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**Mast
Group**

MASTDISCS® *Combi* AmpC, ESβL and Carbapenemase Detection Set

D72C

Intended use

For the detection of extended spectrum beta-lactamase (ESβL) and/or AmpC (including inducible AmpC) enzyme production in Enterobacterales, with confirmation of inducible AmpC and carbapenem resistance status.

FOR IN VITRO DIAGNOSTIC USE ONLY

Contents and Formulation*

6 cartridges per pack, each cartridge containing approximately 50 discs.

Cartridge A	Cefpodoxime 10 µg
Cartridge B	Cefpodoxime 10 µg + ESβL inhibitor
Cartridge C	Cefpodoxime 10 µg + AmpC inhibitor
Cartridge D	Cefpodoxime 10 µg + ESβL inhibitor + AmpC inhibitor
Cartridge E	Cefpodoxime 10 µg + ESβL inhibitor + AmpC inducer
Cartridge F	Penem antibiotic

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

- Using a pure, fresh culture of the test organism, prepare a suspension equivalent in density to a 0.5 McFarland standard in physiological saline.
- 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.
- Using a MAST® DISCMASTER® Dispenser, or alternatively a sterile needle or forceps, place one of each disc onto the inoculated medium, ensuring sufficient space between the discs to allow formation of clearly defined zones of inhibition.
- Incubate at 35 ± 1 °C for 18 ± 2 hours.
- Measure and record the diameter of any zones of inhibition, to the nearest whole millimetre. Record whether microcolonies are present in the zone of inhibition surrounding disc F. 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 D72C calculator. The calculator is available for download and can be accessed via www.mast-group.com, in the registered members section.

Additional interpretation of the zone surrounding Disc F

No zone of inhibition around disc F is indicative of suspected carbapenemase activity. This should be entered into the D72C calculator as 6 mm. A zone of inhibition with microcolonies growing within the inhibition zone may suggest suspected carbapenemase activity (OXA-48 mediated). Measure the zone of inhibition, **ignoring any microcolonies**. Note the presence/absence of microcolonies in the zone by entering Y or N respectively in the appropriate column in the D72C spreadsheet.

It is recommended that differentiation and confirmation of carbapenemase resistance mechanisms is carried out using **MASTDISCS® *Combi Carba plus*** (D73C).

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 from discs A to E against negative control organism *E. coli* ATCC® 25922 should be equal or show no greater difference in diameter than ±2 mm. Disc F should produce a clear zone of inhibition (no microcolonies). Any deviation implies a 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>Escherichia coli</i> NCTC 13351	ESβL Positive
<i>Enterobacter cloacae</i> NCTC 13406	AmpC Positive
<i>Enterobacter cloacae</i> NCTC 13405	Inducible AmpC Positive
<i>Klebsiella pneumoniae</i> NCTC 13438	Suspected carbapenemase
<i>Klebsiella pneumoniae</i> NCTC 13442	Suspected carbapenemase
<i>Escherichia coli</i> ATCC® 25922	Negative

Limitations

D72C is not suitable for use with *Pseudomonas* spp. or *Acinetobacter* spp. To avoid potentially erroneous results do not mix cartridges from different batches of D72C and ensure all discs are tested on the same plate. Organisms producing a fully resistant profile i.e. no zone of inhibition on all discs could indicate demonstration of an MβL or KPC carbapenemase. A small proportion of non-carbapenemase producing Enterobacterales may demonstrate resistance to disc F. The presence of ESβLs may be masked by carbapenemases. Users are obliged to always use the latest version of the D72C calculator. Information of product or calculator updates will be posted on the MAST® website. ESβL and AmpC producing organisms with reduced permeability may produce equivocal results.

References

Bibliography available on request.