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## MASTDISCS® *Combi* *Carba plus* (Enterobacteriaceae)

**D73C.** For the detection of carbapenemase and OXA-48 enzyme production in Enterobacteriaceae.

FOR IN VITRO DIAGNOSTIC USE ONLY

**Contents and Formulation\*:** 5 cartridges, each cartridge containing 50 discs.

**Cartridge A** Penem discs

**Cartridge B** Penem + MβL inhibitor discs

**Cartridge C** Penem + KPC inhibitor discs

**Cartridge D** Penem + AmpC inhibitor discs

**Cartridge E** Temocillin + MβL inhibitor discs

### Storage and shelf life

Store at 2 to 8°C in the containers provided until the expiry date shown on the pack label. Allow to equilibrate to room temperature before opening.

### Precautions

For in vitro diagnostic use only. Observe approved biohazard precautions and aseptic techniques. To be used only by adequately trained and qualified laboratory personnel. Sterilise all biohazard waste before disposal. Refer to Product Safety Data sheet.

### Materials required but not provided

Standard microbiological supplies and equipment such as loops, MAST® culture media, swabs, applicator sticks, incinerators and incubators, etc., as well as serological and biochemical reagents and additives such as blood.

### Procedure

- Using a pure, fresh culture of the test organism (i.e. an organism which presents reduced susceptibility to carbapenem antibiotics), prepare a suspension equivalent in density to a McFarland 0.5 opacity standard.
- Using a sterile swab spread the suspension uniformly across the surface of a susceptibility test agar plate (e.g. MAST® Mueller Hinton Agar DM170).
- Using a sterile needle, forceps or MAST® DISCMASTER Dispenser, place one of each type of **MASTDISCS® *Combi Carba plus*** Detection Set discs onto the inoculated medium, ensuring sufficient space between the discs to allow formation of clearly defined zones of inhibition.
- Incubate at 35 to 37°C for 18 to 24 hours.
- Measure and record the diameter of any zones of inhibition, to the nearest whole millimetre **ignoring any micro-colonies within the zone**. Discs showing no zone of inhibition should be recorded as 6mm.

### Interpretation of results

Compare the zone of inhibition of the penem disc (A) to the zones of inhibition of each of the penem plus inhibitor discs (B, C and D).

If disc **B only** shows a zone difference  $\geq 5\text{mm}$  than disc A (C - A and D - A should be  $< 5\text{mm}$ ), record the organism as demonstrating MβL activity.

If disc **C only** shows a zone difference  $\geq 5\text{mm}$  than disc A (B - A and D - A should be  $< 5\text{mm}$ ), record the organism as demonstrating KPC activity.

If discs C and D both show significant zone differences ( $\geq 5\text{mm}$ ) compared to disc A (B - A should be  $< 4\text{mm}$ ), record the organism as demonstrating AmpC activity coupled with porin loss (impermeability).

If no synergy is obtained between discs A, B, C and D and disc E shows a zone of inhibition of  $\leq 10\text{mm}$ , record the organism as demonstrating OXA-48 activity. **If an equivocal or negative result is generated but resistance to disc A is shown, the organism may still be expressing a carbapenemase enzyme. Molecular testing or MASTDISCS® *ID D74 Indirect Carbapenemase Test (ICT)* can be performed to verify this.**

### Quality control

Check for signs of deterioration. Quality control must be performed 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 combination disc with inhibitor and corresponding penem only disc against control negative *Escherichia coli* (e.g. ATCC® 25922), should be equal or show no greater difference in diameter than  $\pm 2\text{mm}$ . Any greater difference implies malfunction or deterioration. Disc E should be greater than 10mm. Do not use the product if the reactions with the control organisms are incorrect. The list below illustrates a range of performance control strains which the end user can easily obtain.

Test Organism	Result
<i>Klebsiella pneumoniae</i> NCTC 13440	MβL Positive
<i>Klebsiella pneumoniae</i> NCTC 13438	KPC Positive
<i>Klebsiella pneumoniae</i> NCTC 13442	OXA-48 Positive
<i>Escherichia coli</i> ATCC® 25922	Negative

### Limitations

To avoid potentially erroneous results - do not test cartridges from different batches together - batches should never be mixed. D73C is not suitable for detection of carbapenemase production in *Pseudomonas* spp. or *Acinetobacter* spp. D73C may give equivocal results against clinical isolates that have acquired complex co-resident carbapenemase mediated resistance mechanisms.

### References

Bibliography available on request.