP 2" 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)	15-22
S.aureus (*29213*)	15-21
E.faecalis (*29212*)	15-21

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)

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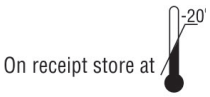

Storage temperature

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Chloramphenicol C 30 mcg SD006

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

Composition

*Ingredients	Concentration
Chloramphenicol	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 ± 2°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*

Antimicrobial agent	Interpretative criteria for	Sensitive mm or more	Intermediate mm	Resistant mm or less
Chloramphenicol 30 mcg	*Enterobacteriaceae, Staphylococcus & Enterococcus spp.*	18	13-17	12
	Haemophilus influenzae & Haemophilus parainfluenzae	29	26-28	25
	Neisseria meningitidis	26	20-25	19
	S.pneumoniae	21	-	20
	Streptococcus spp. Viridians group, Streptococcus spp. beta haemolytic gruop	21	18-20	17

Quality Control:

Appearance: Filter paper discs of 6mm diameter with printed "C 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)	21-27
S.aureus (25923)	19-26
S.aureus (*29213*)	20-28

* = Interpretive criteria & QC ranges as per CLSI & EUCAST standards.

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)

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Storage temperature

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Co-Trimoxazole COT 25 mcg (Trimethoprim/Sulphamethoxazole) (1.25/23.75 mcg) SD010

Co-Trimoxazole (Trimethoprim/Sulphamethoxazole) COT 25 mcg (1.25/23.75mcg) discs are used for antimicrobial susceptibility testing of bacterial cultures as per Bauer-Kirby Method

Composition

*Ingredients	Concentration
Co-Trimoxazole (Trimethoprim/Sulphamethoxazole)	25 mcg/disc (1.25/23.75 mcg)

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 ± 2°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
Co-Trimoxazole (Trimethoprim/ Sulphamethoxazole) 25 mcg (1.25/23.75 mcg)	*Enterobacteriaceae ,Acientobacter, B.cepacia, S.maltophila, Staphylococcus, Haemophilus influenzae & Haemophilus parainfluenzae*	16	11-15	10
	Neisseria meningitidis	30	26-29	25
	S.pneumoniae	19	16-18	15

Quality Control:

Appearance: Filter paper discs of 6mm diameter with printed "COT 25 (1.25/23.75mcg)" 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*)*	24-32
E.faecalis (29212)	>=20
S.pneumoniae (49619)	20-28

* = Interpretive criteria & QC ranges as per CLSI standards.

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)

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CE Marking

Storage temperature

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Doxycycline Hydrochloride DO 30 mcg

SD012

Doxycycline Hydrochloride DO 30 mcg discs are used for antimicrobial susceptibility testing of bacterial cultures as per Kirby-Bauer Method

Composition

*Ingredients	Concentration
Doxycycline Hydrochloride	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 ± 2°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*

Antimicrobial agent	Interpretative criteria for	Sensitive mm or more	Intermediate mm	Resistant mm or less
Doxycycline Hydrochloride 30 mcg	*Enterobacteriaceae*	14	11-13	10
	Acientobacter	13	10-12	9
	Staphylococcus, Enterococcus spp.	16	13-15	12
	S. pneumoniae	28	25-27	24

Quality Control:

Appearance: Filter paper discs of 6mm diameter with printed "DO 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)	23-29

* = Interpretive criteria & QC ranges as per CLSI standards.

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)

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Erythromycin E 15 mcg SD013

Erythromycin E 15 mcg discs are used for antimicrobial susceptibility testing of bacterial cultures as per Kirby-Bauer Method

Composition

*Ingredients	Concentration
Erythromycin	15 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 ± 2°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*

Antimicrobial agent	Interpretative criteria for	Sensitive	Intermediate	Resistant
		mm or more	mm	mm or less
Erythromycin 15 mcg	*Staphylococcus & Enterococcus spp.*	23	14-22	13
	S.pneumoniae, Streptococcus spp. Viridians group, Streptococcus spp. beta haemolytic gruop	21	16-20	15

Quality Control:

Appearance: Filter paper discs of 6mm diameter with printed "E 15" 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)*
S.aureus (25923)	22-30
S.aureus (*29213*)	23-29

* = Interpretive criteria & QC ranges as per CLSI & EUCAST standards

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**

IVD — In vitro diagnostic medical device

CE Marking

Storage temperature

Do not use if package is damaged

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Furazolidone FR 50 mcg SD015

Furazolidone FR 50 mcg discs are used for antimicrobial susceptibility testing of bacterial cultures as per Bauer-Kirby Method

Composition

*Ingredients	Concentration
Furazolidone	50 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 ± 2°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 "FR 50" 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)	20-25
S.aureus (25923)	18-22

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)

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CE — CE Marking

Storage temperature

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Gentamicin GEN 10 mcg SD016

Gentamicin GEN 10 mcg discs are used for antimicrobial susceptibility testing of bacterial cultures as per Bauer-Kirby Method

Composition

*Ingredients	Concentration
Gentamicin	10 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 ± 2°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
Gentamicin 10 mcg	*Enterobacteriaceae, P.aeruginosa, Acientobacter & Staphylococcus*	15	13-14	12

Quality Control:

Appearance: Filter paper discs of 6mm diameter with printed "GEN 10" 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)	19-26
S.aureus (25923)	19-27
P.aeruginosa (27853)	17-23

* = Interpretive criteria & QC ranges as per CLSI standards.

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)

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Metronidazole MT 5 mcg SD020

Metronidazole MT 5 mcg discs are used for antimicrobial susceptibility testing of anaerobic organisms.

Composition

*Ingredients	Concentration
Metronidazole	5 mcg/disc

Susceptibility Test Procedure:

1. Prepare Brucella agar with Hemin and Vitamin K1 supplement with 5 % v/v sterile defibrinated sheep blood (M1039).
2. Inoculum is prepared by picking five or more well isolated colonies of similar morphology from 24 to 48 hours old culture grown on Brucella Blood agar.
3. Colonies are suspended in 5 ml of sterile Brucella Broth or other clear broth 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)
4. Dip a sterile non-toxic cotton swab in the inoculum and swab the prepared culture.
5. Aseptically incorporate the discs in the medium.
6. Incubate immediately at 35 ± 2°C under anaerobic conditions and examine after 24 hours or longer, if necessary.
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:

Brucella Agar Base w/ Hemin and Vitamin K1 is a modified and highly enriched medium, which can be used for the isolation of Brucella and other anaerobic bacteria. The medium contain tryptone, peptone and yeast extract serves as sources of carbon, nitrogen, long chain amino acids and essential growth nutrients including B-complex vitamins. Dextrose serves as a source of energy. Addition of blood provides nutrients and helps to differentiate hemolytic organisms. Presence of hemin and Vitamin K1 supports growth of other fastidious bacteria like Bacteroides species and gram-positive spore bearers like Clostridium species.

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:

Interpret the results on the basis of growth obtained when compared to positive control tubes. Lesser the growth more effective is the antibiotic concentration.

Quality Control:

Appearance: Filter paper discs of 6mm diameter with printed "MT 5" on centre of each side of the disc. **Cultural response**: Avaerage diameter of zone of inhibition observed on Brucella agar with Hemin and Vitamin K1 supplement with 5 % v/v sterile defibrinated sheep blood (M1039). Incubated anaerobically at 35°C for 24 - 48 hours.

Organisms (ATCC)	Std. zone of diameter (mm)
Cl. perfringens (ATCC 12924)	26-34
Cl. perfringens (ATCC 13124)	26-34
B fragilis (ATCC 25285)	38-44

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)

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Storage temperature

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Nalidixic Acid NA 30 mcg SD021

Nalidixic Acid NA 30 mcg discs are used for antimicrobial susceptibility testing of bacterial cultures as per Bauer-Kirby Method

Composition

*Ingredients	Concentration
Nalidixic Acid	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 ± 2°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
Nalidixic Acid 30 mcg	*Enterobacteriaceae*	19	14-18	13

Quality Control:

Appearance: Filter paper discs of 6mm diameter with printed "NA 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)	22-28

* = Interpretive criteria & QC ranges as per CLSI standards.

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)

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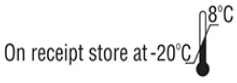

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