Technical Information

Cefoxitin-Cloxacillin CXX 30-200 mcg

Product Code: SD1285

Application: Cefoxitin-Cloxacillin (30-200) mcg is used for screening of AmpC β-lactamases producers.

Composition**

*Antibiotic	Concentration
Cefoxitin	30 mcg
+	
Cloxacillin	200 mcg/disc

Susceptibility Test Procedure:

1. Prepare plates with Mueller Hinton Agar (DM1173/DM2084) 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 (DM1011) and incubate at 35-37°C for2-8 hours until light to moderate turbidity develops. Compare the inoculum turbidity with that of standard 0.5 McFarland (prepared by mixing 0.5 ml of 1.175% barium chloride and 99.5 ml of 0.36N sulfuric acid). Dilute the inoculum or incubate further as necessary to attain comparative turbidity. Alternatively, the inoculum can be standardized by other appropriate optical method (0.08 - 0.13 OD turbid suspension at 625 nm)
3. Dip a sterile non-toxic cotton swab on a wooden applicator into the standardized inoculum and rotate the soaked swab firmly against the upper inside wall of the tube to express excess fluid. Streak the entire agar surface of the plate with the swab three times, turning the plate at 60° angle between each streaking. Allow the inoculum to dry for 5 - 15 minutes with lid in place.
4. Apply the discs using aseptic technique. When using cartridges, the discs can be applied using the specially designed applicator. When the vials are used, apply the discs using sterile forceps.
5. Deposit the discs with centers at least 24 mm apart. For fastidious organisms and for Penicillins and Cephalosporins, the discs should preferably be deposited with centers 30 mm apart.
6. Incubate immediately at 35 ± 2°C and examine after 16-18 hours or longer, if necessary. For fastidious organisms incubate at appropriate temperature and time.
7.Measure the zones showing complete inhibition and record the diameters of the zones to the nearest millimeter using a calibrated instrument like zone scales (PW1096/PW1297)
8. For detection of AmpC test with SD041 (Cefoxitin 30 mcg disc).

Principle

The prevalence of multi drug resistant Gram-negative bacteria has become an issue of concern in past few years. Techniques were developed for detection of ESBL producing strains. However, an ESBL producer may harbour AmpC or Metallo-beta-lactamase or both. AmpC type β-lactamases are commonly isolated from extended-spectrum cephalosporin-resistant Gram-negative bacteria. AmpC β-lactamases (also termed class C or group 1) are typically encoded on the chromosome of many Gram-negative bacteria including *Citrobacter, Serratia* and *Enterobacter* species where its expression is usually inducible; it may also occur on *Escherichia coli* but is not usually inducible, although it can be hyper expressed. AmpC type βlactamases may also be carried on plasmids (1). AmpC β-lactamases, in contrast to ESBLs, hydrolyze broad and extended-spectrum cephalosporins (cephamycins as well as to oxyimino-β-lactams) but are not inhibited by β-lactamase inhibitors such as Clavulanic acid. Currently, no guidelines are available for detection of AmpC β-lactamases, and that some ESBL & AmpC producers will give false negative results with current CLSI methods. For detection of AmpC few inhibitors like Boronic Acid, Cloxacillin etc. are employed (2,3,4). The disc of 30 mcg Cefoxitin containing Cloxacillin 200 mcg are employed by few to detect the presence AmpC (3)

Interpretation

A zone diameter difference of ≥ 4 mm between Cefoxitin 30 mcg discs & Cefoxitin-Cloxacillin 30-200 mcg discs should be interpreted as AmpC positive.

Quality Control

Appearance: Filter paper discs of 6mm diameter with printed "CXX 30-200" on centre of each side of the disc.

Cultural response: Average diameter of zone of inhibition observed on Mueller Hinton Agar (DM1173) after 18 hours incubation at 35-37°C for standard cultures.

Organisms (ATCC)	Std. zone of diameter (mm)*
E.coli (25922)	26-34
S.aureus (25923)	36-50
AmpC positive isolate	≥ 4mm increase in diameter as compared to zone obtained with SD1041 (Cefoxitin 30 mcg)

Further Reading

1. Philippon A, Arlet B, Jacoby GA.2002. "Plasmid-determined AmpC-type β-lactamases" Antimicrob Agents Chemother.. 2002; 46
2. Beesley, T., et.al 1983. The inhibition of class C β-lactamases by boronic acids, Biochem. J. 209:229-233.
3. Jacoby, G.A. 2009. Amp C β-lactamases. Clin. Microbiol. Rev. 22:161-182.
4. Tan, T.Y., et al. 2009. Evaluation of screening methods to detect plasmid-mediated AmpC in Escherichia coli, Klebsiella pneumoniae, and Proteus mirabilis. Antimicrob. Agents Chemother. 53:146-149.
5. Bauer, Kirby, Sherris and Turck, 1966, Am. J. Clin. Path., 45: 493.

Note:

1. Use following media to carry out susceptibility test
2. For rapidly growing aerobic organisms : Mueller Hinton Agar (DM1173/DM2084)
3. for *Haemophilus* spps : Haemophilus Test Agar (DM2259 + MS2117)
4. For *S.pneumoniae* : Muller Hinton Agar supplemented with 5% Sheep Blood
5. For *Neisseria spps* : G.C.Agar +1% defined growth supplement (DM1434 + MS2025)

*** Not for Medicinal Use**

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.

Disclaimer

- User must ensure suitability of the product(s) in their application prior to use.
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