

MASTDISCS®Combi Carbaplast

D73C

Intended use

For the detection of carbapenemase and OXA-48 enzyme production in Enterobacterales.

FOR IN VITRO DIAGNOSTIC USE ONLY

Contents and Formulation*

5 cartridges per pack, each cartridge containing approximately 50 discs:

Cartridge A	Penem
Cartridge B	Penem + M β L inhibitor
Cartridge C	Penem + KPC inhibitor
Cartridge D	Penem + AmpC inhibitor
Cartridge E	Temocillin + M β L inhibitor

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, Mueller-Hinton agar, swabs, forceps, callipers, etc., as well as an incubator capable of maintaining 35 ± 1°C.

Procedure

1. Using a pure, fresh culture of the test organism, prepare a suspension equivalent in density to a 0.5 McFarland standard in physiological saline.
2. Using a sterile swab, spread the suspension uniformly across the surface of a single Mueller Hinton Agar plate in accordance with the European Committee on Antimicrobial Susceptibility Testing (EUCAST) procedure.
3. Using a MAST® DISCMASTER Dispenser, or alternatively a sterile needle or forceps, place one of each disc on to the plate of inoculated medium, ensuring sufficient space between the discs to allow formation of clearly defined zones of inhibition.
4. Incubate at 35 ± 1 °C for 18 ± 2 hours.
5. Measure and record the diameter of any zones of inhibition, to the nearest whole millimetre, **ignoring any microcolonies in the zone**. Discs showing no zone of inhibition should be recorded as 6 mm.

Interpretation of results

To interpret results based on observed zones of inhibition, use the D73C calculator. The calculator is available for download and can be accessed via www.mast-group.com, in the registered members section. Alternatively, results can be interpreted manually by comparing inhibition zone diameters as described below:

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 ≥ 5 mm than disc A (C - A and D - A should be < 5 mm), record the organism as demonstrating M β L activity.

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

If discs C and D both show significant zone differences (≥ 5 mm) compared to disc A (B - A should be < 4 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 < 10 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 the combination discs with inhibitor and corresponding penem only disc against negative control organism *E. coli* ATCC® 25922 should be equal or show no greater difference in diameter than ≥ 2 mm. The zone diameter for disc E should be > 10 mm. Any deviation implies malfunction or deterioration. 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	M β L Positive OXA-48 Positive Negative
<i>Klebsiella pneumoniae</i> NCTC 13438	
<i>Klebsiella pneumoniae</i> NCTC 13442	
<i>Escherichia coli</i> ATCC® 25922	

Limitations

D73C is not suitable for detection of carbapenemase production in *Pseudomonas* spp. or *Acinetobacter* spp. To avoid potentially erroneous results, do not mix cartridges from different batches of D73C and ensure all discs in the set are tested on the same plate. D73C may give equivocal results against clinical isolates that have acquired complex co-resident carbapenemase mediated resistance mechanisms. Users are obliged to always use the latest version of the D73C calculator.

References

Bibliography available on request.