MASTDISCS® Combi

Mast Group

ESBL Detection Set (EUCAST) - D76C

Frequently asked Questions and Answers

What does D76C detect?

D76C detects Extended Spectrum Beta-Lactamases (ESBLs) in Enterobacterales using the disc potencies as recommended by The European Society of Antimicrobial Chemotherapy (EUCAST).

What are the new disc potencies recommended by EUCAST?

The EUCAST standard disc potencies for antimicrobial susceptibility testing (AST) of cefotaxime and ceftazidime, are 5 and 10 µg, respectively. However, for ESBL confirmation, EUCAST (as CLSI) has previously recommended the use of cefotaxime 30µg and ceftazidime 30µg discs with and without clavulanic acid 10µg. EUCAST now recommends the use of combination discs with the same cephalosporin disc content (cefotaxime 5µg and ceftazidime 10µg) for standard disc diffusion and ESBL confirmation[1].

EUCAST has, in a multicenter study[2], compared the performance of previously recommended discs and the proposed new discs. Combination discs containing cefotaxime 5 µg ± CLAV 10 µg and ceftazidime 10 µg ± CLAV 10 µg showed the same sensitivity and specificity as discs containing cefotaxime 30µg and ceftazidime 30µg.

What is an ESBL?

Extended spectrum beta-lactamases (ESBL) are bacterial enzymes which confer resistance to penicillin and extended spectrum cephalosporin antibiotics in members of order Enterobacterales. They commonly express plasmid encoded beta-lactamases e.g. TEM, SHV and CTX-M, enabling the hydrolysis of 3rd generation cephalosporins including cefotaxime, ceftazidime and cefpodoxime. Most ESBLs are susceptible to cephamycins e.g. cefoxitin and cefotetan although there have been reports of resistance to these 2nd generation cephalosporins. Carbapenem antibiotics are usually the treatment of choice for infections due to ESBL producing organisms; although therapy should be monitored to ensure resistance does not arise through porin loss. ESBLs are inhibited by clavulanate.

Why is it important to detect ESBLs?

They hydrolyse broad spectrum antibiotics, which are the first line agents for many critically ill patients. Resistance can be shown against non-beta-lactam antibiotics e.g. aminoglycosides, limiting therapeutic options. Infections caused by such resistant organisms can increase the length of hospital stay and result in intensive care unit (ICU) admission. Inappropriate treatment of these complex infections can increase mortality and morbidity. Rapid detection of these enzymes allows for de-

escalation to more targeted therapy, to conserve carbapenem antibiotics for more serious infections. Hospital outbreaks can be caused by the spread of plasmids, leading to pathogen persistence, which can have a major impact on the financial cost to the healthcare setting (approximately €5000 per infection).

The misuse of carbapenems for the treatment of infection caused by Gram-negative organisms that are harbouring ESBL has led to increased carbapenem resistance. ESBLs are carbapenem susceptible and thereby differentiating carbapenemases from ESBL's and helps facilitate delivery of the appropriate targeted antibiotic therapy.

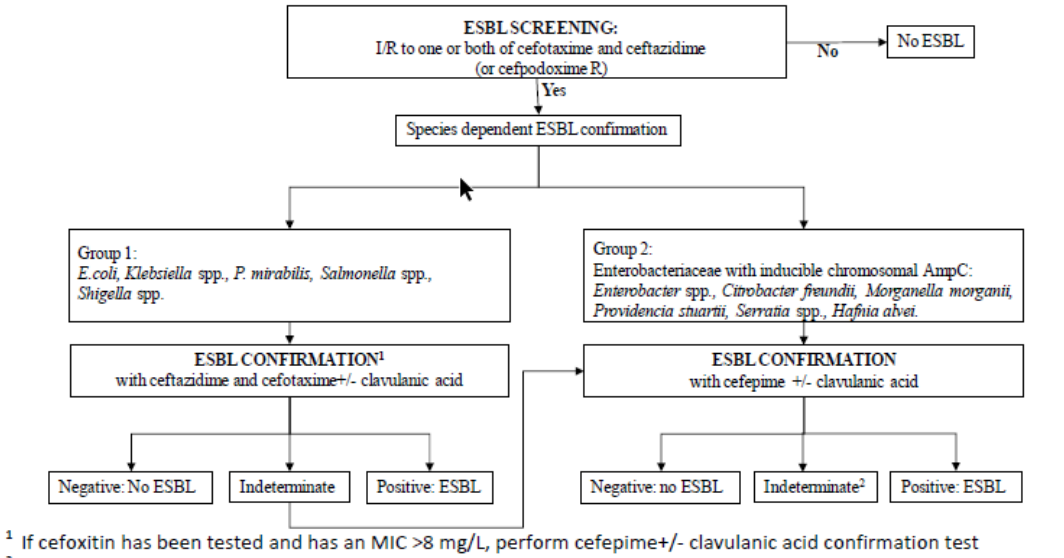
How will this test fit into my routine laboratory?

ESBL detection involves two steps:

1. A screening test with an indicator cephalosporin, to indicate resistance or reduced susceptibility to identify isolates that may be producing ESBL.
2. A confirmatory step to detect the synergy between an oxyimino cephalosporin and clavulanic acid, distinguishing isolates with ESBLs from those that are resistant due to different resistance mechanisms. See Figure 1 below from EUCASTs guidelines for detection of resistance mechanisms[3].

D76C is a confirmatory tests for ESBL isolates found to be resistant to the indicator cephalosporin from the ESBL screening step.

Figure 1 – EUCAST Algorithm for phenotypic detection of ESBLs



[1] If cefoxitin has been tested and has an MIC >8 mg/L, perform cefepime+/- clavulanic acid confirmation test

[2] Cannot be determined as either positive or negative (e.g. if a gradient diffusion strip cannot be read due to growth beyond the MIC range of the strip or there is no clear synergy in combination-disk and double-disk synergy tests). In confirmation with cefepime +/- clavulanic acid is still indeterminate, genotypic testing is required.

What are the limitations of Mast D76C ESBL Detection Set (EUCAST)?

To avoid potentially erroneous results do not mix cartridges from different batches of D76C, only use a combination discs plus clavulanic acid with the corresponding cephalosporin only disc from the same pack/batch. AmpC producing organisms may produce equivocal/false negative results. In Group 2 Enterobacteriaceae (*Enterobacter* spp, *Serratia* spp., *Citrobacter freundii*, *Morganella morganii*, *Providencia* spp, *Hafnia alvei*) a very common mechanism of cephalosporin resistance is derepressed chromosomal AmpC β-lactamase in these species. Since cefepime is stable to AmpC hydrolysis, it can be used in phenotypic testing with clavulanic acid (**MAST**DISCS® *Combi* Cefepime 30 & Cefepime 30/ Clavulanic Acid 10 - D63C).

If carbapenemase is suspected what can I use for detection?

If the interpretation is a suspected carbapenemase, **MAST**DISCS® *Combi Carba plus* (D73C) can be used to confirm carbapenemase production. D73C is a five disc detection set comprising of a penem antibiotic only disc, and with MβL inhibitors, KPC inhibitor and AmpC inhibitor discs. The fifth disc comprises of a temocillin disc with a MβL inhibitor. D73C detects MBL positive strains, KPC positive strains, OXA-48 positive strains and also can differentiate KPC positive isolates from isolates expressing *ampC* coupled with porin loss

What countries are affected?

The majority of countries throughout the world are affected. 'The Europe wide increase of antimicrobial resistance observed in *E. coli* in recent years is continuing unimpeded' (European Antimicrobial Resistance Surveillance Network Annual Report 2010).

What is the pack size?

4 x 50 cartridges sufficient for 50 tests:

- 1 x Cefotaxime 5µg discs
- 1 x Cefotaxime 5µg + Clavulanic acid 10µg discs
- 1 x Ceftazidime 10 µg discs
- 1 x Ceftazidime 10 µg + Clavulanic acid 10 µg discs

What is the shelf life and storage of D76C discs?

Store at 2 -8 ℃ in the containers provided until the expiry date shown on the pack label. Product in a properly maintained dispenser containing adequately charged desiccant is stable at 2-8℃ for 1 month.

Which dispensers do they fit?

Mast D76C will fit any MAST® DISCMASTER dispenser.

References

1. EUCAST technical guidance on the use of the combination disc test (CDT) for confirmation of ESBL in Enterobacterales. New disc potencies for combination discs containing cefotaxime and ceftazidime without and with clavulanic acid February 2019)

2. Can combination discs with EUCAST potencies for cefotaxime and ceftazidime be used for confirmation of ESBL in Enterobacteriaceae? Jenny Åhman, Erika Matuschek, Petra Edquist, Angela Camporeale, Karin Sjöström and Christian G. Giske (presented at ECCMID 2018)

3. EUCAST guidelines for detection of resistance mechanisms and specific resistances of clinical and/or epidemiological importance. Version 2.0, 2017.