TBD 016 – CEPHALOTHIN

INTENDED USE

For antimicrobial susceptibility testing of bacterial cultures by disk diffusion method of Bauer-Kirby.

COMPOSITION

Ingredients	Concentration(s)
Cephalothin	30 mcg /disc

APPEARANCE

Filter Paper disc of 6.0 mm diameter with printed "CEP 30" on centre of both side of each disc.

PRINCIPLE

Disc diffusion test is a qualitative test method. This method is based on the principle that antibiotic-impregnated disk, placed on agar previously inoculated with the test bacterium, picks up moisture and the antibiotic diffuse radially outward through the agar medium producing an antibiotic concentration gradient. The concentration of the antibiotic at the edge of the disk is high and gradually diminishes as the distance from the disk increases to a point where it is no longer inhibitory for the organism, which then grows freely. A clear zone or ring is formed around an antibiotic disk after incubation if the agent inhibits bacterial growth. The disk diffusion method is performed using Mueller-Hinton Agar (MHA), which is the best medium for routine susceptibility tests because it has good reproducibility, low in sulfonamide, trimethoprim, and tetracycline inhibitors, and gives satisfactory growth of most bacterial pathogens.

The purpose of the Antimicrobial susceptibility testing (AST) by Kirby-Bauer disk diffusion susceptibility test is to determine the sensitivity or resistance of pathogenic microbes to various antimicrobial compounds in order to assist the clinical laboratories in selecting treatment options for patients. The findings of these tests are the basis of selection of the most appropriate antimicrobial agent for treatment against the infection.

Till the 1950s, clinical laboratories were lacking in the methodologies for the accurate determination of in vitro responses of organisms to antimicrobial agents. Kirby and co-workers, extensively reviewed the susceptibility testing literature. They consolidated and updated all the previous descriptions of the disk diffusion method in 1960s. 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).

The Clinical Laboratory Standards Institute (CLSI) and The European Committee on Antimicrobial Susceptibility Testing (EUCAST) have published comprehensive documents regarding the disc diffusion systems. Now-a-days, the agar disc diffusion test is a convenient and widely used method for routine antimicrobial susceptibility testing.

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

INSTRUCTION FOR USE

- Prepare sterile media plates of MUELLER HINTON AGAR (TM 339/TM 236) for rapidly growing aerobic organisms as per user's instruction. The agar depth should be 4.0 ± 0.5 mm.
- Inoculate the plates using inoculum of turbidity comparable to that of standard 0.5 McFarland by lawn technique using a sterile cotton swab. Alternatively, the inoculum can be standardized by other appropriate optical method (0.08 - 0.13 OD turbid suspension at 625 nm).
- Allow the inoculum to dry for 5 - 15 minutes with lid in place.

- Apply the antibiotic disc(s) aseptically, using sterile applicator or forceps.
- Place the antimicrobial discs with centers at least 24 mm apart. For fastidious organisms and for Penicillin and Cephalosporins, the discs should preferably be placed with centers 30 mm apart.
- Incubate immediately at 35 ±2°C and examine after 16-20 hours or longer, if necessary. For fastidious organisms incubate at appropriate temperature and time.
- Measure the zone of inhibition and record the diameters of the zones to the nearest millimeter using a calibrated instrument.

INTERPRETATION (AS PER CLSI STANDARDS)

Antimicrobial Agent	Interpretative criteria for	Sensitive (mm or more)	Intermediate (mm)	Resistant (mm or less)
Cephalothin 30 mcg/disc	Enterobacteriaceae & *Staphylococcus* spp.	18	15-17	14

MICROBIOLOGICAL PARAMETERS (AS PER CLSI STANDARDS)

Cultural characteristics observed after inoculating the plates using fresh inoculum of turbidity comparable to that of standard 0.5 McFarland (1-2 x 10^8 CFU/ml) by lawn technique, dispensing antibiotic discs and incubation at 35 ± 2°C for 16 - 20 hours. After incubation, inhibition zone diameter measured in mm.

Microorganism	ATCC	Standard zone of inhibition (diameter in mm)
Escherichia coli	25922	15-21
Staphylococcus aureus	25923	29-37
Pseudomonas aeruginosa	27853	Resistant

PRECAUTIONS

- The agar depth should be 4.0 ± 0.5 mm.
- The inoculum suspension should optimally be used within 15 and always within 60 min of preparation to ensure the correct number of viable cells.
- During inoculation, prohibit the use of flooding the plates.
- Antibiotic disks should be applied within 15 min of inoculation and start incubation within another 15 minutes.
- Do not to exceed the incubation period of 16–20 h, because prolonged incubation often results in indistinct zone edges or colonies within the inhibition zones, which might produce false results.
- A maximum of six disks can be accommodated on a 90-mm circular plate and 12 on a 150-mm circular plate.

PACKAGING

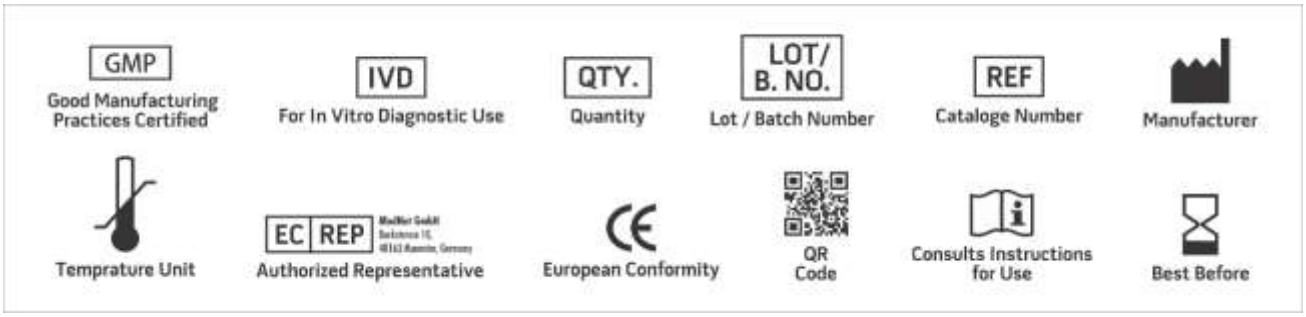
Disc available in three different packaging: Blister pack, Plastic Container and Vial.

STORAGE

Discs should be stored at - 20°C to +8°C under dry conditions, along with the desiccator pouch provided in each individual pack.

REFERENCES

1.Bauer, A.L., Kirby, W.M.M., Sherris, J.C., Turck, M. 1966. Am. J. Clin. Pathol. 45: 493-496.
2. Performance standards of Antimicrobial Disc Susceptibility Tests, CLSI Vol. 32 No.3, Jan 2012



NOTE: Please consult the Material Safety Data Sheet for information regarding hazards and safe handling Practices.

***For Lab Use Only**

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