ROSCO CE

## Insert for Kit 98016

## Total Metallo-β-Lactamase Confirm Kit

**REVISION:** DBV0035P

**DATE OF ISSUE:** 10.02.2017

**LANGUAGE:** English

FOR IN VITRO DIAGNOSTIC USE ONLY

**PRODUCT GROUP:** Kits for beta-lactamase identification

**MANUFACTURE:** ROSCO, Taastrupgaardsvej 30, DK-2630 Taastrup, Denmark.

**INTENDED USE:** Tablets are used for qualitative *in vitro* identification of microbial resistance mechanisms by the agar tablet/disc diffusion method, in order to confirm the mechanism by which the organism has gained resistance to specific antimicrobial agents.

**INTENDED USERS:** To be used only by professionals, qualified laboratory personnel and people trained to work with microbes and disc diffusion testing.

**TEST PRINCIPLE:** Five cartridges of diffusion disc/tablets containing Meropenem 10 µg, Dipicolinic acid (DPA), Imipenem 10 µg, Imipenem + DPA and Imipenem + EDTA.
Enterobacteriaceae, Pseudomonas aeruginosa and Acinetobacter spp. producing Metallo-β-lactamases (MBL), hydrolyses carbapenems efficiently. MBL's are inhibited by Dipicolinic acid (DPA). DPA has no intrinsic antimicrobial activity, as opposed to Ethylene diamine tetra-acetic acid (EDTA), and the results are easily interpreted. The use of two carbapenems in combination with DPA permits the identification of any MBL in any species. Synergy between DPA and Imipenem 10 µg or Meropenem 10 µg is particularly useful, when the isolates show no zone around Imipenem 10 µg and / or Meropenem 10 µg.

**DETAILED INSTRUCTIONS:** ROSCO's detailed *Instruction for Use for Detection of resistance mechanisms* should be available in laboratories working with ROSCO's Diagnostic products.
Latest version of Instruction for Use can be seen in and/or printed out from ROSCO's website www.rosco.dk
*User's Guide* can be obtained free of charge from your local distributor on request, or from ROSCO:
E-mail: info@rosco.dk
Phone: +45 43 99 33 77

**CONTENT AND FORMULATION:** 5 cartridges of tablets, formulated for maximum stability, each containing approximately 50 tablets:

- Meropenem 10 µg, coded MRP10
- Dipicolinic acid, coded DPA
- Imipenem 10 µg, coded IMI10
- Imipenem 10 µg + DPA, coded IM+DP
- Imipenem 10 µg + EDTA, coded IM10E

**STORAGE/HANDLING:**

Store at 2-8 °C until the expiration date shown on the product label. Cartridges should be closed during storage. Always seal the cartridges with the original green lid and never place the dispenser in the refrigerator.

Allow the cartridges to acclimatize at room temperature (30-60 min) before removing the lid. Cartridges may open and close several times during use, without affecting tablets' shelf-life. The long shelf-life is due to the use of crystalline substances.

**PRECAUTIONS:**

For *in vitro* diagnostic use only. Safety precautions should be taken and aseptic techniques should be used when working with potential biohazards. To be used only by adequately trained and qualified laboratory personnel. Sterilize all biohazard waste before disposal. Refer to Product Safety Data Sheet.

**REQUIRED BUT NOT PROVIDED MATERIALS:**

Standard microbial equipment such as loops, culture media, incubator etc. and biochemical reagents.

**PROCEDURE:**

Using a fresh, pure culture prepare a suspension of the organism to be tested equivalent to McFarland 0.5.
Using a sterile swap or Drigalski spatula spread the suspension uniformly over the entire area of a Mueller-Hinton agar plate. Iso-sensitest Agar must not be used (false negative). Using a single tablet dispenser, place one of each tablet on the inoculated agar plate, ensuring sufficient space between individual tablets to allow for proper measurement of inhibition zones. Please notice that DPA Diatabs are placed between Imipenem 10 µg and Meropenem 10 µg at a distance of approx. 5 mm (edge to edge) if the isolates show no inhibition zones around IMP10 and / or MRP10.
If the zones around IMP10 and / or MRP10 are > 15 mm, the DPA is placed at approx. 10 mm (edge to edge).
Incubate at 35±1°C for 18±2 hours (overnight).
Measure and record the diameter of the inhibition zones. No zone around a tablet corresponds to a 9 mm inhibition zone. Record synergism or not between DPA and Imipenem 10 µg and Meropenem 10 µg.

**INTERPRETATION OF RESULTS:**

The results are interpreted by comparing the inhibition zones of the different tablets **Test only Ceftazidime resistant isolates.**

Compare the zone of inhibition around Imipenem 10 µg to that of Imipenem + DPA and Imipenem +EDTA. If all zones are within 3 mm of each other, the organism is not expressing MBL activity.
Look for synergism between DPA and Imipenem 10 µg and Meropenem 10 µg.

**For Enterobacteriaceae:** Compare the zones around Imipenem and Imipenem + DPA. If a difference in zone diameter of ≥ 5 mm (IM+DP-IMI10) is observed, report the isolate as expressing MBL activity. Synergism between DPA and Imipenem 10 µg or Meropenem 10 µg indicates that the isolate is MBL positive.

**For Pseudomonas, aeruginosa/Acinetobacter spp.:** Compare the zones around Imipenem and Imipenem + DPA. If a difference in zone diameter of ≥ 5 mm (IM+DP-IMI10) is observed, report the isolate as expressing MBL activity. MBL detection with the EDTA combination in Pseudomonas aeruginosa and Acinetobacter spp. If a difference in zone diameter is ≥ 8 mm, the isolate is MBL positive.
Do not test Meropenem with non-fermenters.

**For Acinetobacter and oxacillinases:**
Oxacillinases are influenced by EDTA resulting in a weak synergism between Imipenem and EDTA, while DPA has no effect. This can be used to detect oxacillinases in Acinetobacter (see table 3).

**QUALITY CONTROL:**

Use table 1, 2 and 3 to assist in the interpretation (see below).
Although ROSCO produces the most stable diffusion discs (tablets) it is necessary to perform regular quality control. This should be done with at least one organism to demonstrate a positive reaction and at least one organism to demonstrate a negative reaction. Zones of inhibition obtained using the combination tablets plus the carbapenem alone tablet against the negative control (i.e. E. coli ATCC 25922), should be within 3 mm.

*As positive Q. C. stains the following may be used:*

*Klebs. pneumoniae NCTC 13438, MBL positive*
*Klebs. pneumoniae ATCC BAA-2146, MBL positive*

**Table 1: Enterobacteriaceae**

| | | DPA | Imipenem + DPA IM+DP | Imipenem + EDTA IM10E |
|---|---|---|---|---|
| MBL | Meropenem 10 µg MRP10 | Synergism | - | - |
| MBL | Imipenem 10 µg IMI10 | Synergism | ≥ 5 mm | ≥ 8 mm |

**Table 2: Pseudomanas aeruginosa. Acinetobacter spp.**

| | | DPA | Imipenem + DPA IM+DP | Imipenem + EDTA IM10E |
|---|---|---|---|---|
| MBL | Imipenem 10 µg IMI10 | Synergism | ≥ 5 mm **or** | ≥ 8 mm |
| MBL | No zone | | Zone >= 12 mm | |

**Table 3: Acinetobacter and Oxacillinases**

| | | Imipenem + DPA IM+DP | Imipenem + EDTA IM10E |
|---|---|---|---|
| Oxacillinases | Imipenem 10 µg IMI10 | <= 3 mm **and** | 4 - 7 mm |

Non MβL: All zones within 3 mm of each other.

**REFERENCES:**

Dongeun Yong et al: Evaluation of double disk potentiation and disk potentiation tests using Dipicolinic acid for detection of MBL – producing Pseudomonas spp and Acinetobacter spp. J.Clin. Microbiol. 50, 3227-3232, 2012.