Cefepime CPM 30 mcg

Intended Use
Cefepime CPM 30 mcg discs are used for antimicrobial susceptibility testing of bacterial cultures as per Kirby-Bauer Method.

Principle
Antimicrobial Susceptibility Testing (AST) is a laboratory procedure performed by laboratory technician to identify, which antimicrobial regimen is specifically effective for individual patients. The introduction of various antimicrobials for treating variety of infections showed the necessity of performing antimicrobial susceptibility testing as a routine procedure in all microbiology laboratories. antibiotics are generally defined as the substances produced by the microorganism such as Penicillium, which has the ability to kill or inhibit the growth of other microorganisms, mainly bacteria. Antimicrobial Susceptibility Tests (ASTs) basically measures the ability of an antibiotic or other antimicrobial agent to inhibit the *In vitro* microbial growth.
The basic principle of the antibiotic susceptibility testing has been used in microbiology laboratories over 80 years. Till the 1950s, laboratories were lacking in the methodologies and equipment's for the accurate determination of *In vitro* responses of organisms to antimicrobial agents. Bauer *et al.,* began the development of standardized methods for antimicrobial susceptibility testing, using disc diffusion system. Antimicrobial Susceptibility Tests are either quantitative or qualitative.
Clinical laboratories currently employ several methods depending on the laboratory test menu that they provide. These approaches include the disk diffusion and Minimum Inhibitory Concentration (MIC) methods. 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.
Various regulatory agencies and standards-writing organizations, published standardized reference procedures 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) for any antimicrobial testing, Quality control or clinical testing. However, few precautions are to be maintained while handling of the Sensitivity discs, the latest CLSI documents should be consulted for current recommendations.

Susceptibility Test Procedure
Prepare plates with Mueller Hinton Agar (201130650100 / 201130650500, 203130830250 / 203130830100, 203090070015 / 203090070030 & Ready Prepared Plates – 205131790100 / 205131790020) for use in the Kirby-Bauer Method for rapidly growing aerobic organisms. For fastidious organisms such as Streptococci, the agar (201130650100 / 201130650500) is supplemented with 5% sterile, defibrinated blood. For *Haemophilus spp*., Haemophilus Test Medium (for all agents when testing *H. influenzae* or *H. parainfluenzae*) or MH-F agar (MHA with 5% mechanically defibrinated horse blood and 20 µg/mL NAD) (for selected agents when testing *H. influenzae*); for *N. gonorrhoeae*, GC Agar Base (201070010500) with 1% defined growth supplement and for *Streptococcus pneumoniae,* MHA with 5% sheep blood or MH-F agar (MHA with mechanically defibrinated horse blood and 20 µg/mL NAD) are recommended respectively. The medium in the plates should be sterile and have a depth of about 4 mm.

Bacterial Inoculum Preparation
1. Gram staining is done before starting susceptibility testing to confirm the culture purity and to determine appropriate battery of tests.
2. Select 3-4 colonies from subcultured slant or plate and transfer them into 5 mL of sterile 0.85% saline, vortex the suspension and adjust the turbidity to yield a uniform suspension matching 0.5 McFarland standard (O.D at 630 nm range: 0.08-0.130).
   Note: The direct colony suspension method is preferred for *Staphylococcus* spp., *S. pneumoniae* and other Streptococci, *Haemophilus* spp. and *N. gonorrhoeae*. OR
   3-5 well-isolated colonies should be selected and transferred to 4-5 mL of a suitable broth using an inoculating needle.

3. Incubate the broth (usually 2-6 hours) at 35°C-37°C until it achieves or exceeds the turbidity of the 0.5 McFarland's barium sulphate standard. This results in a suspension containing approximately 1-2 X 10^8 cfu/mL.
   Note: Overnight broth cultures should not be used as inoculum.
4. Adjust the turbidity to the barium sulphate standard. For the diluents use sterile broth or sterile saline. The turbidity of the standard and the test inoculums should be compared by holding both tubes in front of a white background with finely divided lines or by use of a photometric device.
5. Within 15 minutes of adjusting the turbidity of the inoculum, immerse a sterile cotton swab into the properly diluted inoculum and rotate it firmly several times against the upper inside wall of the tube to express excess fluid.
6. Inoculate the entire agar surface of the plate three times, rotating the plate 60° between streaking to obtain even inoculation. Swab the rim of the agar bed too.
7. The lid may be left ajar for 3-5 minutes and the plate held at room temperature for not more than 15 minutes to allow the surface moisture to be absorbed before applying the antibiotic discs.
8. Apply discs by means of an antimicrobial disc dispenser, aseptically, at least 24 mm apart. Preferably, deposit Penicillin and Cephalosporin discs not more than 10 mm from the edge of the Petri dish, and their centers at least 30 mm apart. Avoid placing such discs adjacent to one another. Tap the discs with some sterile needle or forceps after placing them on the agar for complete contact with the medium surface.
9. Within 15 minutes of applying the discs, invert the plates and incubate at 35°C-37°C. With non-fastidious organisms the plates should not be incubated under an increased concentration of carbon dioxide.
   Note: For *Staphylococcus* spp., testing at temperatures above 35°C may not detect Methicillin-resistant Staphylococci (MRS); for *N. gonorrhoeae*, incubate at 35°C-37°C [do not exceed 37°C]. *Haemophilus* spp., *N. gonorrhoeae*, *S. pneumoniae* and other Streptococci should be incubated in an atmosphere enriched with 5% CO_2.
10. Examine plates after 18-24 hours of incubation. 20-24 hours for *N. gonorrhoeae, S. pneumoniae* and other Streptococci). A full 24 hours of incubation is recommended to detect methicillin/ oxacillin/ vancomycin-resistant Staphylococci in *Staphylococcus* spp., cefoxitin in *Staphylococcus* spp. (except from *S. aureus, S. lugdunensis, S. pseudintermedius* and *S. schleiferi*), and *Enterococcus* spp. in vancomycin resistance. The diameters of the zones of complete inhibition are measured, as determined by gross visual inspection.

Zones are measured to the nearest whole millimeter. For further details in measuring zones of inhibition, consult the reference. If only isolated colonies grow, the inoculum is too light and the test should be repeated. Zones around discs containing different drugs are not comparable for the purpose of comparing activity of drugs. See the Zone Diameter Interpretive Chart, which gives expected values from testing common aerobes.

Interpretation

Antimicrobial Agent	Interpretative criteria for	Resistant (mm / ≤)	Intermediate (mm)	Sensitive (mm / ≥)	SDD*
Cefepime 30 mcg	*Enterobacterales*	18	-	25	19-24
	Acinetobacter spp.	14	15-17	18	-
	Pseudomonas aeruginosa	14	15-17	18	-
	Haemophilus influenzae & *Haemophilus parainfluenzae*	-	-	26	-
	Neisseria gonorrhoeae	-	-	31	-
	Streptococcus spp. β-hemolytic Group	-	-	24	-
	Streptococcus spp. Viridans Group	21	22-23	24	-

***SDD: Susceptible-Dose Dependent**

Quality Control

Appearance: Filter paper disc of 6 mm diameter with printed ''CPM 30 mcg'' on each side of the disc.
Cultural response: Average diameter of zone of inhibition observed on Mueller Hinton Agar for 18-24 hours incubation at 35°C-37°C for standard cultures.

Organism (ATCC)	Standard Diameter of zone of inhibition in mm
Escherichia coli (25922)	31-37
Staphylococcus aureus subsp. aureus (25923)	23-29
Pseudomonas aeruginosa Strain Boston 41501 (27853)	25-31
Haemophilus influenzae (49247)	25-31
Streptococcus pneumoniae (49619)	28-35
Neisseria gonorrhoeae (49226)	37-46
Escherichia coli (35218)	31-27

Storage and Shelf-life:

Discs in routine use should be stored at 2°C-8°C. Longer term storage should be at -20°C-8°C.

References:

1. Bauer, Kirby, Sherris and Turck, 1966, Am. J. Clin. Path., 45: 493
2. Performance standards of Antimicrobial Disc Susceptibility Tests, CLSI Vol. 34, Feb 2024.
3. Data on file: Microxpress®, A Division of Tulip Diagnostics (P) Ltd.

Product Presentation:

Cat No.	Product description	Pack Size
206030460250	Antimicrobial Susceptibility Disc	5 Carts (5 x 50 disc)
206030460500	Antimicrobial Susceptibility Disc	5 Vials (5 x 100 disc)
206030460100	Antimicrobial Susceptibility Disc	Single Vial (1 x 100 Disc)