ConfirmID
Carbapenemase/Metallo-β-Lactamase Confirmative Identification Pack
FOR IN VITRO DIAGNOSTIC USE ONLY

PRODUCT GROUP: ROSCO Neo-Sensitabs™

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.

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

PRINCIPLE OF THE TEST:

Four cartridges of tablets containing 10 µg Meropenem (diffusible amount) but three cartridges in addition contain inhibitors of different β-lactamases. If an organism shows reduced susceptibility to Carbapenems there can be three likely reasons:

1. The organism hyper-produces AmpC. Because of the slow hydrolyses of carbapenems by the AmpC enzyme, the AmpC is probably coupled to other resistance mechanism like efflux pumps, porin loss or other β-lactamases. The AmpC enzyme is inhibited by Cloxacillin. The Cloxacillin is used to distinguish between AmpC and KPC since both are inhibited by Boronic Acid. Thus a difference (≥ 5mm) in zones between Meropenem and Meropenem + Cloxacillin indicates AmpC activity.

2. The organism produces a Metallo β-lactamase that hydrolyses carbapenems efficiently. MBLs are inhibited by Dipicolinic Acid and a difference in zone size (≥ 5mm) between Meropenem and Meropenem + DPA indicates the presence of a MBL. DPA has no (as opposed to EDTA) intrinsic antimicrobial activity and thus the results with this compound are more easily interpret.

3. The organism produces a KPC enzyme. KPC enzymes are inhibited by Boronic Acid. However, Boronic Acid also inhibits the AmpC and in order to raise the specificity of the Kit, the Cloxacillin combination is included to distinguish between the two. So a zone difference (≥ 5mm) with Meropenem + Boronic Acid but no difference (<3mm) with the Meropenem + Cloxacillin indicates the presence of a KPC enzyme.

DETAILED INSTRUCTIONS:

ROSCO’s detailed Instruction for Use for DIATABS should be available in each laboratory working with ROSCO’s DIATABS. Last edition of Instruction for Use can be seen in and/or printed out from ROSCO’s website [www.rosco.dk](http://www.rosco.dk). Here more detailed information can also be found in ROSCO’s User’s Guide for DIATAB in English.
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:
4 cartridges of tablets, formulated for maximum stability, each containing approximately 50 tablets:
1. Meropenem 10 µg, coded MRP10
2. Meropenem 10 µg + Boronic Acid (KPC and AmpC inhibitor), coded MR+BO
3. Meropenem 10 µg + Cloxacillin (AmpC inhibitor), coded MR+CL
4. Meropenem 10 µg + Dipicolinic acid (Metallo-β-Lactamase inhibitor), coded MR+DP

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.

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 Mueller Hinton susceptibility agar plate (i.e. ROSCO Square Plates).
3. Using a forceps or a 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. Notice that more than one ConfirmID Kit can be tested on the same plate.
4. Incubate at 35±1°C for 18±2 hours (overnight).
5. Measure and record the diameter of the inhibition zones. No zone around a tablet corresponds to a 9 mm inhibition zone.

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 10 µg + inhibitor tablets. If all zones are within 2mm 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 zone around Meropenem 10 µg + Cloxacillin (MR+CL). If the zone for the combination tablet is ≥ 5mm than the single disc and the values in step 3) and 4) ≤ 3mm, the organism is demonstrating AmpC activity alone. The AmpC is probably hyper-produced and/or coupled with porin loss and/or efflux pumps.
3. Measure the inhibition zone around Meropenem 10 µg (MRP10) and compare with the zone around Meropenem 10 µg + Boronic Acid (MR+BO). If the zone for the
combination tablet is ≥ 5mm than the single disc and the values in step 2) and 4) ≤ 3mm, the organism is demonstrating KPC activity alone.

4. Measure the inhibition zone around Meropenem 10 µg (MRP10) from Meropenem 10 µg + DPA (MR+DP). If MR+DP – MRP10 ≥ 5mm and the values in step 2) and 3) is ≤ 3mm, the organism is positive for Metallo-β-Lactamase activity only.

5. It is possible for an organism to be positive for more than one resistance mechanism. So if, for example, if the value in step 4) is ≥ 5mm and in step 3) is also ≥ 5 mm, then the organism is both positive for MBL and KPC activity, although in several cases the MBL may mask the KPC making it difficult to detect in the presence of an MBL. No combination of resistance mechanisms is impossible and more than one ConfirmID™ can be tested on the same plate.

6. Use table 1 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 alone tablet against the negative control (i.e. E. coli ATCC 25922), should be within 3 mm.

Table 1:

<table>
<thead>
<tr>
<th>AmpC + porin loss</th>
<th>Meropenem + Boronic MR+BO</th>
<th>≤ 3mm</th>
<th>≥ 5mm AND</th>
<th>≤ 3mm</th>
<th>≥ 5mm</th>
</tr>
</thead>
<tbody>
<tr>
<td>KPC</td>
<td>Meropenem 10 µg MRP10</td>
<td>≥ 5mm</td>
<td>≤ 3mm</td>
<td>≤ 3mm</td>
<td></td>
</tr>
<tr>
<td>MβL</td>
<td>Meropenem 10 µg MRP10</td>
<td>≤ 3mm</td>
<td>≥ 5mm</td>
<td>≤ 3mm</td>
<td></td>
</tr>
</tbody>
</table>

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

REFERENCES: www.rosco.dk