ENGLISH

KPC&MBL&OXA-48 disc kit (acc. to EUCAST)

Disc tests for confirmation of carbapenemase-producing Enterobacteriaceae.

DESCRIPTION

Carbapenemases are β-lactamases that hydrolyze penicillins, in most cases cephalosporins and to various degree carbapenems. The most important carbapenemases are categorized as three types of enzymes:

1. *Klebsiella pneumoniae* carbapenemases (KPC) belonging to the Ambler class A of β-lactamases;
2. VIM, IMP and NDM belonging to the class B metallo β-lactamases (MBL);
3. Class D β-lactamases of OXA-48-like enzymes.

Since the 1990s, the increased consumption of carbapenems to treat infections caused by extended-spectrum β-lactamases (ESBL) producing bacteria has in turn contributed to the dramatic increase in carbapenem-resistant Enterobacteriaceae (CRE). Bacterial acquisition of carbapenemases is crucial to the emergence of CRE. Indeed, the corresponding genes are mostly plasmid-located and associated with various mobile genetic structures and may confer resistance to virtually all β-lactams. Infections with carbapenemase-producing Enterobacteriaceae are associated with high mortality posing a serious threat especially to hospitalized patients. Carbapenemases are considered to be of high epidemiological importance and one of the most important concerns is the spread in the community, particularly in *E. coli*. Adequate treatment and control of CRE infections is predicted upon their accurate and prompt diagnosis from patient samples in the clinical laboratory.
Carbapenem resistance can also arise in both ESBL and AmpC-producing organisms (Ambler class A and C, respectively) by mutation that regulate the synthesis of porins contained in the outer membrane.

Carbapenem MICs for carbapenemase-producing enterobacteriaceae may be below the clinical breakpoints, however, the epidemiological cut-off (ECOFF) defined by EUCAST can be used to detect carbapenemase-producers. Meropenem, offering the best compromise between sensitivity and specificity, is recommended for screening.
Following detection of reduced susceptibility to carbapenems, a phenotypic method for confirmation of carbapenemase-production should be applied. Meropenem discs supplemented with different inhibitors of carbapenemase are used to evaluate carbapenem-resistant strains.

CONTENTS OF THE PACKAGES

5 x 50 discs cartridges, each packaged in a “blister” with a dryer.

METHOD PRINCIPLE

Enterobacteriaceae suspected to be producers of carbapenemases may be confirmed by using meropenem and evaluating the synergistic effects when combined with the following inhibitors:

- **Phenylboronic acid** inhibits class A carbapenemase;
- **EDTA** inhibits class B carbapenemase;
- **Cloxacillin**, which inhibits AmpC β-lactamases, permits to differentiate between AmpC hyperproduction plus porin loss and carbapenemase-production.

There is no currently available inhibitor for class D carbapenemases. However, when there is no synergy of meropenem with any of the above mentioned inhibitors, 30 µg **Temocillin** disc (TMO) can be used in order to differentiate between ESBL plus porin loss and OXA-48 like enzymes.

For each combined disc test (CDT), discs containing meropenem alone and in combination with a carbapenemases inhibitor are applied. The inhibition zone around the meropenem disc combined with inhibitor is compared with the zone around the 10 µg meropenem disc (MRP).

GATHERING AND KEEPING SAMPLES

The colonies that are to be subjected to the susceptibility test are taken up by culture media that have been previously swabbed with the sample under examination.

TEST PROCEDURE

1. Using a fresh, pure culture prepare a suspension of the test organism equal to 0.5 McFarland Standard.
2. Using a sterile cotton swab, spread the adjusted suspension over the entire area of a Mueller Hinton agar plate.
3. Apply Meropenem, Meropenem + EDTA, Meropenem + Phenylboronic acid, Meropenem + Cloxacillin, on the inoculated plate, ensuring sufficient space between individual discs to allow for proper measurement of inhibition zones.
4. Incubate at 36±1°C for 18-24 hours.

EVALUATING THE RESULTS

At the end of the incubation period, measure the inhibition halos and interpret as indicated in the table below.

Interpretative Table. Synergy of meropenem with carbapenem inhibitors for confirmation of CRE.

Increase in inhibition zone of meropenem with the following inhibitor			Temocillin (TMO)*	β-lactamases
Phenylboronic acid (MR+BO)	EDTA (MR+ED)	Cloxacillin (MR+CL)		
≥ **4 mm**	< 5 mm	< 5 mm	---	KPC
< 4 mm	≥ **5 mm**	< 5 mm	---	MBL
≥ **4 mm AND**	< 5 mm	≥ **5 mm**	---	AmpC + porin loss or efflux
< 4 mm	< 5 mm	< 5 mm	**< 11 mm**	OXA-48-like

*Temocillin susceptibility test is recommended only in cases where no synergy is detected.

QUALITY CONTROL

Each production batch of discs is subjected to the quality control with the following bacterial strains:

Microorganism		
Klebsiella pneumoniae	ATCC® 700603	Negative control
Klebsiella pneumoniae	ATCC® BAA-2146	MBL positive
Klebsiella pneumoniae	ATCC® BAA-1705	KPC positive
Klebsiella pneumoniae	NCTC 13442	OXA-48 positive

LIMITS

Diffusion susceptibility tests use an *in vitro* technique and cannot therefore reproduce the extremely complex *in vivo* conditions. Nevertheless, it is a useful and important tool that helps the clinician choose the correct therapy. Many variable factors influence the final result of the diffusion susceptibility test. The main ones are: the culture medium used, impregnation of the discs, inoculation of the medium, temperature, time and incubation atmosphere of the plates, pre-incubation and pre-diffusion conditions, depth of the medium, etc.

PRECAUTIONS

The discs cannot be classified as being hazardous according to current legislation but fall within the specific field of application where a safety data sheet must be supplied because they can cause phenomena of sensitization in sensitive subjects if they come into contact with the skin.
The discs are disposable products. They are only for diagnostic *in vitro* use and are intended for professional use. They must be used in the laboratory by properly trained operators using approved aseptic and safety methods for pathogenic agents.

STORAGE

Store the unopened blister at -20°C to +8°C till the expiry date. Allow unopened cartridge to come to room temperature before removing it from the blister for minimising condensation on the discs. Leftover discs from an opened cartridge should be stored at 2-8°C for no more than 7 days. Return unused discs to the refrigerator as soon as the application of the discs has been completed. Dispose of expire discs.

ELIMINATING USED MATERIAL

After use, the discs and the material that comes into contact with the sample must be decontaminated and disposed of in accordance with current laboratory techniques for the decontamination and disposal of potentially infected material.

REFERENCES

- EUCAST guidelines for detection of resistance mechanisms and specific resistances of clinical and/or epidemiological importance. Version 1.0, 2013.

PRESENTATION

DESCRIPTION		PACKAGING	REF
Meropenem 10 µg	MRP	50 Discs	99007
Meropenem + EDTA	MR+ED	50 Discs	
Meropenem + Phenylboronic acid	MR+BO	50 Discs	
Meropenem + Cloxacillin	MR+CL	50 Discs	
Temocillin 30 µg	TMO	50 Discs	

TABLE OF SYMBOLS

LOT Batch code	**IVD** *In Vitro* Diagnostic Medical Device	Manufacturer	Use by	Fragile, handle with care
REF Catalogue number	Temperature limitation	Contains sufficient for <n> tests	Caution,consult accompanying documents	Do not reuse

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