Insert for Kit 98025

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

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LANGUAGE: English

FOR IN VITRO DIAGNOSTIC USE ONLY

PRODUCT GROUP: Kits for beta-lactamase identification

MANUFACTURE: ROSCO Diagnostica Aps, 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:

- KPC (Class A) , Metallo-beta-lactamases (MBL) and Oxacillinases in Pseudomonas aeruginosa and Acinetobacter spp.
- Not to be used with Enterobacterales (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 KPC(Class A). 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.

3. The organism produces an oxacillinase.Oxacillinases are detected comparing the inhibition zones around Imipenem 10 ug with those of IMP+DPA and IMP+EDTA.Oxacillinases show some synergism between IMP and EDTA (+4-7 mm compared to IMI10),but no synergism between IMP+DPA(<= 3mm).Bedenic et al(6) detected false MBL positives when using EDTA

as chelating agent for Acinetobacter baumannii.The use of both DPA and EDTA in combination with IMP,differentiate between oxacillinases and MBLs.

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-diagnostica.com
User's Guide can be obtained free of charge from your local distributor on request, or from ROSCO:
E-mail: : info@rosco-diagnostica.com
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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,before placing them 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.

Shelf-life of the product is **at least 2 years** from the date of manufacture.Due to the use of crystalline antimicrobials,cartridges may be opened and closed several times during use,without affecting the shelf-life of the product.On the opposite,paper disks based products (amorphous antimicrobials),after opening the cartridge,have a shelf-life of **a few weeks** at 2-8 C.

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 and Acinetobacter*

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 (Class A) 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
   - 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(MBL positive)

Note: **Test only Ceftazidime resistant isolates**. Otherwise false MBL-positive isolates may be obtained (if Ceftazidime sensitive),for ex with Klebsiella oxytoca.

Heinrichs et al (5) showed that Imipenem + DPA (but not Meropenem + DPA) should 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).
Pilate et al (7) using the kit 98025,in the period of Oct 2018 to March 2019,tested 109 meropenem non-susceptible P. aeruginosa isolates.From them 10 strains (9.2 %) produced a carbapenemase (9 VIM and 1 NDM) and were all detected by kit 98025.No false positives or negatives were found.
Grill et al(10)found a phenotypic algorithm that could indicate carbapenemase production in Ps. aeruginosa.Strains showing Imipenem R,Ceftazidime R and Cefepime R are most probably carbapenemase producers.
Bedenic et al (6) found a false detection of MBLs in Acinetobacter baumanni,when using Imipenem + EDTA.Oxacillinases were false detected as MBLs.

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. .Oxacillinases are detected comparing the inhibition zones around Imipenem 10 ug with those of IMP+DPA and IMP+EDTA.(8).Ps aeruginosa producing oxacillinases,show resistance to **(CAZ)**.Besides,there is synergism between IMI10 and CAZ.

Oxacillinases and Acinetobacter: Oxacillinase-positive strains of Acinetobacter show imipenem MICs >= 8-16 ug/ml,corresponding to zones of inhibition <= 14 mm around Imipenem 10 ug Neo-sensitabs.

3. Oxacillinases positive strains show some synergism between IMP and EDTA (4-7 mm larger zone than IMI10),but no synergism between IMP + DPA (<= 3 mm).
4. If the isolate possess *both* **oxacillinases and KPC(Class A**),it will be observed synergism between IMP and Boronic acid (zone >= 4 mm larger)and weak synergism between IMP and EDTA (4-7 mm)and *no synergism between IMP and DPA*(<= 3 mm).
5. If the isolate possesses *both* **oxacillinase+MBLs**,use Aztreonam 30 ug Neo-sensitabs and place it approx. 10 mm from IMI10.Lack of synergism between AZT and IMI10 (no ghost zone),together with synergism IMP/EDTA (>= 8 mm) and IMP+DPA (>= 5 mm) indicates the presence of both MBL and oxacillinase.
   Use the table (see below) to assist in the interpretation.
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Please Notice:
Multiresistant Ps. aeruginosa GES variants (GES-2,GES-5,GES-6),that show **resistance** to Ceftolozane+Tazobactam may have a resistance profile similar to a carbapenemase,but they may show a negative result(for Class A carbapenemase) in kit 98025 and other phenotypic methods such as Carba NP or Rapid Blue Carb.
They show a positive result to Rapid ESBL kit 98022 (expression of ESBL-phenotype).

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 ATTC 10141, MBL positive (can be used for both *Ps. aeruginosa and Acinetobacter)*
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, 3846-3848,2013..
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, 3227-3232,2012.
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.

6. Bedenic B et al : False positive phenotypic detection of MBLs in Acinetobacter baumannii.Acta Clin Croat.**58**,113-118,2019.
7. Pilate T et al : Detection of carbapenemase production in P. aeruginosa in a tertiary care center.University Hospitals Leuven(Belgium).Royal Belgian Sciety of Laboratory Medicine 2019.
8. Hojabri Z et al : Evaluation of the commercial combined disk test and MIC determination for detection of carbapenemase producers among gram-negative bacilli isolated in a region with high prevalence of OXA-48 and NDM.Intern.Microbiology 327320102,August 2018.
9. Recommendations for the detection of carbapenemases in multiresistant Ps aeruginosa and Acinetobacter spp in Belgian Laboratories.7/6 2018(flemish).
10. Gill Ch et al : Evaluation of a phenotypic algorithm to detect carbapenemase testing in Ps. aeruginosa in a multicenter cohort. Microb. Drug Resist **27**(9)Sept. 2021.

Table for results' interpretation.

ZONE DIFFERENCE AGAINST IMIPENEM 10 µG					BETA LACTAMASE
IMP/AZT	IMPBO	IPCX4	IM+DP	IMI10E	
		>= 5 mm			No Carbapenemase production
No SYN	>= 4 mm	<= 3 mm			KPC (Class A)
Synergism			>= 5 mm or ghost zone	>= 8 mm	MBL
No SYN			<= 3 mm	4-7 mm	Oxacillinase
		DUAL BETA-LACTAMASES			
No SYN	<=3 mm	<= 3mm	>= 5mm or ghost zone	>=8 mm	Oxacillinase + MBL
No SYN	>= 4 mm	<= 3 mm	<= 3 mm	4-7 mm	Oxacillinase + KPC(Class A)
No SYN	>= 4 mm	<= 3 mm	>= 5 mm	>= 8 mm	KPC + MBL
No SYN	>=4 mm	<=3mm	>= 5mm	>=8 mm	GES+MBL(Cefta+Clav SYN

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
AZT: Aztreonam 30 ug