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MASTDISCS® *Combi*
AmpC and ESβL Detection Set

D68C

Intended use
For the detection of AmpC and/or extended spectrum beta-lactamase (ESβL) enzyme production in Enterobacterales.

FOR IN VITRO DIAGNOSTIC USE ONLY

Contents and Formulation*
4 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

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 onto 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. 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 D68C 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 in the sequence described below:-

Step 1 - Compare the zone of inhibition of the cefpodoxime disc (**A**) to the zones of inhibition of each of the cefpodoxime plus inhibitor discs (**B, C, and D**). If all zones are within 2mm of each other, record the organism as demonstrating neither ESBL nor AmpC activity.

Step 2 - Subtract **A** from **B,** and **C** from **D.** If each of **B** – **A** and **D** – **C** ≥ 5mm **AND** when compared, the differences in zone diameter between discs **B** and **D** and discs **A** and **C** are 4mm or less then the organism is demonstrating ESBL activity alone.

Step 3 – Subtract **B** from **D** and **A** from **C**. If each of **D** – **B** and **C** – **A** ≥ 5mm **AND** when compared the differences in zone diameter between discs **A** and **B** and discs **C** and **D** are 4mm or less then the organism is demonstrating AmpC activity alone

Step 4 – Subtract **C** from **D.** If **D** – **C** ≥ 5mm **AND** when compared the differences in zone diameter between discs **A** and **B** is 4mm or less then the organism is demonstrating ESBL and AmpC combined activity.

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 against a negative control organism *E. coli* (ATCC® 25922) should be equal or show no greater difference in diameter than ±2mm. Any greater difference 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
Escherichia coli NCTC 13351	ESβL Positive
Escherichia coli NCTC 13352	ESβL Positive
Escherichia coli NCTC 13353	ESβL Positive
Enterobacter cloacae NCTC 13406	AmpC Positive
Escherichia coli ATCC® 25922	ESβL and AmpC Negative

Limitations
D68C is not suitable for use with *Pseudomonas* spp. or *Acinetobacter* spp. To avoid potentially erroneous results do not mix cartridges from different batches of D68C and ensure all discs in the set 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 production, which could also be masking concurrent ESβL or AmpC expression. Users are obliged to always use the latest version of the D68C calculator.

References
Bibliography available on request.

IFU179 GB 03/21 V13

*Formulation may change to meet performance criteria