**Insert for KPC/Metallo-B-Lactamase Confirm Kit 98006**
**Insert for Triple disk 98010 (old no. 68912)**
**Insert for KPC/MBL and OXA-48 Confirm Kit 98015 (EUCAST)**

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**KPC/Metallo-β-Lactamase Confirm Kit**
**KPC/Metallo-beta-lactamase and OXA-48 Confirm Kit**

FOR IN VITRO DIAGNOSTIC USE ONLY

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

**MANUFACTURER:** A/S ROSCO, Stensmosevej 24, DK-2620 Albertslund, 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, MBL and OXA-48 in **Enterobacteriaceae**, and MBL in **Bacteroides spp**(12).
Do not use with Pseudomonas or Acinetobacter (use kit 98025 instead).

**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) 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). In addition the KPC/MBL and OXA-48 Confirm Kit (98015) also contains one cartridge of 30 µg Temocillin tablets to detect OXA-48 or similar producing isolates.
If an organism shows reduced susceptibility to Carbapenems there can be four 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 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 Phenylboronic 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 Phenylboronic Acid. However, Phenylboronic 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 + Phenylboronic 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 no zone of inhibition with Temocillin 30 µg 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. The Temocillin test is only valid for Enterobacteriaceae.

**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 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:**

4 or 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 (AmpC inhibitor), coded MRPCX
4. Meropenem 10 µg + Dipicolinic acid (Metallo-β-Lactamase inhibitor), coded MRPDP.
5. Temocillin 30 ug (only in the OXA-48 Confirm kit 98015)

**STORAGE/HANDLING:**

**Store at 2-8 °C** 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. Cartridges may be opened and closed several times during use, without affecting the shelf-life of the tablets. Always seal the cartridges with the original green lid,before placing them in the refrigerator.When stored at 2-8 C the cartridges should be allowed to acclimatize before openenig.
**Shelf-life : of the product is at least 2 years from the date of manufacture,d**ue to the use of crystalline antimicrobials.On the opposite paper disk based products(amorphous),after opening of the cartridge have a shelf-life of **1 month** at 2-8 C

**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 + 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 to the zones around the two combination tablets; Meropenem + Cloxacillin (MRPCX) and Meropenem + Phenylboronic (MRPBO):If the zone around MRPCX is ≥ 5mm, and the zone around MRPBO is ≥ 4mm in comparison to the single disc respectively, 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 zones around Meropenem + Phenylboronic Acid (MRPBO) *and* Meropenem + Cloxacillin (MPRCX):If the zone around MRPBO is ≥ 4mm and the zone around MRPCX 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 MRPCX: If the zone around MRPBO disc is ≥ 4 mm than the zone around the MRPCX disc then the isolate is KPC positive.
4. Measure the inhibition zone around Meropenem 10 µg (MRP10) and Meropenem + DPA (MRPDP): If the zone around MRPDP 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.
5. Some isolates showing Meropenem MIC's ≤ 0.25 µg/ml (zone of inhibition on Meropenem 10 µg > 25 mm) may produce carbapenemases (metallo β lactamases VIM-1 or similar) that may be difficult to detect with the KPC, MBL Confirm kit. In order to detect this isolates, use Imipenem 10µg and Meropenem 10 µg and please a Dipicolinic acid (DPA) Diatabs in between, at a distance of 10 mm from tablet edge to tablet edge. Synergism (phantom zone) between DPA and Imipenem and/or Meropenem indicates a MBL.
6. Look at the zone around Temocillin 30 µg Neo-Sensitabs. If there is no zone of inhibition, the strain is presumptively OXA-48 (or similar) positive. In 80% of the cases OXA-48 is accompanied by a CTX-M, ESBL. It can be detected using CAZ/Clavulanate or Meropenem + Tazobactam.
7. 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 the value in step 3) is ≥ 4 mm, 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.MBL+ OXA-48 : will show synergism(ghost zone) between TEMO and Aztreonam 30 ug,placed approx. 10 mm from each other.
8. 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 + Phenylboronic + Dipicolinic (98010))that permits the identification of both enzymes (inhibition zones compared with Meropenem + DPA and Meropenem + Phenylboronic, respectively) The triple disk (98010) is available for detection of KPC+MBL in the same isolate (table 2).(14,15)

**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:*
*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*
*As negative Q.C strain the following may be used:*
*Klebs. pneumoniae ATCC 700603*

**Table 1: Enterobacteriaceae**

| | | Meropenem + Phenylboronic MRPBO | Meropenem + DPA MRPDP | Meropenem + Cloxacillin MRPCX | Temocilin 30 µg |
|---|---|---|---|---|---|
| AmpC + porin loss | Meropenem 10 µg MRP10 | ≥ 4mm and | ≤ 3 mm | ≥ 5mm | > 14mm |
| ESBL + porin loss (a) | Meropenem 10 µg MRP10 | ≤ 3 mm | ≤ 3 mm | ≤ 3 mm | > 14mm |
| KPC | Meropenem 10 µg MRP10 | ≥ 4mm | ≤ 3 mm | ≤ 3 mm | Variable |
| | Meropenem + Cloxacillin (MRPCX) | ≥ 4mm | - | - | - |
| MβL | Meropenem 10 µg MRP10 | < 4mm | ≥ 5mm | ≤ 3 mm | Variable |
| OXA-48 and similars | Meropenem 10 ug MRP10 | ≤ 3 mm | ≤ 3 mm | ≤ 3 mm | <=14 mm |
| OXA-48 + ESBL (a) | Meropenem 10 ug MRP10 | ≤ 3 mm | ≤ 3 mm | ≤ 3 mm | <=14 mm |

(a) : Synergism CAZ / Clavulanate.

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

**Table 2: Enterobacteriaceae**

| | | Triple Disk MER + DPA + BO | Temo 30 ug |
|---|---|---|---|
| KPC | MRPDP | ≥ 4 mm | |
| MBL | MRPBO | ≥ 4 mm | |
| KPC + MBL | MRPDP<br>MRPBO | ≥ 4 mm and<br>≥ 4 mm | |
| MBL + OXA-48 | MRPDP | <= 3mm | <= 14 mm |
| Syn TEMO/Aztreonam | MRPBO | >= 4 mm | <= 14 mm |

**Table 3 : Anaerobes ( B. fragilis)**

| | | MRPBO | MRPDP |
|---|---|---|---|
| **MBL** | Meropenem 10 ug MRP10 | < 4 mm | >= 5 mm |

Pantel et al (7) evaluated the performance of the 98015 kit for detection of carbapenemase-producing Enterobacteriaceae and found sensitivity of 98.8 % and specificity of 93.1 %. The Rosco test detected all class A and D carbapenemases including 5 OXA-48 with low imipenem MICs (0.25 – 0.38 ug/ml) as well as 16 MBL producers (NDM and VIM types). The assay is easy to implement (less than 30 min preparation) and at low cost. The interpretation is easy and co-expression of several carbapenemases can be detected.

Willey et al (8) compared Temocillin 30 ug Neo-Sensitabs with the corresponding MAST Disks for detecting Temocillin resistance as a marker for Class D carbapenemase. The authors concluded that the ROSCO Neo-Sensitabs had a sensitivity of 100 % and a specificity of 100 %, while the MAST discs (3 different lots) showed a sensitivity of 100 % but a specificity of 71-82 %. The lower specificity of the paper disks may be explained by the instability of temocillin in paper disks, where it is present in an **amorphous** form, while it is **crystalline** in the Neo-Sensitabs.

Dortet et al (9) evaluated the algorithm proposed by the SFM(CA) for the screening of carbapenemase producers in Enterobacteriaceae.With the use of Temocillin 30ug(TEMO), Ticarcillin + Clav 75+10 ug(TCC) and Imipenem 10 ug(IMI10)is this possible. Enterobacteriaceae strains showing zones >= 15 mm with TEMO and TCC and >= 22 mm with IMI10 **are non-carbapenemase** producers.

Hammerum et al (10) report for the first time in Denmark an outbreak of NDM-1 producing Citrobacter freundii and in vivo spread to other Enterobacteriaceae (using the 98015 kit).

Karatuna et al (11) compared Mast D7OC and Rosco 98015 kit for detection of carbapenemases in Enterobacteriaceae.For isolates carrying OXA-48 + MBL the Mast disks detected 75 % and the Rosco 98015 kit detected 100 % of the double enzymes.

Schensen et al (12) used the KPC/MBL Confirm kit (98006) for phenotypic detection of the cfiA metallo-beta.lactamase in Bacteroides fragilis.They conclude that the Rosco KPC/MBL Confirm kit detected all MBL-producing B. fragilis with a zone difference breakpoint of 4 mm.

Haldorsen et al (13) conclude that: the overall results support the Rosco KPC/MBL/OXA-48 kit as a reliable tool for identification of the major carbapenemase classes.

Hopkins et al(16) recommends the use of temocillin MIC >= 64 ug/ml (Temo 30 ug zone <= 14 mm) as breakpoint for detection of OXA-48-like Enterobacteriaceae.

**REFERENCES:**


1) Kemble S et al: Validation of Rosco Diagnostica Diffusion discs for identification of carbapenem resistance mechanisms in a clinical laboratory. Presentation 1394 IDWeek.Philadelphia,USA,2014
2) Giske CG et al: A sensitive and specific phenotypic assay for detection of MBL and KPC in K. pneumoniae with the use of meropenem disks supplemented with phenylboronic acid, dipicolinic acid and cloxacillin. Clin Microbiol Infect **17**, 552-556, 2011.
3) Ambrettii et al: Evaluation of phenotypic and genotypic approaches for the detection of Class A and Class B carbapenemases in Enterobacteriaceae. Microbial Drug Res **19**,212-215, 2013.
4) Miriagou V et al: Combined disk methods for the detection of KPC and or VIM positive K. pneumonia: improving reliability for the double carbapenemase producers. Clin Microbiol Infect E412-E415, 2013.
5) Giakkoupi P et al: Evaluation of the modified KPC+MBL Confirm kit for the phenotypic detection of Class A and B carbapenemases in K. pneumoniae isolates. Presented at the ECCMID 2012.
6) Pillai P et al: Triple disk assay for phenotypic detection of predominant carbapenemases. Indian J Med Research **138**, 1025-1026, 2013.
7) Pantel A. et al: Evaluation of two phenotypic screening tests for carbapenemase-producing Enterobacteriaceae. J Clin Microbiol **53**, 3359-3362, 2015.
8) Willey BM et al: Comparison of ROSCO tablets and MAST disks for detecting Temocillin resistance as a marker for Class D and B carbapenemase-producing organisms. ICAAC 2015, September 17-21, Presentation D-184.
9) Dortet L et al: Prospective evaluation of an algorithm for the phenotypic screening of carbapenemase-producing Enterobacteriaceae. J Antimicr Chemother oct 12, 2015 (ahead of print).
10) Hammerum AM et al : Use of WGS data for investigation of a long term NDM-1 producing Citrobacter freundii outbreak and secondary in vivo spread of ndm-1 gene to E. coli,K. pneumoniae and K. oxytoca.J. Antimicr Chemother **71**,3117-3124,2016.
11) Karatuna O et al : Evaluation of the performances of Mast and Rosco phenotypic carbapenemase detection kits for E. coli and K. pneumoniae isolates carrying carbapenemase genes.Poster EV0433,ECCMID 2016.
12) Schwensen SA et al : Phenotypic detection of the cfiA MBL in Bacteroides fragilis with the meropenem-EDTA double-ended Etest and the Rosco KPC/MBL Confirm kit.J Antimicr Chemother **72**,437-440,2017.
13) Haldorsen BC et al : Performance of the Rosco KPC/MBL/OXA-48 kit in phenotypic confirmation of carbapenemases in Enterobacteriaceae : a Nordic multi-laboratory study. 29th ECCMID,April 13-16,2019.Presentation P1276.
14) Bou Casals J et al : Phenotypic method for the detection of MBLs and KPC carbapenemases in the same isolate of Enterobacteriaceae.ECCMID 2011.Presentation P-697.
15) Bou Casals J : Stable Meropenem/inhibitor combinations for phenotypic detection of KPC,MBL and KPC+MBL in Klebsiella pneumoniae.ECCMID 2012,Presentation D-674.
16) Hopkins KL et al : Evaluation of temocillin and meropenem MICs as diagnostic markers for OXA-48 like carbapenemases.J Antimicr Chemother **74**,3641-3643,December 2019.