Insert for KPC/Metallo-B-Lactamase Confirmation kit
Insert for KPC/MBL and OXA-48 Confirmation kit

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KPC/Metallo-β-Lactamase Confirmation kit (98006)
KPC/Metallo-beta-lactamase and OXA-48 Confirmation kit (98015)
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.

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 hydrolysis of carbapenems by the AmpC enzyme, the AmpC is probably coupled to other resistance mechanisms 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 interpreted.

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 (≥ 4mm) with Meropenem + Boronic Acid but no difference (<4mm) with the Meropenem + Cloxacillin indicates the presence of a KPC enzyme.

4. The Enterobacteriaceae produce an oxacillinase (OXA-48 or similar). Negative results of all synergy tests and, Temocillin 30 ug: no zone of inhibition, is presumptive of an OXA-48 or similar. Besides, these isolates are highly resistant to Piperacillin + tazobactam. Please notice if both meropenem and all combinations show no zone of inhibition, the Temocillin test is invalid and the result inconclusive.

DETAILED INSTRUCTIONS: ROSCO’s detailed Instruction for Use for Detection of resistance mechanisms should be available in each laboratory working with ROSCO’s Diagnostic products.
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 DIATABS 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 MRPBO
3. Meropenem 10 µg + Cloxacillin (AmpC inhibitor), coded MRPCX
4. Meropenem 10 µg + Dipicolinic acid (Metallo-β-Lactamase inhibitor), coded MRPDP.
5. Temocillin 30 µg (only in the OXA-48 Confirm kit)

**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. Never place the dispenser in the refrigerator.

**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.
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. Notice that more than one Confirm 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 3mm 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 (MRPCX). If the zone for the combination tablet is ≥ 5mm than the single disc and the zone around Meropenem + Boronic is >= 4mm, 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 (MRPBO) and Meropenem 10 + Cloxacillin (MRPCX). If the zone for the MRPBO is ≥ 4mm and the zone around MRPCX is < 5mm than the single disc and the values in step 2) and 4) < 5mm, the organism is demonstrating KPC activity alone.

4. Measure the inhibition zone around Meropenem 10 µg (MRP10) from Meropenem 10 µg + DPA (MRPDP). If MRPDP – MRP10 ≥ 5mm and the values in step 2) and 3) is < 5mm, 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 ≥ 4 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 Confirm kit can be tested on the same plate.

6. Use table 1 to assist in the interpretation
Isolates processing both KPC and MBL in the same isolate have been described in Greece and Germany. Rosco Diagnostica has developed a triple combination tablet: Meropenem + Boronic + Dipicolinic that permits the identification of both enzymes (inhibition zones compared with Meropenem + DPA and Meropenem + Boronic, respectively)
The triple disk (68912) is available for detection of KPC+MBL in the same isolate.

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.

As positive Q. C. strains the following may be used:
Klebs. pneumoniae NCTC 13438, KPC positive
Klebs. pneumoniae NCTC 13439, MBL positive
Klebs. pneumoniae ATCC BAA-1705, KPC positive
Klebs. pneumoniae ATCC BAA-2146, MBL positive
<table>
<thead>
<tr>
<th></th>
<th>Meropenem + Boronic MR+BO</th>
<th>Meropenem + DPA MRDP</th>
<th>Meropenem + Cloxacillin MRPCX</th>
<th>Temocillin 30 µg</th>
</tr>
</thead>
<tbody>
<tr>
<td>AmpC + porin loss</td>
<td>≥ 4mm and ≤ 3mm</td>
<td>≥ 5mm</td>
<td>≥ 12mm</td>
<td></td>
</tr>
<tr>
<td>KPC</td>
<td>≥ 4mm</td>
<td>≤ 3 mm</td>
<td>≤ 3 mm</td>
<td>≥ 12mm</td>
</tr>
<tr>
<td>MβL</td>
<td>&lt; 4mm</td>
<td>≥ 5mm</td>
<td>≤ 3 mm</td>
<td>≥ 12mm</td>
</tr>
<tr>
<td>OXA-48 and similars</td>
<td>≤ 3 mm</td>
<td>≤ 3 mm</td>
<td>≤ 3 mm</td>
<td>No zone of inhibition</td>
</tr>
</tbody>
</table>

Neither AmpC, KPC nor MβL: All zones within 3 mm of each other.
OXA-48 (K. pneumoniae) show negative results with KPC+MBL Confirm kit, but it is Temocillin resistant (no zone around Temocillin 30 ug Neo-Sensitabs).

REFERENCES: www.rosco.dk