

## Imipenem –EDTA IE 10-750 mcg

SD282

Imipenem–EDTA (10-750 mcg) is used for screening of Metallo-β-lactamases producers.

### Composition

*Ingredients	Concentration
Imipenem	10mcg/disc
+	
EDTA	750 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 ± 2°C and examine after 16-18 hours or longer, if necessary. For fastidious organisms incubate at appropriate temperature and time.
7. Measure the zones showing complete inhibition and record the diameters of the zones to the nearest millimeter using a calibrated instrument like zone scales (PW096/PW297)

### Principle and Interpretation:

The introduction of carbapenems into clinical practice represented a great advance for the treatment of serious bacterial infections caused by beta-lactam resistant bacteria. Due to their broad spectrum of activity and stability to hydrolysis by most beta-lactamases, the carbapenems have been the drug of choice for treatment of infections caused by penicillin-or cephalosporin-resistant Gram-negative bacilli especially, extended spectrum β-lactamase (ESBL) producing Gram-negative infections. However, Carbapenem resistance has been observed frequently in non fermenting bacilli *Pseudomonas aeruginosa* and *Acinetobacter* spp. Resistance to carbapenems is due to carbapenem hydrolyzing enzymes-carbapenemase among the others. These carbapenemase are class B metallo β –lactamases. Metallo beta lactamase (MBL) belongs to a group β -lactamase which requires divalent cations of zinc as co-factors for enzyme activity. These have potent hydrolyzing activity not only against carbapenem but also against other β -lactam antibiotics. The genes responsible for MBL production are horizontally transferable via plasmids and can rapidly spread to other bacteria. The genes responsible for MBL production may be chromosomally or plasmid mediated and hence pose a threat of spread of resistance by gene transfer among the Gram-negative bacteria. Thus, MBL-producing *Pseudomonas aeruginosa* isolates have been reported to be important causes of nosocomial infections . The appearance of MBL genes and their spread among bacterial pathogens is a matter of concern with regard to the future of antimicrobial chemotherapy.

Various methods have been recommended for screening MBL. These include the modified Hodge test, double disc synergy test using Imipenem and EDTA discs or ceftazidime and EDTA discs, EDTA impregnated Imipenem discs.

### Interpretation

A zone diameter difference of  $\geq 7$  mm between Imipenem discs & Imipenem plus EDTA discs should be interpreted as Metallo- $\beta$ -Lactamase positive.

### Quality Control:

**Appearance:** Filter paper discs of 6mm diameter with printed "IE 10/750" 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)
<i>E. coli</i> (25922)	25-31
<i>P. aeruginosa</i> (27853)	19-27

### 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. Prospective evaluation of Imipenem/EDTA combined disc & E-test for detection of Metallo- $\beta$ -Lactamase producing *P.aeruginosa*; L.Berges *et.al.* Journal of Antimicrobial Chemotherapy; February 2007
2. Metallo- $\beta$ -lactamase-producing clinical isolates of *Acinetobacter* species and *Pseudomonas aeruginosa* from intensive care unit patients of a tertiary care hospital; S Irfan *et.al.*; Indian J Med Microbiol 2008;26:243-245
3. Imipenem-EDTA disk method for differentiation of metallo-beta-lactamase-producing clinical isolates of *Pseudomonas* spp. and *Acinetobacter* spp.; Yong D, *et.al.* J Clin Microbiol 2002; 40:3798-801.
4. Performance standards of Antimicrobial Disc Susceptibility Tests, CLSI Vol. 32 No.3, Jan 2012.
5. Bauer, Kirby, Sherris and Turck, 1966, Am. J. Clin. Path., 45: 493

\* Not for Medicinal Use

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