Insert for Kit 98025 (Version 2)

KPC, MBL and Oxacillinase detection in *Pseudomonas aeruginosa and Acinetobacter spp.*

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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. The kit is intended for detection of:

- Klebsiella pneumoniae carbapenemase (KPC) and Metallo-beta-lactamase (MBL) in Pseudomonas aeruginosa
- Metallo-beta-lactamase (MBL) and Oxacillinase in Acinetobacter spp.
- Not to be used with Enterobacteriaceae (use kit 98015 instead).

Mueller-Hinton agar should be used for the test.

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 tablets containing Imipenem 10µg and Imipenem in combination with inhibitors of different β-lactamases. Inhibitors are added to differentiate isolates with resistance mechanisms from those without resistance mechanisms (see explanation below). Reduced susceptibility to carbapenems is observed when:

1. The organism produces a metallo-beta-lactamase (MBL) that hydrolyses carbapenems efficiently. MBLs are inhibited by dipicolinic acid (DPA) and EDTA. Synergy (ghost zone) between imipenem and DPA or/and EDTA indicates the presence of a MBL. If here is no zone around Imipenem 10µg, synergy should be checked at a closer distance between Imipenem 10µg and Imipenem + DPA.

2. The organism produces a Klebsiella pneumoniae carbapenemase (KPC). KPC enzymes are inhibited by Phenylboronic Acid. However, Phenylboronic Acid inhibits also the AmpC (class C cephalosporinases). In order to raise Kit's specificity, Cloxacillin High (AmpC inhibitor) is included to distinguish between these two. Thus, different inhibition zone using Imipenem + Phenylboronic Acid and Imipenem + Cloxacillin High indicates the presence of a KPC enzyme.

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:

- Imipenem 10µg, coded IMI10
- Imipenem 10µg + Phenylboronic Acid (KPC and AmpC inhibitor), coded IMPBO
- Imipenem 10µg + Cloxacillin High (AmpC inhibitor), coded IPCX4
- Imipenem 10µg + Dipicolinic acid, coded IM+DP
  Imipenem + 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:

Biochemical reagents and standard microbial equipment such as loops, culture media, incubator etc.

PROCEDURE:

1. Using a fresh, pure culture prepare a suspension of the testing organism, 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 agar plate. Note: Iso-sensitest Agar should not be used (false negative).

3. Using a single dispenser, place one tablet of each in the inoculated agar plate, ensuring sufficient space between individual tablets (allow proper measurement of inhibition zones). Note: in case the isolate do not show any inhibition zone around IMI10, the test should be repeated. IMI10 and IM+DP should be placed at a distance of approx. 5mm (synergy).

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 9mm inhibition zone.

7. Record synergism (ghost zone) or no-synergism between Imipenem 10µg and Imipenem + DPA, when the isolate shows no zone (9mm) around Imipenem 10µg.

INTERPRETATION OF RESULTS:

Compare the inhibition zones of the different tablets to interpret the results.

For *Pseudomonas aeruginosa*:

1. Measure the inhibition zone around Imipenem 10µg (IMI10) and compare it with the zones around Imipenem + Phenylboronic Acid (IMPBO) and Imipenem + Cloxacillin High (IPCX4). If the zone difference around

- IMPBO and IMI10 is ≥4mm
- IPCX4 and IMI10 is <3mm

the organism demonstrates KPC activity.

Note: If IPCX4 zone is ≥5mm than IMI10, the strains do not produce carbapenemase. (Do not test with the kit).

2. Measure the inhibition zones around Imipenem 10µg (IMI10) and compare it with Imipenem + DPA (IM+DP) and Imipenem + EDTA (IM10E). If the zone difference around
   - IM+DP and IMI10 is ≥5mm
     and/or
   - IM10E and IMI10 is ≥8mm

   the isolate produces a Metallo-beta-lactamase.

   If there is no IMI10 inhibition zone (9mm) and

   - IM+DP inhibition zone is ≥12mm
   - IM10E inhibition zone is ≥13mm

   Synergism is indicated, thus a positive result.

Note: Test only Ceftazidime resistant isolates. Otherwise false MBL-positive isolates may be obtained (if Ceftazidime sensitive).

Heinrichs et al (5) showed that Imipenem + DPA (but not Meropenem + DPA) could be used for detecting MBLs in Pseudomonas aeruginosa, with a sensitivity of 99 % and a specificity of 95 %. Meropenem + DPA should be used for detecting MBLs in Enterobacteriaceae (kit 98015).

For *Acinetobacter spp.*

1. Same method as applies for the detection of MBLs in Ps. Aeruginosa.
2. Oxacillinases are influenced by EDTA resulting in a weak synergism between Imipenem + EDTA, while DPA has no effect. This can be used for oxacillinase detection from Acinetobacter spp.
   Use the table (see below) to assist in the interpretation

QUALITY CONTROL:

Quality control should be conducted with at least one organism to demonstrate a positive reaction and at least one organism to demonstrate a negative reaction. See below possible strains for positive and negative control:

Pseudomonas aeruginosa ATCC 10145/CCUG 59626, MBL positive
Pseudomonas aeruginosa ATTC 10141, MBL positive (can be used for both *Ps. aeruginosa and Acinethobacter)*
Klebsiella pneumoniae CCUG 58547, MBL positive
Klebsiella pneumoniae NCTC 13439, MBL positive
Klebsiella pneumoniae CCUG 56233, KPC positive
Klebsiella pneumoniae NCTC 13438, KPC positive/MBL negative

REFERENCES:

1. J. Bou Casals (2012) Stable combination discs of imipenem and dipicolinic acid, for phenotypic detection of metallo-beta lactamases in Pseudomonas aeruginosa and Acinetobacter spp. ECCMID. Presentation 304. Available at: https://www.escmid.org/escmid_publications/escmid_elibrary/material/?mid=3521
2. Fournier D., Garnier P., Jeannot K., Mille A., Gomez A. S. and Plésiat P. (2013) A Convenient Method To Screen for Carbapenemase-Producing Pseudomonas aeruginosa. J. Clin. Microbiol. vol. 51, no. 11, 3846-3848.

3. Fournier D., Garnier P., Jeannot K. and Plésiat P. (2013) Evaluation of nine phenotypic tests for the detection of metallo beta-lactamase-producing Pseudomonas aeruginosa. ECCMID eP689. Available at: https://www.escmid.org/escmid_publications/escmid_elibrary/material/?mid=6253
4. Yong D., Lee Y., Jeong S. H., Lee K. and Chong Y. (2012) Evaluation of Double-Disk Potentiation and Disk Potentiation Tests Using Dipicolinic Acid for Detection of Metallo-β-Lactamase-Producing Pseudomonas spp. and Acinetobacter spp. J. Clin. Microbiol. vol. 50, no. 10, 3227-3232.
5. Heinrichs A et al: Evaluation of several phenotypic methods for the detection of carbapenemase-producing P. aeruginosa Eur J Clin Microbiol Infect Dis 34, 1467-1474, 2015.

Table for results' interpretation.

Bacterium	Zone difference against Imipenem 10 µg (IMI10)				β-lactamase
	IMPBO	IPCX4	IM+DP	IM10E	
Ps. aeruginosa		≥5 mm			No carbapenemase production
	≥4 mm	≤3 mm			KPC
			≥5 mm or ghost zone	≥8 mm	MLB
Acinetobacter spp.			≥5 mm or ghost zone	≥8 mm	MLB
			≤3 mm	4-7 mm	Oxacillinase

Abbreviations

IMI10: Imipenem 10µg
IMPBO: Imipenem 10µg + Phenylboronic Acid
IPCX4: Imipenem 10µg + Cloxacillin High
IM+DP: Imipenem 10µg + Dipicolinic acid
IM10E: Imipenem + EDTA
KPC: Klebsiella pneumoniae carbapenemase
MBL: Metallo-beta-lactamase