IMPREGNATED DISKS FOR DETECTING METALLO-β-LACTAMASE (MBL)

50-DISK CARTRIDGES

SCREENING TEST FOR DETECTING METALLO-β-LACTAMASE

IVD CE

2012/10

1- CLINICAL INTEREST

Carbapenems (Imipenem, Meropenem, Ertapenem, etc.) are the antibiotics of choice for treating infections caused by multi-resistant Gram-negative bacteria (Acinetobacter, Pseudomonas, Enterobacteria, etc.). Two types of enzymes hydrolysing carbapenem activity (carbapenemases) have been described: serine carbapenemases and metallo-β-lactamases (MBL) (5). They both provide these molecules with resistance. The detection of metallo-β-lactamase (MBL) producing strains makes it possible to adapt antibiotic treatment, if necessary, and to implement precautionary measures to prevent possible nosocomial infections and the spreading of the strains in question (4).

2- PRINCIPLE

Metallo-β-lactamases (MBL) are enzymes that belong to class B of Ambler's molecular classification (1, 4). Their activity is dependent upon the presence of divalent cations such as zinc. Thus, the activity of MBLs can be inhibited by chelating agents such as EDTA (ethylenediaminetetraacetic acid), which binds to zinc cations.

The technique, for a bacterial strain identified on the species level, consists in comparing the *in vitro* activity of a disk impregnated with an antibiotic belonging to the carbapenem class (Imipenem or Meropenem) and a disk impregnated with the same molecules combined with a metallo-β-lactamase enzyme inhibitor: EDTA.

With bacteria producing this enzyme, the *in vitro* activity of the disk combining the carbapenem molecule and EDTA is augmented compared to that of the disk with carbapenem alone. EDTA blocks MBL action. The antibiotic can then act on the bacteria.

The choice of the carbapenem and EDTA concentration comes from scientific publications (6) and has been validated.

3- PRESENTATION

6.5-mm disks produced using high-quality absorbent paper are impregnated with an antibiotic/inhibitor solution at precise concentrations.

The disks are clearly identified with a 3-letter symbol printed on each side of the disk.

Name	Disk load	Symbol	Packaging	Product code
Imipenem+EDTA	10 / 930	EIP	1X50 disks	67936
Meropenem+EDTA	10 / 930	EME	1X50 disks	67966

4- STORAGE

The expiry date only applies to disks contained in their intact cartridges stored in compliance with the manufacturer's instructions. The expiry date and the batch number can be found on each packaging component (cartridge and container).

- Disk cartridges must be stored in their container in a dry place between +2 and +8 °C.
- Leave the containers at room temperature before opening them (up to one hour); return the cartridges to a dry place between +2 and +8 °C once the disks have been distributed.
- Do not use disks whose expiry date has passed.
- Do not use disk cartridges that have been left at room temperature for more than 8 hours or those that have undergone several temperature variations (from the cold room to the laboratory), otherwise check their acceptable performance level before continuing use.
- If the cartridges are stored in the distributor after depositing, the distributor must be stored in a dry place at +2 to +8 °C with dessicants inside.
- Disk stability in open cartridges placed in distributors stored between +2 and +8 °C with dessicants has been validated in routine conditions. It is indicated in weeks in the following pictogram:

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5- REQUIRED MATERIAL NOT SUPPLIED

- Disk distributor: 7 disks, product code 50294
- Disk distributor: 12-16 disks, product code 50295
- Culture medium for the antibiotic sensitivity test: Mueller Hinton
- Bacterial strains needed for quality control to validate the reagents
- Opacity control equivalent to the 0.5 Mac Farland standard or any other process for verifying bacterial opacity
- Laboratory equipment for antibiotic sensitivity tests using the agar diffusion method

6- PRECAUTIONS FOR USE

Follow the directions for use from the applicable CLSI recommendations, (7). The results obtained with the impregnated disks depend not only on the load contained in the disk, but also on an appropriate inoculum, recommended mediums, storage conditions and other factors.
Follow the applicable techniques and precautions for protection against microbiological hazards at all times. After use, sterilise the cultures and all contaminated equipment.

7- OPERATING PROCEDURE

The disks should not be used for tests directly on biological samples.

Preparation of the inoculum:
Refer to the applicable recommendations from the CLSI/NCCLS for obtaining a 0.5 McFarland bacterial inoculum from a pure, fresh culture, CLSI (7). Identifying the strain is an important element for the result. The presence of metallo-β-lactamase is only described for the following strains of bacterial species: Enterobacteria, Pseudomonas, Acinetobacter and Chryseobacterium (4, 5).

Inoculating the Mueller-Hinton medium:
Refer to the technique described by the CLSI (7). The incubation time and temperature also depend on the applicable recommendations: 16-20 hours at 35-37°C are usually considered satisfactory conditions.

Disk depositing:
The choice of testing one combination over another can be made depending on the identification of the bacterial species, but given the sensitivity and specificity performances, the result of the screening is optimised by testing both combinations respectively using disks with carbapenem alone.

- Deposit the 10 µg Imipenem disk (product code 66568) and the **Imipenem + EDTA** disk (product code 67936) on the surface of an adequate medium dish (Mueller-Hinton) inoculated with a pure bacterial strain.
- Deposit the 10 µg Meropenem disk (product code 67048) and the **Meropenem + EDTA** disk (product code 67966) on the surface of an adequate medium dish (Mueller-Hinton) inoculated with a pure bacterial strain.

The disk of carbapenem alone and its combination with EDTA should be tested with the same bacterial inoculum in order to calculate a consistent difference in diameters.
A minimum distance of 3 cm (centre to centre) between the disks is recommended.

8- INTERPRETATION OF THE RESULTS

Precisely measure the diameters of the zones of inhibition observed for each of the disks deposited:

- **Meropenem +EDTA (EME)** and Meropenem alone **(MEM)**
- **Imipenem +EDTA (EIP)** and Imipenem alone **(IPM)**
- The bacterial strain is interpreted as potentially producing metallo-β-lactamase if the difference in diameters between the disks combined and the disks alone is greater than or equal to 4 mm.
- Any positive result means a presumed presence of metallo-β-lactamase, but is not enough to confirm this resistance phenotype. The only confirmation technique is genotyping for the strain studied. Determining the MIC (Minimum Inhibitory Concentration) of the carbapenem molecule alone and carbapenem + EDTA backs up the result obtained with the disks.

9- PERFORMANCES

An external evaluation covering a panel of genotyped strains has established the detection threshold for metallo-β-lactamase producing strains, the sensitivity of different disk combinations and the specificity for each.

The panel of bacterial strains tested included 28 genotyped metallo-β-lactamase positive strains and 43 genotyped metallo-β-lactamase negative strains:

Strains tested	**MBL + (n = 28)**	**MBL – (n = 43)**			
		Oxacillinase	Class A carbapenemases	Other mechanisms	Negative control
Enterobacteria	10	3	7	2	
Pseudomonas spp. *Chryseobacterium* spp. *Stenotrophomonas maltophilia*	15		2	2	10
Acinetobacter spp.	3	12			5
Total	**28**	**15**	**9**	**4**	**15**

The sensitivity and specificity performances with the two different combinations are given in the table below:

Enterobacteria strains	**% sensitivity**	**% specificity**
Imipenem+EDTA disks	40	100
Meropenem+EDTA disks	80	85

Pseudomonas/Acinetobacter strains	**% sensitivity**	**% specificity**
Imipenem+EDTA disks	72	97
Meropenem+EDTA disks	100	35

Given the results obtained for sensitivity and specificity, testing is recommended for the two disk combinations, **Imipenem+EDTA and Meropenem+EDTA**, to search for metallo-β-lactamase for strains of Pseudomonas/Acinetobacter and Enterobacteria. The screening performances are thus improved.

The table below proposes decision-making assistance for interpreting this screening:

	Result with the IPM+EDTA disk	**Result with the MEM+EDTA disk**	**Presence of MBL**
Enterobacteria	+	+	Very high probability
	-	-	Low probability
	+	-	Very high probability
	-	**+**	High probability
Acinetobacter/ *Pseudomonas aeruginosa*	**+**	**+**	Very high probability
	-	-	No probability
	+	-	High probability
	-	**+**	Low probability

Positive or negative results are obtained using the difference in diameters obtained by simultaneously testing a disk with carbapenem alone and its combination with EDTA.

10- IN-HOUSE QUALITY CONTROL

The performances of the **Meropenem + EDTA** and **Imipenem+EDTA** disks are systematically controlled by testing the following strains:

- Recombinant strain *Escherichia coli* Vim-4 referenced by Bio-Rad. The acceptance criterion is a difference in diameters at least equal to 4 mm between the Meropenem and **Meropenem + EDTA** disks and between the Imipenem and Imipenem+EDTA disks.
- *Escherichia coli* ATCC 25922 strain.

11- MANUFACTURER QUALITY CONTROL

All products manufactured and sold by Bio-Rad are covered by a quality assurance system from the reception of the raw materials to the sale of the finished products. Each batch of finished products undergoes quality control and is not put on the market unless it complies with the acceptance criteria. Documentation relative to the production and control of each batch is kept by the manufacturer.

12- LIMITS OF USE

The bacterial strain must be identified; the presence of metallo-β-lactamases has not been proven for Gram-positive bacteria.
The performances of the combined carbapenem + EDTA disks were determined using disks with carbapenemes alone from the same supplier (10 µg Imipenem, product code 66568, and 10 µg Meropenem, product code 67048). The single and combined disks from a given supplier must be tested.
Confirmation of a positive screening result by genotype is necessary to precisely identify the nature of the enzyme responsible for the carbapeneme resistance.
The presence of micro-colonies in the zone of inhibition should not be taken into account when reading the diameter and interpreting the results.
False positive reactions have been observed due to the permeabilising effect of EDTA itself (3, 4).

13- BIBLIOGRAPHICAL REFERENCES

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