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Mast Diagnostic

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MAST[®] /D Indirect Carbapenemase Test (ICT)

D74

Intended use

A screening test for the detection of carbapenemase production in Enterobacterales, *Pseudomonas* and *Acinetobacter* spp.

FOR IN VITRO DIAGNOSTIC USE ONLY

Contents and Formulation

25 foil packets, each packet containing 1 x D74 paper device. Each device comprises two number coded 8mm tips (one to be used as a control and the other to be used for the test organism), and a single 6mm tip containing a penem indicator antibiotic (labelled "X").

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 reporter organism, prepare a suspension equivalent in density to a McFarland 0.5 opacity standard. Use *Escherichia coli* ATCC[®] 25922 as the reporter organism when testing Enterobacteriaceae and *Acinetobacter* spp. Use *Klebsiella pneumoniae* ATCC[®] 700603 as the reporter organism when testing *Pseudomonas* spp.
- Using a sterile swab spread the suspension uniformly across the surface of a susceptibility test agar plate (e.g. MAST[®] Mueller Hinton Agar DM170D), as shown in Fig.1.
- Using sterile forceps remove D74 ICT from its foil packet and place onto a sterile surface with tips 1, 2 and X uppermost.
- Using a pure, fresh culture of *Escherichia coli* ATCC[®] 25922 (this is to be used as a negative control) remove ¼ to ½ of a 10µl loopful of this organism. Proceed to apply, by rubbing, colonies onto tip "1" of D74 ICT covering as much of the area as possible (as shown in Fig. 2).
- much of the area as possible (as shown in Fig. 2).
 Apply test organism to tip "2" of D74 ICT using the same method described in step 4 (as shown in Fig. 3).
- 6. Using sterile forceps, place D74 ICT onto the inoculated plate, ensuring Tips 1 and 2 are in direct contact with the medium i.e. organism-coated side facing down and the MAST[®] ICT text facing up. Ensure D74 ICT has fully adhered to the surface of the plate (as shown in Fig 4).
- Incubate at 35°C to 37°C aerobically for 16 to 20 hours when testing Enterobacterales and *Acinetobacter* spp. Incubate at 35°C to 37°C anaerobically for 16 to 20 hours when testing *Pseudomonas* spp.
- 8. Read the plates from the front with the lid removed, by observing the shape of the zone of inhibition created by the indicator tip "X" for distortion in proximity to tip "3" and tip "4" i.e. flattening of the normally circular zone.

Interpretation of results

Positive for carbapenemase production

Distortion of the zone of inhibition around tip "X" (indicator antibiotic) in proximity to tip "3" indicates a positive result (Carbapenemase production). Tip "X" in proximity to tip "4" (negative control) should always show an undistorted circular zone. It is recommended that positive results are confirmed using molecular testing methods.

Negative for carbapenemase production

No distortion (a circular zone of inhibition) in proximity to tip "3" indicates a negative result.

Quality control

Check for signs of deterioration. Quality control must be performed with at least one organism to demonstrate a negative reaction and at least one representative organism to demonstrate a positive reaction. For example, if the test organism is *Pseudomonas* spp., *P. aeruginosa* NCTC 13437 is an appropriate positive control, whereas if the test organism is an Enterobacterales, any of the three *K. pneumoniae* QC organisms listed below could be used. 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	Positive (MβL)
<i>Klebsiella pneumoniae</i> NCTC 13438	Positive (KPC)
<i>Klebsiella pneumoniae</i> NCTC 13442	Positive (OXA-48)
<i>Acinetobacter baumanii</i> NCTC 13301	Positive (OXA-23)
<i>Pseudomonas aeruginosa</i> NCTC 13437	Positive (MβL)
<i>Escherichia coli</i> ATCC [®] 25922	Negative

Limitations

D74 is a screening test for carbapenemase production and cannot distinguish between different carbapenemase enzymes. For Enterobacterales, it is recommended that **MAST**DISCS[®] *Combi* Carba plus (D73C) is used as a follow on test for this purpose.

All positive results obtained when testing *Pseudomonas* and *Acinetobacter* spp. should be confirmed using molecular methods as recommended by the EUCAST guidelines on the detection of resistant mechanisms (available on the EUCAST website).

References

Bibliography available on request.





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Fig. 4

Fig. 2



Fig. 5

Fig. 3



Fig. 6

