Insert for KPC/MBL in P. aeruginosa/Acinetobacter Confirm Kit (98020)
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KPC/MBL in P. aeruginosa/Acinetobacter Confirm Kit
FOR IN VITRO DIAGNOSTIC USE ONLY

PRODUCT GROUP: Kits for beta-lactamase identification

MANUFACTURER: ROSCO Diagnostica A/S, 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. The kit is intended for detection of the carbapenemases; KPC and MBL in Pseudomonas aeruginosa and MBL in Acinetobacter spp..

INTENDED USERS: Only to be used by professionals and people trained to work with microbes and disc diffusion testing.

PRINCIPLE OF THE TEST:

Five cartridges of tablets containing Meropenem 10 µg and Imipenem 10 µg (diffusible amount) alone and in combination with inhibitors of different β-lactamases.
Inhibitors are added to differentiate between isolates with and without resistance mechanisms (see explanation below).
Reduced susceptibility to Carbapenems is observed when:

1. The organism produces a Metallo β-lactamase that hydrolyses carbapenems efficiently. MBLs are inhibited by Dipicolinic Acid (DPA) and a difference in zone size between Imipenem and Imipenem + DPA indicates the presence of a MBL.
2. Synergy between DPA and Imipenem 10 µg and / or Meropenem 10 µg is particularly useful, when the isolates show no zone around Imipenem 10 µg and / or Meropenem 10 µg.
3. The organism produces a KPC enzyme. KPC enzymes are inhibited by Phenylboronic Acid. However, Phenylboronic Acid also inhibits the AmpC and in order to raise the specificity of the Kit, the Cloxacillin High combination is included to distinguish between the two. Thus, a zone difference with Meropenem + Phenylboronic Acid but no difference with the Meropenem + Cloxacillin High indicates the presence of a KPC enzyme.
4. The synergistic performance of DPA is improved by the use of **Mc Conkey agar,** instead of Mueller – Hinton. Therefore, use **Mc Conkey agar.**

DETAILED INSTRUCTIONS: ROSCO's detailed Instruction for Use for *Detection of resistance mechanisms* should be available in laboratories working with ROSCO's *Diagnostic products.*
Lastest edition of Instruction for Use can be seen in and/or printed out from ROSCO's website www.rosco.dk.
Instructions for Use and User's Guide can be obtained free of charge from your local distributor on request, or from ROSCO Diagnostica A/S:
E-mail: info@rosco.dk or
Fax: +45 43 52 73 74

CONTENT AND FORMULATION:

5 cartridges of tablets, formulated for maximum stability, each containing approximately 50 tablets:

1. Meropenem 10 µg, coded MRP10
2. Meropenem 10 µg + Phenylboronic Acid (KPC and AmpC inhibitor), coded MRPBO
3. Meropenem 10 µg + Cloxacillin High (AmpC inhibitor), coded MRCXH
4. Imipenem 10 µg, coded IMI10
5. Dipicolinic acid (DPA)

STORAGE/HANDLING: Store at 2-8 °C in the box provided or unopened cartridges until the expiry date shown on the product label. Allow the cartridges to acclimatize to room temperature for 30-60 minutes before the lid is removed from the cartridge. Once a cartridge has been opened and in particular when placed in a dispenser, it should be kept at room temperature for up to 2 months. If necessary, when in use for a longer period of time than 2 months, the cartridges can be stored at 2-8 °C. Always seal the cartridges with the original green lid, and never place the dispenser in the refrigerator. When stored at 2-8 °C the cartridges should be allowed to acclimatize, as described above, before use.

PRECAUTIONS:

For *in vitro* diagnostic use only. Safety precautions should be taken and aseptic techniques 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.

MATERIALS REQUIRED BUT NOT PROVIDED:

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

PROCEDURE:

1. Using a fresh, pure culture prepare a suspension of the organism to be tested equivalent to McFarland 0.5
2. Using a sterile swap or Drigalski spatula spread the suspension uniformly over the entire area of a **Mc Conkey agar** plate. Iso-sensitest Agar must not be used (false negative)
3. 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.
4. 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) when the isolate shows no zone around Imipenem 10 µg and Meropenem 10 µg. If the zones around Imipenem 10 µg and / or Meropenem 10 µg are > 15 mm, the DPA Diatabs is placed at approx. 10 mm (edge to edge).
5. Incubate at 35±1°C for 18±2 hours (overnight).
6. Measure and record the diameter of the inhibition zones. No zone around a tablet corresponds to a 9 mm inhibition zone.
7. Record synergism or not between DPA and Imipenem 10 µg and / or Meropenem 10 µg.

INTERPRETATION OF RESULTS:

The results are interpreted by comparing the inhibition zones of the different tablets

1. Compare the zone of inhibition of the Meropenem 10 µg tablet to the zones of inhibition of each of the Meropenem + inhibitor tablets, the same applies to Imipenem 10 µg. If all zones are within 2 mm of each other, record the organism as neither expressing KPC nor MBL activity.

2. Measure the inhibition zone around Meropenem 10 µg (MRP10) and compare with the zones around Meropenem + Phenylboronic Acid (MRPBO) *and* Meropenem + Cloxacillin High (MPCXH):
   If the zone around MRPBO is ≥ 4mm and the zone around MRCXH is < 3mm in comparison to the single disc, the organism demonstrates KPC activity.
   And/or
   Compare the zones around the two combination discs MRPBO and MRCXH:
   If the zone around MRPBO disc is ≥ 4 mm than the zone around the MRCXH disc then the isolate is KPC positive.

3. Measure the inhibition zone around Imipenem 10 µg (IMI10) and Imipenem + DPA (IM+DP):
   If the zone around IM+DP is ≥ 5mm in comparison to the single disc, the organism is positive for Metallo-β-Lactamase activity.
   Test only Ceftazidime resistant isolates. False MBL positive may be obtained with Ceftazidime sensitive isolates.

4. See if there is synergism between DPA and Imipenem 10 µg and / or Meropenem 10 µg.

5. Use table 1 and 2 to assist in the interpretation.

QUALITY CONTROL:

Although ROSCO Diagnostica A/S produces, by far, 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 tablet alone against the negative control (i.e. *E. coli* ATCC 25922), should be within 3 mm.

As positive Q. C. strains the following may be used:
P. aeruginosa ATTC 10145/CCUG 59626, MBL positive
K. pneumonia CCUG 58547, MBL positive
K. pneumoniae NCTC 13439, MBL positive
K. pneumonia CCUG 56233, KPC positive
K. pneumoniae NCTC 13438, KPC positive

Table 1:

	Mc Conkey agar		Meropenem + Phenylboronic MRPBO	Meropenem + Cloxacillin High MRCXH	DPA
KPC	P. aeruginosa	Meropenem 10 µg MRP10	≥ 4mm **and**	< 3 mm	Synergism
		Meropenem + Cloxacillin High MRCXH	≥ 4 mm		
MβL	P. aeruginosa	Imipenem 10 µg IMI10	-	-	Synergism
	Acinetobacter		-	-	Synergism

Neither AmpC, KPC nor MβL: All zones within 2 mm of each other.

So far there is little or no experience in the detection of KPC's in Acinetobacter spp., consequently it is uncertain whether this kit will be able to detect it.

REFERENCES: www.rosco.dk

1. 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.