

Technical Data

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
<i>Bifidobacterium infantis</i> ATCC 25962	50-100	good-luxuriant
<i>Bifidobacterium bifidum</i> ATCC 15696	50-100	good-luxuriant
<i>Bifidobacterium breve</i> 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

3. Atlas R. M. 2004, 3rd Edi. Handbook of Microbiological Media, Parks, L. C. (Ed.), CRC Press, Boca Raton.4.

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

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

6.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.

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HiCromeTM Enterococcus faecium Agar Base

M1580

Intended use

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 p	arameters

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

HiCromeTM 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 β -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 ; 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

pН

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
<i>Escherichia coli</i> ATCC 25922 (00013*)	>=10 ⁴	inhibited	0%	
Enterococcus faecalis ATCC 29212 (00087*)	50-100	luxuriant	>=50%	blue
<i>Enterococcus faecium</i> 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 15-30°C in a tightly closed container and the prepared medium at 2-8°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition Seal the container tightly after use. Product performance is best if used within stated expiry period.

Disposal

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

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.
- 4. Willinger B. and Manafi M., 1995, Lett. Appl. Microbiol., 20:300-302.
- 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.
- 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.

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Technical Data

Urea Indole Medium

M1784

To differentiate micro-organisms especially *Enterobacteriaceae* on the basis of their ability to hydrolyze urea and indole production

Composition**

Ingredients	Gms / Litre
L- Tryptophan	3.000
Sodium chloride	5.000
Potassium phosphate, monobasic	1.000
Potassium phosphate, dibasic	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 distilled water. Dissolve the medium completely and sterilize by filtration . DO NOT AUTOCLAVE. Aseptically, dispense into sterile tubes.

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 Kovacs reagent, decolourizes the medium, due to drop in pH.

L- Trypytophan is an essential amino acid and is converted to skatole and indole, which is detected by the addition of Kovacs 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 alkalinility so developed imparts pink colour to the medium (2).

Quality Control

AppearanceLight yellow to light pink homogeneous free flowing powderColour and Clarity of prepared mediumYellow to light orange coloured clear solutionReactionReactionReaction of 3.00% w/v aqueous solution at 25°C. pH : 6.8 ± 0.2 pH6.60-7.00Cultural ResponseCultural characteristics observed after an incubation at 35-37°C for 18-24 hours.Cultural ResponseOrganismInoculumGrowthUrease

(CFU)

Escherichia coli ATCC 25922	50-100	luxuriant	Negative reaction,no
Proteus mirabilis ATCC 12453	50-100	luxuriant	change Positive reaction, Pink
Proteus vulgaris ATCC 13315	50-100	luxuriant	Positive reaction, Pink
Salmonella Typhimurium ATCC 14028	50-100	luxuriant	Negative reaction,no change

Storage and Shelf Life

Store below 8°C in tightly closed container and prepared medium at 2-8°C. Use before expiry period on the label

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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Technical Data

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 TM	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 performanc	e 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 5μ g/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 susceptibility testing of discs.

HM infusion B and AcicaseTM 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 $35 \pm 2^{\circ}$ 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 $35 \pm 2^{\circ}$ 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

pН

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)
<i>Candida albicans</i> 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
<i>Candida tropicalis</i> 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.
- Method for Antifungal Disk Diffusion Susceptibility 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.
- 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	0.5	1	2	3	4
Standard					
Approximate	1.5	3	6	9	12
Corresponding					
suspension x					
10 ⁸ CFU/ml					

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.

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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 $35 \pm 2^{\circ}$ C and examine after 16-18 hours or longer, if necessary. For fastidious organisms incubate at appropriate temperature and time.
- 7. Measure the zones showing complete inhibition and record the diameters of the zones to the nearest millimeter using a calibrated instrument like zone scales (PW096/PW297)

Principle:

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

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

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

Interpretation:

Use following interpretive criteria for susceptibility categorization*

Antimicrobial agent	Interpretative criteria for	Sensitive mm or more	Intermediate mm	Resistant mm or less
	Enterobacteriaceae	17	14-16	13
	Staphylococcus	29	-	28
Ampicillin AMP	Enterococcus spp.	17	-	16
10mcg	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
<i>S.aureus</i> (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
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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 $35 \pm 2^{\circ}$ C and examine after 16-18 hours or longer, if necessary. For fastidious organisms incubate at appropriate temperature and time.
- 7. Measure the zones showing complete inhibition and record the diameters of the zones to the nearest millimeter using a calibrated instrument like zone scales (PW096/PW297)

Principle:

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

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

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

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
<i>E.faecalis</i> (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)

* Not for Medicinal Use



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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 $35 \pm 2^{\circ}$ C and examine after 16-18 hours or longer, if necessary. For fastidious organisms incubate at appropriate temperature and time.
- 7. Measure the zones showing complete inhibition and record the diameters of the zones to the nearest millimeter using a calibrated instrument like zone scales (PW096/PW297)

Principle:

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

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

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

Interpretation:

Use following interpretive criteria for susceptibility categorization*

		Sensitive	Intermediate	Resistant
Antimicrobial agent	Interpretative criteria for	mm or more	mm	mm or less
	Enterobacteriaceae, Staphylococcus & Enterococcus spp.	18	13-17	12
	Haemophilus influenzae & Haemophilus parainfluenzae	29	26-28	25
Chloramphenicol 30 mcg	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
- Performance standards of Antimicrobial Disc Susceptibility Tests, M100S, 32nd Ed., CLSI Vol. 42 No.2, Feb-2022.
- 3. EUCAST, Breakpoint tables for interpretation of MIC's & zone diameters, version 12.0, valid from 01.01.2022.

Note :

Use following media to carry out susceptibility test For rapidly growing aerobic organisms : Mueller Hinton Agar (M173/M1084) For *Haemophilus* spps : Haemophilus Test Agar (M1259 + FD117) For *S.pneumoniae* : Muller Hinton Agar supplemented with 5% Sheep Blood For Neisseria spps : G.C.Agar +1% defined growth supplement (M434 + FD025)

* Not for Medicinal Use





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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 Co-Trimoxazole (Trimethoprim/Sulphamethoxazole) Concentration 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 $35 \pm 2^{\circ}$ C and examine after 16-18 hours or longer, if necessary. For fastidious organisms incubate at appropriate temperature and time.
- 7. Measure the zones showing complete inhibition and record the diameters of the zones to the nearest millimeter using a calibrated instrument like zone scales (PW096/PW297)

Principle:

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

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

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

Interpretation:

Use following interpretive criteria for susceptibility categorization*

		Sensitive	Intermediate	Resistant
Antimicrobial agent	Interpretative criteria for	mm or more	mm	mm or less
Enterobacteriaceae ,Acientobacter, B.cepacia, S.maltophila, Staphylococcus, Haemophilus influenzae & Haemophilus parainfluenzae	16	11-15	10	
Sulphamethoxazole) 25 mcg	Neisseria meningitidis	30	26-29	25
(1.23/23.73 mcg)	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
<i>S.aureus (</i> 25923 <i>)</i>	24-32
<i>E.faecalis</i> (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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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 $35 \pm 2^{\circ}$ C and examine after 16-18 hours or longer, if necessary. For fastidious organisms incubate at appropriate temperature and time.
- 7. Measure the zones showing complete inhibition and record the diameters of the zones to the nearest millimeter using a calibrated instrument like zone scales (PW096/PW297)

Principle:

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

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

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

Interpretation:

Use following interpretive criteria for susceptibility categorization*

		Sensitive	Intermediate	Resistant
Antimicrobial agent	Interpretative criteria for	mm or more	mm	mm or less
Doxycycline	Enterobacteriaceae	14	11-13	10
Hydrochloride	Acientobacter	13	10-12	9
30 mcg	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)

* Not for Medicinal Use



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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 $35 \pm 2^{\circ}$ C and examine after 16-18 hours or longer, if necessary. For fastidious organisms incubate at appropriate temperature and time.
- 7. Measure the zones showing complete inhibition and record the diameters of the zones to the nearest millimeter using a calibrated instrument like zone scales (PW096/PW297)

Principle:

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

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

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

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

Interpretation:

Use following interpretive criteria for susceptibility categorization*

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



DB Maarn The Netherlands, www.cepartner 4u.eu

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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

*IngredientsConcentrationFurazolidone50 mcg/disc

Susceptibility Test Procedure:

- 1. Prepare plates with Mueller Hinton Agar (M173/M1084) for rapidly growing aerobic organisms as per Bauer-Kirby Method. The medium in the plates should be sterile and should have a depth of about 4 mm.
- 2. Inoculate 4-5 similar colonies with a wire, needle or loop to 5 ml Tryptone Soya Broth (M011) and incubate at 35-37°C for2-8 hours until light to moderate turbidity develops. Compare the inoculum turbidity with that of standard 0.5 McFarland (prepared by mixing 0.5 ml of 1.175% barium chloride and 99.5 ml of 0.36N sulfuric acid). Dilute the inoculum or incubate further as necessary to attain comparative turbidity. Alternatively, the inoculum can be standardized by other appropriate optical method (0.08 0.13 OD turbid suspension at 625 nm)
- 3. Dip a sterile non-toxic cotton swab on a wooden applicator into the standardized inoculum and rotate the soaked swab firmly against the upper inside wall of the tube to express excess fluid. Streak the entire agar surface of the plate with the swab three times, turning the plate at 60° angle between each streaking. Allow the inoculum to dry for 5 15 minutes with lid in place.
- 4. Apply the discs using aseptic technique. When using cartridges, the discs can be applied using the specially designed applicator. When the vials are used, apply the discs using sterile forceps.
- 5. Deposit the discs with centers at least 24 mm apart. For fastidious organisms and for Penicillins and Cephalosporins, the discs should preferably be deposited with centers 30 mm apart.
- 6. Incubate immediately at $35 \pm 2^{\circ}$ C and examine after 16-18 hours or longer, if necessary. For fastidious organisms incubate at appropriate temperature and time.
- 7. Measure the zones showing complete inhibition and record the diameters of the zones to the nearest millimeter using a calibrated instrument like zone scales (PW096/PW297)

Principle:

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

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

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

Quality Control:

Appearance: Filter paper discs of 6mm diameter with printed "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
<i>S.aureus</i> (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
- Performance standards of Antimicrobial Disc Susceptibility Tests, M100S, 32nd Ed., CLSI Vol. 42 No.2, Feb-2022.
- 3. EUCAST, Breakpoint tables for interpretation of MIC's & zone diameters, version 12.0, valid from 01.01.2022.

Note :

Use following media to carry out susceptibility test

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

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

For S.pneumoniae : Muller Hinton Agar supplemented with 5% Sheep Blood

For Neisseria spps : G.C.Agar +1% defined growth supplement (M434 + FD025)

* Not for Medicinal Use



In vitro diagnostic medical device CE Marking

Storage temperature



Do not use if package is damaged

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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 $35 \pm 2^{\circ}$ C and examine after 16-18 hours or longer, if necessary. For fastidious organisms incubate at appropriate temperature and time.
- 7. Measure the zones showing complete inhibition and record the diameters of the zones to the nearest millimeter using a calibrated instrument like zone scales (PW096/PW297)

Principle:

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

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

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

Interpretation:

Use following interpretive criteria for susceptibility categorization*

		Sensitive	Intermediate	Resistant
Antimicrobial agent	Interpretative criteria for	mm or more	mm	mm or less
Gentamicin	Enterobacteriaceae, P.aeruginosa,	15	12 14	12
10 mcg	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
<i>S.aureus</i> (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
- Performance standards of Antimicrobial Disc Susceptibility Tests, M100S, 32nd Ed., CLSI Vol. 42 No.2, Feb-2022.
- 3. EUCAST, Breakpoint tables for interpretation of MIC's & zone diameters, version 12.0, valid from 01.01.2022.

Note :

Use following media to carry out susceptibility test For rapidly growing aerobic organisms : Mueller Hinton Agar (M173/M1084) For *Haemophilus* spps : Haemophilus Test Agar (M1259 + FD117) For *S.pneumoniae* : Muller Hinton Agar supplemented with 5% Sheep Blood For Neisseria spps : G.C.Agar +1% defined growth supplement (M434 + FD025)

* Not for Medicinal Use

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IVD

Storage temperature



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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 $35 \pm 2^{\circ}$ 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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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 $35 \pm 2^{\circ}$ C and examine after 16-18 hours or longer, if necessary. For fastidious organisms incubate at appropriate temperature and time.
- 7. Measure the zones showing complete inhibition and record the diameters of the zones to the nearest millimeter using a calibrated instrument like zone scales (PW096/PW297)

Principle:

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

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

Use following interpretive criteria for susceptibility categorization*

Antimicrobial agent Interpretative criteria for mm or more	mm	mm or less
Nalidixic Acid 30 mcgEnterobacteriaceae19	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
- 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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Nystatin NS 100 Units

SD025

Nystatin NS 100 Units discs are used for antimicrobial susceptibility testing of fungal cultures

Composition

*Ingredients	Concentration		
Nystatin	100 Units/disc		

Susceptibility Test Procedure:

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 $35 \pm 2^{\circ}$ C. Colonies are suspended in 5ml of sterile 0.85% Saline.
- 2. Vortex the resulting suspension and adjust the turbidity to yield 1×10^6 5 x 10^6 cells /ml (i.e. 0.5 McFarland standard).

Test Procedure:

- 1. Prepare plates with Muller Hinton Agar + 2% Glucose + 0.5 mcg/ml Methylene Blue Dye (M1825) 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.
- 4. Invert the plates and place in an incubator set to $35 \pm 2^{\circ}$ 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.

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.

- 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 "NS 100" on centre of each side of the disc.

Cultural response: Average diameter of zone of inhibition observed on Muller Hinton Agar + 2% Glucose + 0.5 mcg/ml Methylene Blue Dye after 24-48 hours incubation at 35-37°C for standard cultures.

Organisms (ATCC)	Std. zone of diameter (mm)		
<i>C.albicans</i> (90028)*	19-27		
C.parapsilosis (22019)*	16-25		
C.tropicalis (750)*	16-21		
C.krusei(6528)*	15-20		
C.albicans(10231)	15-23		
S.cerevisiae(9763)	17-25		

* = Q.C. Strains recommended by CLSI

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. Method for Antifungal Disk Diffusion Susceptibility Testing of Yeasts; Approved Guidelines-Second edition Vol.29 No.17, August- 2009 CLSI document M44-A2. For more details refer to this volume

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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Polymyxin-B PB 300 Units

SD029

Polymyxin-B PB 300 Units discs are used for antimicrobial susceptibility testing of of bacterial cultures as per Bauer-Kirby Method

Composition

*Ingredients	Concentration		
Polymyxin-B	300 Units/disc		

Susceptibility Test Procedure:

- 1. Prepare plates with Mueller Hinton Agar (M173/M1084) for rapidly growing aerobic organisms as per Bauer-Kirby Method. The medium in the plates should be sterile and should have a depth of about 4 mm.
- 2. Inoculate 4-5 similar colonies with a wire, needle or loop to 5 ml Tryptone Soya Broth (M011) and incubate at 35-37°C for2-8 hours until light to moderate turbidity develops. Compare the inoculum turbidity with that of standard 0.5 McFarland (prepared by mixing 0.5 ml of 1.175% barium chloride and 99.5 ml of 0.36N sulfuric acid). Dilute the inoculum or incubate further as necessary to attain comparative turbidity. Alternatively, the inoculum can be standardized by other appropriate optical method (0.08 0.13 OD turbid suspension at 625 nm)
- 3. Dip a sterile non-toxic cotton swab on a wooden applicator into the standardized inoculum and rotate the soaked swab firmly against the upper inside wall of the tube to express excess fluid. Streak the entire agar surface of the plate with the swab three times, turning the plate at 60° angle between each streaking. Allow the inoculum to dry for 5 15 minutes with lid in place.
- 4. Apply the discs using aseptic technique. When using cartridges, the discs can be applied using the specially designed applicator. When the vials are used, apply the discs using sterile forceps.
- 5. Deposit the discs with centers at least 24 mm apart. For fastidious organisms and for Penicillins and Cephalosporins, the discs should preferably be deposited with centers 30 mm apart.
- 6. Incubate immediately at $35 \pm 2^{\circ}$ C and examine after 16-18 hours or longer, if necessary. For fastidious organisms incubate at appropriate temperature and time.
- 7. Measure the zones showing complete inhibition and record the diameters of the zones to the nearest millimeter using a calibrated instrument like zone scales (PW096/PW297)

Principle:

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

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

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Interpretation:

Use following interpretive criteria for susceptibility categorization*

		Sensitive	Intermediate	Resistant
Antimicrobial agent	Interpretative criteria for	mm or more	mm	mm or less
Polymyxin-B 300 Units	P.aeruginosa	12	-	11

Quality Control:

Appearance: Filter paper discs of 6mm diameter with printed "PB 300" 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)	13-19
P.aeruginosa (27853)	14-18

* = 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)

* Not for Medicinal Use In vitro diagnostic medical



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Rifampicin RIF 5mcg

SD030

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

Composition

*Ingredients	Concentration		
Rifampicin	5mcg /disc		

Susceptibility Test Procedure:

- 1. Prepare plates with Mueller Hinton Agar (M173/M1084) for rapidly growing aerobic organisms as per Bauer-Kirby Method. The medium in the plates should be sterile and should have a depth of about 4 mm.
- 2. Inoculate 4-5 similar colonies with a wire, needle or loop to 5 ml Tryptone Soya Broth (M011) and incubate at 35-37°C for2-8 hours until light to moderate turbidity develops. Compare the inoculum turbidity with that of standard 0.5 McFarland (prepared by mixing 0.5 ml of 1.175% barium chloride and 99.5 ml of 0.36N sulfuric acid). Dilute the inoculum or incubate further as necessary to attain comparative turbidity. Alternatively, the inoculum can be standardized by other appropriate optical method (0.08 0.13 OD turbid suspension at 625 nm)
- 3. Dip a sterile non-toxic cotton swab on a wooden applicator into the standardized inoculum and rotate the soaked swab firmly against the upper inside wall of the tube to express excess fluid. Streak the entire agar surface of the plate with the swab three times, turning the plate at 60° angle between each streaking. Allow the inoculum to dry for 5 15 minutes with lid in place.
- 4. Apply the discs using aseptic technique. When using cartridges, the discs can be applied using the specially designed applicator. When the vials are used, apply the discs using sterile forceps.
- 5. Deposit the discs with centers at least 24 mm apart. For fastidious organisms and for Penicillins and Cephalosporins, the discs should preferably be deposited with centers 30 mm apart.
- 6. Incubate immediately at $35 \pm 2^{\circ}$ C and examine after 16-18 hours or longer, if necessary. For fastidious organisms incubate at appropriate temperature and time.
- 7. Measure the zones showing complete inhibition and record the diameters of the zones to the nearest millimeter using a calibrated instrument like zone scales (PW096/PW297)

Principle:

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

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

Use following interpretive criteria for susceptibility categorization*

		Sensitive	Intermediate	Resistant
Antimicrobial agent	Interpretative criteria for	mm or more	mm	mm or less
Rifampicin	Staphylococcus, Enterococcus , Haemophilus influenzae & Haemophilus parainfluenzae	20	17-19	16
5mcg	Neisseria meningitidis	25	20-24	19
	S.pneumoniae	19	17-18	16

Quality Control:

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

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

Organisms (ATCC)	Std. zone of diameter (mm)*
E.coli (25922)	8-10
S.aureus (25923)	26-34

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

Storage and Shelf-life:

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

References:

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

Note :

Use following media to carry out susceptibility test

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

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

For S.pneumoniae : Muller Hinton Agar supplemented with 5% Sheep Blood

For Neisseria spps : G.C.Agar +1% defined growth supplement (M434 + FD025)

* Not for Medicinal Use





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Streptomycin S 10 mcg

SD031

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

Composition

Ingredients	Concentration
Streptomycin	10mcg /disc

Susceptibility Test Procedure:

- 1. Prepare plates with Mueller Hinton Agar (M173/M1084) for rapidly growing aerobic organisms as per Bauer-Kirby Method. The medium in the plates should be sterile and should have a depth of about 4 mm.
- 2. Inoculate 4-5 similar colonies with a wire, needle or loop to 5 ml Tryptone Soya Broth (M011) and incubate at 35-37°C for2-8 hours until light to moderate turbidity develops. Compare the inoculum turbidity with that of standard 0.5 McFarland (prepared by mixing 0.5 ml of 1.175% barium chloride and 99.5 ml of 0.36N sulfuric acid). Dilute the inoculum or incubate further as necessary to attain comparative turbidity. Alternatively, the inoculum can be standardized by other appropriate optical method (0.08 0.13 OD turbid suspension at 625 nm)
- 3. Dip a sterile non-toxic cotton swab on a wooden applicator into the standardized inoculum and rotate the soaked swab firmly against the upper inside wall of the tube to express excess fluid. Streak the entire agar surface of the plate with the swab three times, turning the plate at 60° angle between each streaking. Allow the inoculum to dry for 5 15 minutes with lid in place.
- 4. Apply the discs using aseptic technique. When using cartridges, the discs can be applied using the specially designed applicator. When the vials are used, apply the discs using sterile forceps.
- 5. Deposit the discs with centers at least 24 mm apart. For fastidious organisms and for Penicillins and Cephalosporins, the discs should preferably be deposited with centers 30 mm apart.
- 6. Incubate immediately at $35 \pm 2^{\circ}$ C and examine after 16-18 hours or longer, if necessary. For fastidious organisms incubate at appropriate temperature and time.
- 7. Measure the zones showing complete inhibition and record the diameters of the zones to the nearest millimeter using a calibrated instrument like zone scales (PW096/PW297)

Principle:

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

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

Use following interpretive criteria for susceptibility categorization*

		Sensitive	Intermediate	Resistant
Antimicrobial agent	Interpretative criteria for	mm or more	mm	mm or less
Streptomycin 10mcg	Enterobacteriaceae	15	12-14	11

Quality Control:

Appearance: Filter paper discs of 6mm diameter with printed "S 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)	12-20
<i>S.aureus</i> (25923)	14-22

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

Storage and Shelf-life:

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

References:

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

Note :

Use following media to carry out susceptibility test For rapidly growing aerobic organisms : Mueller Hinton Agar (M173/M1084) For *Haemophilus* spps : Haemophilus Test Agar (M1259 + FD117) For *S.pneumoniae* : Muller Hinton Agar supplemented with 5% Sheep Blood For Neisseria spps : G.C.Agar +1% defined growth supplement (M434 + FD025)



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Amikacin AK 30 mcg

SD035

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

Composition

, Ingredients	Concentration
"Amikacin	30mcg/disc

Susceptibility Test Procedure:

- 1. Prepare plates with Mueller Hinton Agar (M173/M1084) for rapidly growing aerobic organisms as per Kirby-Bauer Method. The medium in the plates should be sterile and should have a depth of about 4 mm.
- 2. Inoculate 4-5 similar colonies with a wire, needle or loop to 5 ml Tryptone Soya Broth (M011) and incubate at 35-37°C for2-8 hours until light to moderate turbidity develops. Compare the inoculum turbidity with that of standard 0.5 McFarland (prepared by mixing 0.5 ml of 1.175% barium chloride and 99.5 ml of 0.36N sulfuric acid). Dilute the inoculum or incubate further as necessary to attain comparative turbidity. Alternatively, the inoculum can be standardized by other appropriate optical method (0.08 0.13 OD turbid suspension at 625 nm)
- 3. Dip a sterile non-toxic cotton swab on a wooden applicator into the standardized inoculum and rotate the soaked swab firmly against the upper inside wall of the tube to express excess fluid. Streak the entire agar surface of the plate with the swab three times, turning the plate at 60° angle between each streaking. Allow the inoculum to dry for 5 15 minutes with lid in place.
- 4. Apply the discs using aseptic technique. When using cartridges, the discs can be applied using the specially designed applicator. When the vials are used, apply the discs using sterile forceps.
- 5. Deposit the discs with centers at least 24 mm apart. For fastidious organisms and for Penicillins and Cephalosporins, the discs should preferably be deposited with centers 30 mm apart.
- 6. Incubate immediately at $35 \pm 2^{\circ}$ C and examine after 16-18 hours or longer, if necessary. For fastidious organisms incubate at appropriate temperature and time.
- 7. Measure the zones showing complete inhibition and record the diameters of the zones to the nearest millimeter using a calibrated instrument like zone scales (PW096/PW297)

Principle:

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

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

Use following interpretive criteria for susceptibility categorization*

Antimicrobial		Sensitive	Intermediate	Resistant
agent	Interpretative criteria for	mm or more	mm	mm or less
Amikacin 30mcg	Enterobacteriaceae, P.aeruginosa, Acientobacter ,Staphylococcus spp	17	15-16	14

Quality Control:

Appearance: Filter paper discs of 6mm diameter with printed "AK 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)	19-26
<i>S.aureus</i> (25923)	20-26
P.aeruginosa (27853)	18-26
<i>S.aureus</i> (29213)	18-24

*= 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
- Performance standards of Antimicrobial Disc Susceptibility Tests, M100S, 32nd Ed., CLSI Vol. 42 No.2, Feb-2022.
- 3. EUCAST, Breakpoint tables for interpretation of MIC's & zone diameters, version 12.0, valid from 01.01.2022.

Note :

Use following media to carry out susceptibility test

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

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

For S.pneumoniae : Muller Hinton Agar supplemented with 5% Sheep Blood

For Neisseria spps : G.C.Agar +1% defined growth supplement (M434 + FD025)

* Not for Medicinal Use

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Tetracycline TE 30 mcg

SD037

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

Composition

*Ingredients	Concentration		
Tetracycline	30 mcg /disc		

Susceptibility Test Procedure:

- 1. Prepare plates with Mueller Hinton Agar (M173/M1084) for rapidly growing aerobic organisms as per Kirby-Bauer Method. The medium in the plates should be sterile and should have a depth of about 4 mm.
- 2. Inoculate 4-5 similar colonies with a wire, needle or loop to 5 ml Tryptone Soya Broth (M011) and incubate at 35-37°C for2-8 hours until light to moderate turbidity develops. Compare the inoculum turbidity with that of standard 0.5 McFarland (prepared by mixing 0.5 ml of 1.175% barium chloride and 99.5 ml of 0.36N sulfuric acid). Dilute the inoculum or incubate further as necessary to attain comparative turbidity. Alternatively, the inoculum can be standardized by other appropriate optical method (0.08 0.13 OD turbid suspension at 625 nm)
- 3. Dip a sterile non-toxic cotton swab on a wooden applicator into the standardized inoculum and rotate the soaked swab firmly against the upper inside wall of the tube to express excess fluid. Streak the entire agar surface of the plate with the swab three times, turning the plate at 60° angle between each streaking. Allow the inoculum to dry for 5 15 minutes with lid in place.
- 4. Apply the discs using aseptic technique. When using cartridges, the discs can be applied using the specially designed applicator. When the vials are used, apply the discs using sterile forceps.
- 5. Deposit the discs with centers at least 24 mm apart. For fastidious organisms and for Penicillins and Cephalosporins, the discs should preferably be deposited with centers 30 mm apart.
- 6. Incubate immediately at $35 \pm 2^{\circ}$ C and examine after 16-18 hours or longer, if necessary. For fastidious organisms incubate at appropriate temperature and time.
- 7. Measure the zones showing complete inhibition and record the diameters of the zones to the nearest millimeter using a calibrated instrument like zone scales (PW096/PW297)

Principle:

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

- However few precautions are to be maintained while handling of the Sensitivity discs,
 - On receipt the discs are to be immediately stored at the recommended temperature.
 - Medium preparation, Inoculum preparation and incubation to be done as specified.

Use following interpretive criteria for susceptibility categorization*

		Sensitive	Intermediate	Resistant
Antimicrobial Agent	Interpretative criteria for	mm or more	mm	mm or less
	Enterobacteriaceae, Acientobacter	15	12-14	10
	Staphylococcus, Enterococcus spp. & Neisseria meningitidis	19	15-18	14
Tetracycline TE 30 mcg	Haemophilus influenzae & Haemophilus parainfluenzae	29	26-28	25
	Neisseria gonorhoeae	38	31-27	30
	S.pneumoniae	28	25-27	24
	Streptococcus spp. Beta haemolytic group & Viridians group	23	19-22	18

Quality Control:

Appearance: Filter paper discs of 6mm diameter with printed "TE 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)	18-25
<i>S.aureus</i> (25923)	24-30
S.aureus (29213)*	23-31

* = Interpretive criteria & QC ranges as per 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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Cefotaxime CTX 30 mcg (Cephotaxime)

Cefotaxime (Cephotaxime) CTX 30 mcg discs are used for antimicrobial susceptibility testing of bacterial cultures as per Kirby-Bauer Method

Composition

Ingredients	Concentration
Cefotaxime	30 mcg/disc
(Cephotaxime)	

Susceptibility Test Procedure:

- 1. Prepare plates with Mueller Hinton Agar (M173/M1084) for rapidly growing aerobic organisms as per Kirby-Bauer Method. The medium in the plates should be sterile and should have a depth of about 4 mm.
- 2. Inoculate 4-5 similar colonies with a wire, needle or loop to 5 ml Tryptone Soya Broth (M011) and incubate at 35-37°C for2-8 hours until light to moderate turbidity develops. Compare the inoculum turbidity with that of standard 0.5 McFarland (prepared by mixing 0.5 ml of 1.175% barium chloride and 99.5 ml of 0.36N sulfuric acid). Dilute the inoculum or incubate further as necessary to attain comparative turbidity. Alternatively, the inoculum can be standardized by other appropriate optical method (0.08 0.13 OD turbid suspension at 625 nm)
- 3. Dip a sterile non-toxic cotton swab on a wooden applicator into the standardized inoculum and rotate the soaked swab firmly against the upper inside wall of the tube to express excess fluid. Streak the entire agar surface of the plate with the swab three times, turning the plate at 60° angle between each streaking. Allow the inoculum to dry for 5 15 minutes with lid in place.
- 4. Apply the discs using aseptic technique. When using cartridges, the discs can be applied using the specially designed applicator. When the vials are used, apply the discs using sterile forceps.
- 5. Deposit the discs with centers at least 24 mm apart. For fastidious organisms and for Penicillins and Cephalosporins, the discs should preferably be deposited with centers 30 mm apart.
- 6. Incubate immediately at $35 \pm 2^{\circ}$ C and examine after 16-18 hours or longer, if necessary. For fastidious organisms incubate at appropriate temperature and time.
- 7. Measure the zones showing complete inhibition and record the diameters of the zones to the nearest millimeter using a calibrated instrument like zone scales (PW096/PW297)

Principle:

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

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

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

SD040

Use following interpretive criteria for susceptibility categorization*

		Sensitive	Intermediate	Resistant
Antimicrobial agent	Interpretative criteria for	mm or more	mm	mm or less
	Enterobacteriaceae	26	23-25	22
	P.aeruginosa, Acientobacter & Staphylococcus	23	15-22	14
Cefotaxime	Haemophilus influenzae & Haemophilus parainfluenzae	26	-	-
(Cephotaxime)	Neisseria meningitidis	34	-	-
CTX 50 meg	Neisseria gonorhoeae	31	-	-
	Streptococcus spp. Viridians group	28	26-27	25
	Streptococcus spp. beta haemolytic gruop	24	-	-

Quality Control:

Appearance: Filter paper discs of 6mm diameter with printed "CTX 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.

35
31
22

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

Storage and Shelf-life:

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

References:

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

(6

CE Marking

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Cefoxitin (Cephoxitin) CX 30 mcg

SD041

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

Composition

*Ingredients

Cefoxitin	Concentration
(Cephoxitin)	30 mcg/disc

Susceptibility Test Procedure:

- 1. Prepare plates with Mueller Hinton Agar (M173/M1084) for rapidly growing aerobic organisms as per Kirby- Bauer Method. The medium in the plates should be sterile and should have a depth of about 4 mm.
- 2. Inoculate 4-5 similar colonies with a wire, needle or loop to 5 ml Tryptone Soya Broth (M011) and incubate at 35-37°C for2-8 hours until light to moderate turbidity develops. Compare the inoculum turbidity with that of standard 0.5 McFarland (prepared by mixing 0.5 ml of 1.175% barium chloride and 99.5 ml of 0.36N sulfuric acid). Dilute the inoculum or incubate further as necessary to attain comparative turbidity. Alternatively, the inoculum can be standardized by other appropriate optical method (0.08 0.13 OD turbid suspension at 625 nm)
- 3. Dip a sterile non-toxic cotton swab on a wooden applicator into the standardized inoculum and rotate the soaked swab firmly against the upper inside wall of the tube to express excess fluid. Streak the entire agar surface of the plate with the swab three times, turning the plate at 60° angle between each streaking. Allow the inoculum to dry for 5 15 minutes with lid in place.
- 4. Apply the discs using aseptic technique. When using cartridges, the discs can be applied using the specially designed applicator. When the vials are used, apply the discs using sterile forceps.
- 5. Deposit the discs with centers at least 24 mm apart. For fastidious organisms and for Penicillins and Cephalosporins, the discs should preferably be deposited with centers 30 mm apart.
- 6. Incubate immediately at $35 \pm 2^{\circ}$ C and examine after 16-18 hours or longer, if necessary. For fastidious organisms incubate at appropriate temperature and time.
- 7. Measure the zones showing complete inhibition and record the diameters of the zones to the nearest millimeter using a calibrated instrument like zone scales (PW096/PW297)

Principle:

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

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

Use following interpretive criteria for susceptibility categorization*

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

Quality Control:

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

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

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

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

Storage and Shelf-life:

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

References:

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

Note :

Use following media to carry out susceptibility test For rapidly growing aerobic organisms : Mueller Hinton Agar (M173/M1084) For *Haemophilus* spps : Haemophilus Test Agar (M1259 + FD117) For *S.pneumoniae* : Muller Hinton Agar supplemented with 5% Sheep Blood For Neisseria spps : G.C.Agar +1% defined growth supplement (M434 + FD025) * Not for Medicinal Use



In vitro diagnostic medical device

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Tobramycin TOB 10 mcg

SD044

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

Composition

*IngredientsConcentrationTobramycin10 mcg /disc

Susceptibility Test Procedure:

- 1. Prepare plates with Mueller Hinton Agar (M173/M1084) for rapidly growing aerobic organisms as per Bauer-Kirby Method. The medium in the plates should be sterile and should have a depth of about 4 mm.
- 2. Inoculate 4-5 similar colonies with a wire, needle or loop to 5 ml Tryptone Soya Broth (M011) and incubate at 35-37°C for2-8 hours until light to moderate turbidity develops. Compare the inoculum turbidity with that of standard 0.5 McFarland (prepared by mixing 0.5 ml of 1.175% barium chloride and 99.5 ml of 0.36N sulfuric acid). Dilute the inoculum or incubate further as necessary to attain comparative turbidity. Alternatively, the inoculum can be standardized by other appropriate optical method (0.08 0.13 OD turbid suspension at 625 nm)
- 3. Dip a sterile non-toxic cotton swab on a wooden applicator into the standardized inoculum and rotate the soaked swab firmly against the upper inside wall of the tube to express excess fluid. Streak the entire agar surface of the plate with the swab three times, turning the plate at 60° angle between each streaking. Allow the inoculum to dry for 5 15 minutes with lid in place.
- 4. Apply the discs using aseptic technique. When using cartridges, the discs can be applied using the specially designed applicator. When the vials are used, apply the discs using sterile forceps.
- 5. Deposit the discs with centers at least 24 mm apart. For fastidious organisms and for Penicillins and Cephalosporins, the discs should preferably be deposited with centers 30 mm apart.
- 6. Incubate immediately at $35 \pm 2^{\circ}$ C and examine after 16-18 hours or longer, if necessary. For fastidious organisms incubate at appropriate temperature and time.
- 7. Measure the zones showing complete inhibition and record the diameters of the zones to the nearest millimeter using a calibrated instrument like zone scales (PW096/PW297)

Principle:

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

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

Use following interpretive criteria for susceptibility categorization*

		Sensitive	Intermediate	Resistant
Antimicrobial agent	Interpretative criteria for	mm or more	mm	mm or less
Tobramycin 10 mcg	Enterobacteriaceae, P.aeruginosa, Acientobacter & Staphylococcus	15	13-14	12

Quality Control:

Appearance: Filter paper discs of 6mm diameter with printed "TOB 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)	18-26
<i>S.aureus</i> (25923)	19-29
P.aeruginosa (27853)	19-25

* = 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)

* Not for Medicinal Use



On receipt store at -20°C

Do not use if package is damaged

Storage temperature



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Cefazolin CZ 30 mcg

SD047

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

Composition

*Ingredients	Concentration
Cefazolin	30 mcg/disc

Susceptibility Test Procedure:

- 1. Prepare plates with Mueller Hinton Agar (M173/M1084) for rapidly growing aerobic organisms as per Kirby- Bauer Method. The medium in the plates should be sterile and should have a depth of about 4 mm.
- 2. Inoculate 4-5 similar colonies with a wire, needle or loop to 5 ml Tryptone Soya Broth (M011) and incubate at 35-37°C for2-8 hours until light to moderate turbidity develops. Compare the inoculum turbidity with that of standard 0.5 McFarland (prepared by mixing 0.5 ml of 1.175% barium chloride and 99.5 ml of 0.36N sulfuric acid). Dilute the inoculum or incubate further as necessary to attain comparative turbidity. Alternatively, the inoculum can be standardized by other appropriate optical method (0.08 0.13 OD turbid suspension at 625 nm)
- 3. Dip a sterile non-toxic cotton swab on a wooden applicator into the standardized inoculum and rotate the soaked swab firmly against the upper inside wall of the tube to express excess fluid. Streak the entire agar surface of the plate with the swab three times, turning the plate at 60° angle between each streaking. Allow the inoculum to dry for 5 15 minutes with lid in place.
- 4. Apply the discs using aseptic technique. When using cartridges, the discs can be applied using the specially designed applicator. When the vials are used, apply the discs using sterile forceps.
- 5. Deposit the discs with centers at least 24 mm apart. For fastidious organisms and for Penicillins and Cephalosporins, the discs should preferably be deposited with centers 30 mm apart.
- 6. Incubate immediately at $35 \pm 2^{\circ}$ C and examine after 16-18 hours or longer, if necessary. For fastidious organisms incubate at appropriate temperature and time.
- 7. Measure the zones showing complete inhibition and record the diameters of the zones to the nearest millimeter using a calibrated instrument like zone scales (PW096/PW297)

Principle:

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

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

		a	T	D
		Sensitive	Intermediate	Resistant
Antimicrobial agent	Interpretative criteria for	mm or more	mm	mm or less
Cefazolin	Enterobacterales	23	20-22	19
30 mcg	Enterobacterales (uncomplicated UTI's)	15	-	14
	Staphylococcus spp	18	15-17	14

Use following interpretive criteria for susceptibility categorization*

Quality Control:

Appearance: Filter paper discs of 6mm diameter with printed "CZ 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)	29-35

* = 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



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Clindamycin CD 2 mcg

SD051

Clindamycin CD 2 mcg discs are used for antimicrobial susceptibility testing of of bacterial cultures as per Bauer-Kirby Method

Composition

, Ingredients	Concentration
Clindamycin	2 mcg/disc

Susceptibility Test Procedure:

- 1. Prepare plates with Mueller Hinton Agar (M173/M1084) for rapidly growing aerobic organisms as per Bauer-Kirby Method. The medium in the plates should be sterile and should have a depth of about 4 mm.
- 2. Inoculate 4-5 similar colonies with a wire, needle or loop to 5 ml Tryptone Soya Broth (M011) and incubate at 35-37°C for2-8 hours until light to moderate turbidity develops. Compare the inoculum turbidity with that of standard 0.5 McFarland (prepared by mixing 0.5 ml of 1.175% barium chloride and 99.5 ml of 0.36N sulfuric acid). Dilute the inoculum or incubate further as necessary to attain comparative turbidity. Alternatively, the inoculum can be standardized by other appropriate optical method (0.08 0.13 OD turbid suspension at 625 nm)
- 3. Dip a sterile non-toxic cotton swab on a wooden applicator into the standardized inoculum and rotate the soaked swab firmly against the upper inside wall of the tube to express excess fluid. Streak the entire agar surface of the plate with the swab three times, turning the plate at 60° angle between each streaking. Allow the inoculum to dry for 5 15 minutes with lid in place.
- 4. Apply the discs using aseptic technique. When using cartridges, the discs can be applied using the specially designed applicator. When the vials are used, apply the discs using sterile forceps.
- 5. Deposit the discs with centers at least 24 mm apart. For fastidious organisms and for Penicillins and Cephalosporins, the discs should preferably be deposited with centers 30 mm apart.
- 6. Incubate immediately at $35 \pm 2^{\circ}$ C and examine after 16-18 hours or longer, if necessary. For fastidious organisms incubate at appropriate temperature and time.
- 7. Measure the zones showing complete inhibition and record the diameters of the zones to the nearest millimeter using a calibrated instrument like zone scales (PW096/PW297)

Principle:

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

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

Use following interpretive criteria for susceptibility categorization*

		Sensitive	Intermediate	Resistant
Antimicrobial agent	Interpretative criteria for	mm or more	mm	mm or less
	Staphylococcus	21	15-20	14
2 mcg	S.pneumoniae, Streptococcus spp. Viridians group, Streptococcus spp. beta haemolytic gruop	19	16-18	15

Quality Control:

Appearance: Filter paper discs of 6mm diameter with printed "CD 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.

me of diameter (mm).
30

* = 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)

* Not for Medicinal Use

In vitro diagnostic medical device

CE Marking



(E

Storage temperature



Do not use if package is damaged



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Norfloxacin NX 10 mcg

SD057

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

Composition

*Ingredients Concentration

Norfloxacin 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 for 2-8 hours until light to moderate turbidity develops. Compare the inoculum turbidity with that of standard 0.5 McFarland (prepared by mixing 0.5 ml of 1.175%barium chloride and 99.5 ml of 0.36N sulfuric acid). Dilute the inoculum or incubate further as necessary to attain comparative turbidity. Alternatively, the inoculum can be standardized by other appropriate optical method (0.08 0.13 OD turbid suspension at 625 nm)
- 3. Dip a sterile non-toxic cotton swab on a wooden applicator into the standardized inoculum and rotate the soaked swab firmly against the upper inside wall of the tube to express excess fluid. Streak the entire agar surface of the plate with the swab three times, turning the plate at 60° angle between each streaking. Allow the inoculum to dry for 5 15 minutes with lid in place.
- 4. Apply the discs using aseptic technique. When using cartridges, the discs can be applied using the specially designed applicator. When the vials are used, apply the discs using sterile forceps.
- 5. Deposit the discs with centers at least 24 mm apart. For fastidious organisms and for Penicillins and Cephalosporins, the discs should preferably be deposited with centers 30 mm apart.
- 6. Incubate immediately at $35 \pm 2^{\circ}$ C and examine after 16-18 hours or longer, if necessary. For fastidious organisms incubate at appropriate temperature and time.
- 7. Measure the zones showing complete inhibition and record the diameters of the zones to the nearest millimeter using a calibrated instrument like zone scales (PW096/PW297)

Principle:

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

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

Use following interpretive criteria for susceptibility categorization*

		Sensitive	Intermediate	Resistant
Antimicrobial agent	Interpretative criteria for	mm or more	mm	mm or less
Norfloxacin	Enterobacteriaceae, P.aeruginosa,	17	12.16	12
10 mcg	Staphylococcus & Enterococcus	17	13-10	12

Quality Control:

Appearance: Filter paper discs of 6mm diameter with printed "NX 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)	28-35
S.aureus (25923)	17-28
P.aeruginosa (27853)	22-29
S.aureus (29213)	18-24
E.faecalis (29212)	16-22

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

Storage and Shelf-life:

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

References:

- 1. Bauer, Kirby, Sherris and Turck, 1966, Am. J. Clin. Path., 45: 493
- 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

In vitro diagnostic medical

For Neisseria spps : G.C.Agar +1% defined growth supplement (M434 + FD025)

* Not for Medicinal Use

IVD

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device CE Marking

On receipt store at /

Storage temperature



Do not use if package is damaged



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Ciprofloxacin CIP 5 mcg

SD060

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

Composition

*Ingredients	Concentration		
Ciprofloxacin	5 mcg/disc		

Susceptibility Test Procedure:

- 1. Prepare plates with Mueller Hinton Agar (M173/M1084) for rapidly growing aerobic organisms as per Bauer-Kirby Method. The medium in the plates should be sterile and should have a depth of about 4 mm.
- 2. Inoculate 4-5 similar colonies with a wire, needle or loop to 5 ml Tryptone Soya Broth (M011) and incubate at 35-37°C for2-8 hours until light to moderate turbidity develops. Compare the inoculum turbidity with that of standard 0.5 McFarland (prepared by mixing 0.5 ml of 1.175% barium chloride and 99.5 ml of 0.36N sulfuric acid). Dilute the inoculum or incubate further as necessary to attain comparative turbidity. Alternatively, the inoculum can be standardized by other appropriate optical method (0.08 0.13 OD turbid suspension at 625 nm)
- 3. Dip a sterile non-toxic cotton swab on a wooden applicator into the standardized inoculum and rotate the soaked swab firmly against the upper inside wall of the tube to express excess fluid. Streak the entire agar surface of the plate with the swab three times, turning the plate at 60° angle between each streaking. Allow the inoculum to dry for 5 15 minutes with lid in place.
- 4. Apply the discs using aseptic technique. When using cartridges, the discs can be applied using the specially designed applicator. When the vials are used, apply the discs using sterile forceps.
- 5. Deposit the discs with centers at least 24 mm apart. For fastidious organisms and for Penicillins and Cephalosporins, the discs should preferably be deposited with centers 30 mm apart.
- 6. Incubate immediately at $35 \pm 2^{\circ}$ C and examine after 16-18 hours or longer, if necessary. For fastidious organisms incubate at appropriate temperature and time.
- 7. Measure the zones showing complete inhibition and record the diameters of the zones to the nearest millimeter using a calibrated instrument like zone scales (PW096/PW297)

Principle:

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

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

		Sensitive	Intermediate	Resistant
Antimicrobial agent	Interpretative criteria for	mm or more	mm	mm or less
	<i>Enterobacteriaceae other than S.</i> Typhi and extraintestinal <i>Salmonella</i> spp., <i>P.aeruginosa</i> , <i>Acientobacter, Staphylococcus & Enterococcus</i>	21	16-20	15
Ciprofloyacin	For S. Typhi and extraintestinal Salmonella spp.	31	21-30	20
5 mcg	Haemophilus influenzae & Haemophilus parainfluenzae	21	-	-
	Neisseria meningitidis	35	33-34	32
	Neisseria gonorhoeae	41	28-40	27

Use following interpretive criteria for susceptibility categorization*

Quality Control:

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

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

Organisms (ATCC)	Std. zone of diameter (mm)*
E.coli (25922)	29-37
<i>S.aureus</i> (25923)	22-30
P.aeruginosa (27853)	25-33
<i>S.aureus</i> (29213)	21-27
<i>E.faecalis</i> (29212)	19-25

* = 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)

* Not for Medicinal Use

CE Marking

In vitro diagnostic medical

device

Storage temperature



On receipt store at -20°C

Do not use if package is damaged

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Cefuroxime CXM 30 mcg

SD061

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

Composition

*Ingredients	Concentration		
Cefuroxime	30 mcg/disc		

Susceptibility Test Procedure:

- 1. Prepare plates with Mueller Hinton Agar (M173/M1084) for rapidly growing aerobic organisms as per Bauer-Kirby Method. The medium in the plates should be sterile and should have a depth of about 4 mm.
- 2. Inoculate 4-5 similar colonies with a wire, needle or loop to 5 ml Tryptone Soya Broth (M011) and incubate at 35-37°C for2-8 hours until light to moderate turbidity develops. Compare the inoculum turbidity with that of standard 0.5 McFarland (prepared by mixing 0.5 ml of 1.175% barium chloride and 99.5 ml of 0.36N sulfuric acid). Dilute the inoculum or incubate further as necessary to attain comparative turbidity. Alternatively, the inoculum can be standardized by other appropriate optical method (0.08 0.13 OD turbid suspension at 625 nm)
- 3. Dip a sterile non-toxic cotton swab on a wooden applicator into the standardized inoculum and rotate the soaked swab firmly against the upper inside wall of the tube to express excess fluid. Streak the entire agar surface of the plate with the swab three times, turning the plate at 60° angle between each streaking. Allow the inoculum to dry for 5 15 minutes with lid in place.
- 4. Apply the discs using aseptic technique. When using cartridges, the discs can be applied using the specially designed applicator. When the vials are used, apply the discs using sterile forceps.
- 5. Deposit the discs with centers at least 24 mm apart. For fastidious organisms and for Penicillins and Cephalosporins, the discs should preferably be deposited with centers 30 mm apart.
- 6. Incubate immediately at $35 \pm 2^{\circ}$ C and examine after 16-18 hours or longer, if necessary. For fastidious organisms incubate at appropriate temperature and time.
- 7. Measure the zones showing complete inhibition and record the diameters of the zones to the nearest millimeter using a calibrated instrument like zone scales (PW096/PW297)

Principle:

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

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

		Sensitive	Intermediate	Resistant
Antimicrobial agent	Interpretative criteria for	mm or more	mm	mm or less
	Enterobacteriaceae & Staphylococcus	18	15-17	14
Cefuroxime 30 mcg	Haemophilus influenzae & Haemophilus parainfluenzae	20	17-19	16
	Neisseria gonorhoeae	31	26-30	25

Use following interpretive criteria for susceptibility categorization*

Quality Control:

Appearance: Filter paper discs of 6mm diameter with printed "CXM 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)	20-26
S.aureus (25923)	27-35

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

Storage and Shelf-life:

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

References:

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

Note :

Use following media to carry out susceptibility test For rapidly growing aerobic organisms : Mueller Hinton Agar (M173/M1084) For *Haemophilus* spps : Haemophilus Test Agar (M1259 + FD117) For *S.pneumoniae* : Muller Hinton Agar supplemented with 5% Sheep Blood For Neisseria spps : G.C.Agar +1% defined growth supplement (M434 + FD025)

* Not for Medicinal Use




Ceftazidime CAZ 30 mcg

SD062

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

Composition

*Ingredients	Concentration
Ceftazidime	30 mcg/disc

Susceptibility Test Procedure:

- 1. Prepare plates with Mueller Hinton Agar (M173/M1084) for rapidly growing aerobic organisms as per Bauer-Kirby Method. The medium in the plates should be sterile and should have a depth of about 4 mm.
- 2. Inoculate 4-5 similar colonies with a wire, needle or loop to 5 ml Tryptone Soya Broth (M011) and incubate at 35-37°C for2-8 hours until light to moderate turbidity develops. Compare the inoculum turbidity with that of standard 0.5 McFarland (prepared by mixing 0.5 ml of 1.175% barium chloride and 99.5 ml of 0.36N sulfuric acid). Dilute the inoculum or incubate further as necessary to attain comparative turbidity. Alternatively, the inoculum can be standardized by other appropriate optical method (0.08 0.13 OD turbid suspension at 625 nm)
- 3. Dip a sterile non-toxic cotton swab on a wooden applicator into the standardized inoculum and rotate the soaked swab firmly against the upper inside wall of the tube to express excess fluid. Streak the entire agar surface of the plate with the swab three times, turning the plate at 60° angle between each streaking. Allow the inoculum to dry for 5 15 minutes with lid in place.
- 4. Apply the discs using aseptic technique. When using cartridges, the discs can be applied using the specially designed applicator. When the vials are used, apply the discs using sterile forceps.
- 5. Deposit the discs with centers at least 24 mm apart. For fastidious organisms and for Penicillins and Cephalosporins, the discs should preferably be deposited with centers 30 mm apart.
- 6. Incubate immediately at $35 \pm 2^{\circ}$ C and examine after 16-18 hours or longer, if necessary. For fastidious organisms incubate at appropriate temperature and time.
- 7. Measure the zones showing complete inhibition and record the diameters of the zones to the nearest millimeter using a calibrated instrument like zone scales (PW096/PW297)

Principle:

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

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

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

Use following interpretive criteria for susceptibility categorization*

Quality Control:

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

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

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

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

Storage and Shelf-life:

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

References:

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

Note :

Use following media to carry out susceptibility test For rapidly growing aerobic organisms : Mueller Hinton Agar (M173/M1084) For *Haemophilus* spps : Haemophilus Test Agar (M1259 + FD117) For *S.pneumoniae* : Muller Hinton Agar supplemented with 5% Sheep Blood For Neisseria spps : G.C.Agar +1% defined growth supplement (M434 + FD025)

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Ceftazidime CAZ 10 mcg

SD062A

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

Composition

*Ingredients Concentration

Ceftazidime 10 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 for 2-8 hours until light to moderate turbidity develops. Compare the inoculum turbidity with that of standard 0.5 McFarland (prepared by mixing 0.5 ml of 1.175% barium chloride and 99.5 ml of 0.36N sulfuric acid). Dilute the inoculum or incubate further as necessary to attain comparative turbidity. Alternatively, the inoculum can be standardized by other appropriate optical method (0.08 0.13 OD turbid suspension at 625 nm)
- 3. Dip a sterile non-toxic cotton swab on a wooden applicator into the standardized inoculum and rotate the soaked swab firmly against the upper inside wall of the tube to express excess fluid. Streak the entire agar surface of the plate with the swab three times, turning the plate at 60° angle between each streaking. Allow the inoculum to dry for 5 15 minutes with lid in place.
- 4. Apply the discs using aseptic technique. When using cartridges, the discs can be applied using the specially designed applicator. When the vials are used, apply the discs using sterile forceps.
- 5. Deposit the discs with centers at least 24 mm apart. For fastidious organisms and for Penicillins and Cephalosporins, the discs should preferably be deposited with centers 30 mm apart.
- 6. Incubate immediately at $35 \pm 2^{\circ}$ C and examine after 16-18 hours or longer, if necessary. For fastidious organisms incubate at appropriate temperature and time.
- 7. Measure the zones showing complete inhibition and record the diameters of the zones to the nearest millimeter using a calibrated instrument like zone scales (PW096/PW297)

Principle:

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

- However few precautions are to be maintained while handling of the Sensitivity discs,
 - On receipt the discs are to be immediately stored at the recommended temperature.
 - Medium preparation, Inoculum preparation and incubation to be done as specified.

Quality Control:

Appearance: Filter paper discs of 6mm diameter with printed "CAZ 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)	23 - 29
P.aeruginosa (27853)	21 - 27
K.pneumoniae (700603)	6 - 12

* = QC ranges as per EUCAST standards

Storage and Shelf-life:

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

References:

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

Note :

Use following media to carry out susceptibility test

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

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

For S.pneumoniae : Muller Hinton Agar supplemented with 5% Sheep Blood

For Neisseria spps : G.C.Agar +1% defined growth supplement (M434 + FD025)

* Not for Medicinal Use





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Disclaimer :

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

SD063

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

Composition

*Ingredients Amoxyclav **Concentration** 30mcg/disc

Susceptibility Test Procedure:

(Amoxycillin/Clavulanic acid)

- 1. Prepare plates with Mueller Hinton Agar (M173/M1084) for rapidly growing aerobic organisms as per Bauer-Kirby Method. The medium in the plates should be sterile and should have a depth of about 4 mm.
- 2. Inoculate 4-5 similar colonies with a wire, needle or loop to 5 ml Tryptone Soya Broth (M011) and incubate at 35-37°C for2-8 hours until light to moderate turbidity develops. Compare the inoculum turbidity with that of standard 0.5 McFarland (prepared by mixing 0.5 ml of 1.175% barium chloride and 99.5 ml of 0.36N sulfuric acid). Dilute the inoculum or incubate further as necessary to attain comparative turbidity. Alternatively, the inoculum can be standardized by other appropriate optical method (0.08 0.13 OD turbid suspension at 625 nm)
- 3. Dip a sterile non-toxic cotton swab on a wooden applicator into the standardized inoculum and rotate the soaked swab firmly against the upper inside wall of the tube to express excess fluid. Streak the entire agar surface of the plate with the swab three times, turning the plate at 60° angle between each streaking. Allow the inoculum to dry for 5 15 minutes with lid in place.
- 4. Apply the discs using aseptic technique. When using cartridges, the discs can be applied using the specially designed applicator. When the vials are used, apply the discs using sterile forceps.
- 5. Deposit the discs with centers at least 24 mm apart. For fastidious organisms and for Penicillins and Cephalosporins, the discs should preferably be deposited with centers 30 mm apart.
- 6. Incubate immediately at $35 \pm 2^{\circ}$ C and examine after 16-18 hours or longer, if necessary. For fastidious organisms incubate at appropriate temperature and time.
- 7. Measure the zones showing complete inhibition and record the diameters of the zones to the nearest millimeter using a calibrated instrument like zone scales (PW096/PW297)

Principle:

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

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

Use following interpretive criteria for susceptibility categorization*

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

Quality Control:

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

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

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

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

Storage and Shelf-life:

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

References:

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

Note :

Use following media to carry out susceptibility test

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

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

For S.pneumoniae : Muller Hinton Agar supplemented with 5% Sheep Blood

For Neisseria spps : G.C.Agar +1% defined growth supplement (M434 + FD025)

* Not for Medicinal Use

In vitro diagnostic medical device CE Marking

On receipt store at /



Do not use if package is damaged

Storage temperature

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Disclaimer :

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Ceftriaxone CTR 30 mcg

SD065

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

Composition

*Ingredients	Concentration
Ceftriaxone	30 mcg/disc

Susceptibility Test Procedure:

- 1. Prepare plates with Mueller Hinton Agar (M173/M1084) for rapidly growing aerobic organisms as per Bauer-Kirby Method. The medium in the plates should be sterile and should have a depth of about 4 mm.
- 2. Inoculate 4-5 similar colonies with a wire, needle or loop to 5 ml Tryptone Soya Broth (M011) and incubate at 35-37°C for2-8 hours until light to moderate turbidity develops. Compare the inoculum turbidity with that of standard 0.5 McFarland (prepared by mixing 0.5 ml of 1.175% barium chloride and 99.5 ml of 0.36N sulfuric acid). Dilute the inoculum or incubate further as necessary to attain comparative turbidity. Alternatively, the inoculum can be standardized by other appropriate optical method (0.08 0.13 OD turbid suspension at 625 nm)
- 3. Dip a sterile non-toxic cotton swab on a wooden applicator into the standardized inoculum and rotate the soaked swab firmly against the upper inside wall of the tube to express excess fluid. Streak the entire agar surface of the plate with the swab three times, turning the plate at 60° angle between each streaking. Allow the inoculum to dry for 5 15 minutes with lid in place.
- 4. Apply the discs using aseptic technique. When using cartridges, the discs can be applied using the specially designed applicator. When the vials are used, apply the discs using sterile forceps.
- 5. Deposit the discs with centers at least 24 mm apart. For fastidious organisms and for Penicillins and Cephalosporins, the discs should preferably be deposited with centers 30 mm apart.
- 6. Incubate immediately at $35 \pm 2^{\circ}$ C and examine after 16-18 hours or longer, if necessary. For fastidious organisms incubate at appropriate temperature and time.
- 7. Measure the zones showing complete inhibition and record the diameters of the zones to the nearest millimeter using a calibrated instrument like zone scales (PW096/PW297)

Principle:

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

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

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Use following interpretive criteria for susceptibility categorization*

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

Quality Control:

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

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

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

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

Storage and Shelf-life:

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

References:

- 1. Bauer, Kirby, Sherris and Turck, 1966, Am. J. Clin. Path., 45: 493
- 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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Cefoperazone CPZ 75 mcg

SD072

Cefoperazone CPZ 75 mcg discs are used for antimicrobial susceptibility testing of bacterial cultures as per Bauer-Kirby Method

Composition

*Ingredients	Concentration
Cefoperazone	75 mcg/disc

Susceptibility Test Procedure:

- 1. Prepare plates with Mueller Hinton Agar (M173/M1084) for rapidly growing aerobic organisms as per Bauer-Kirby Method. The medium in the plates should be sterile and should have a depth of about 4 mm.
- 2. Inoculate 4-5 similar colonies with a wire, needle or loop to 5 ml Tryptone Soya Broth (M011) and incubate at 35-37°C for2-8 hours until light to moderate turbidity develops. Compare the inoculum turbidity with that of standard 0.5 McFarland (prepared by mixing 0.5 ml of 1.175% barium chloride and 99.5 ml of 0.36N sulfuric acid). Dilute the inoculum or incubate further as necessary to attain comparative turbidity. Alternatively, the inoculum can be standardized by other appropriate optical method (0.08 0.13 OD turbid suspension at 625 nm)
- 3. Dip a sterile non-toxic cotton swab on a wooden applicator into the standardized inoculum and rotate the soaked swab firmly against the upper inside wall of the tube to express excess fluid. Streak the entire agar surface of the plate with the swab three times, turning the plate at 60° angle between each streaking. Allow the inoculum to dry for 5 15 minutes with lid in place.
- 4. Apply the discs using aseptic technique. When using cartridges, the discs can be applied using the specially designed applicator. When the vials are used, apply the discs using sterile forceps.
- 5. Deposit the discs with centers at least 24 mm apart. For fastidious organisms and for Penicillins and Cephalosporins, the discs should preferably be deposited with centers 30 mm apart.
- 6. Incubate immediately at $35 \pm 2^{\circ}$ C and examine after 16-18 hours or longer, if necessary. For fastidious organisms incubate at appropriate temperature and time.
- 7. Measure the zones showing complete inhibition and record the diameters of the zones to the nearest millimeter using a calibrated instrument like zone scales (PW096/PW297)

Principle:

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

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

Use following interpretive criteria for susceptibility categorization*

		Sensitive	Intermediate	Resistant
Antimicrobial agent	Interpretative criteria for	mm or more	mm	mm or less
Cefoperazone 75 mcg	Enterobacteriaceae, P.aeruginosa	21	16-20	15

Quality Control:

Appearance: Filter paper discs of 6mm diameter with printed "CPZ 75" 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)	28-34
S.aureus (25923)	24-33
P.aeruginosa (27853)	23-29

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

Storage and Shelf-life:

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

References:

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

Note :

Use following media to carry out susceptibility test

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

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

For S.pneumoniae : Muller Hinton Agar supplemented with 5% Sheep Blood

For Neisseria spps : G.C.Agar +1% defined growth supplement (M434 + FD025)



In vitro diagnostic medical device

CE Marking



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Imipenem IPM 10 mcg

SD073

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

Composition

*Ingredients	Concentration		
Imipenem	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 $35 \pm 2^{\circ}$ C and examine after 16-18 hours or longer, if necessary. For fastidious organisms incubate at appropriate temperature and time.
- 7. Measure the zones showing complete inhibition and record the diameters of the zones to the nearest millimeter using a calibrated instrument like zone scales (PW096/PW297)

Principle:

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

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

		Sensitive	Intermediate	Resistant
Antimicrobial agent	Interpretative criteria for	mm or more	mm	mm or less
Imipenem 10 mcg	Enterobacteriaceae	23	20-22	19
	P.aeruginosa	19	16-18	15
	Acientobacter, Staphylococcus	16	14-15	13
	Haemophilus influenzae & Haemophilus parainfluenzae	16	-	-

Use following interpretive criteria for susceptibility categorization*

Quality Control:

Appearance: Filter paper discs of 6mm diameter with printed "IPM 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)	26-32
P.aeruginosa (27853)	20-28
E.faecalis (29212)	24-30

* = 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)



Disclaimer :

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