

AmpC, ESBL and Carbapenemase Detection Set (D72C)

Frequently asked Questions and Answers

What does D72C detect?

D72C detects ESBL positive strains, AmpC (derepressed/hyperproduced, plasmid mediated and inducible) positive strains, co-production of AmpC and ESBL enzymes and also screens for the production of carbapenemase enzymes.

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.

What is an AmpC?

AmpC beta-lactamases are bacterial enzymes that hydrolyse 3rd generation extended spectrum cephalosporins and cephamycins engendering resistance to these categories of antibiotics. Such enzymes are produced as a result of hyper-production or induction of a chromosomally encoded AmpC enzyme, by the acquisition of a plasmid-mediated *ampC* gene (MOX, FOX, DHA, ACC, CIT, EBC, and CMY) or by the derepression of a chromosomal *ampC* gene. Carbapenem antibiotics are usually the treatment of choice for infections due to AmpC producing organisms although, like with ESBL infections, therapy should be monitored to ensure resistance does not arise through porin loss. Unlike ESBLs, AmpCs are not inhibited by clavulanate, but they are inhibited by class C inhibitors such as cloxacillin and boronic acid.

What is the importance of the different types of *ampC* expression?

Enterobacterales that acquire plasmids include *Escherichia coli*, *Klebsiella* spp., *Proteus mirabilis*, *Salmonella* spp. and *Shigella* spp. These are responsible for the rapid dissemination of resistance, and global clonal spread. Chromosomal *ampC* genes mostly occur in the following members of the order Enterobacterales - *Enterobacter* spp., *Citrobacter freundii*, *Morganella morganii*, *Providencia* spp., *Hafnia alvei* and *Serratia* spp. These can either be derepressed when the *ampC* gene is deregulated to actively produce the enzyme. Alternatively, they can occur as inducible strains which can be induced by various agents to produce the enzyme, and can mutate into derepressed strains. *E. coli* also has an intrinsic *ampC* gene, however this cannot be induced, but it can hyperproduce AmpC enzyme through mutation. Wild-type strains produce a basal level only of the enzyme which does not result in cephalosporin resistance. Whether AmpC enzymes are hyperproduced or result from plasmid encoded or derepressed *ampC* genes, they are all equally as significant, clinically. Inducible AmpCs are difficult to detect as they may look susceptible on the initial antibiogram, however if treated with a cephalosporin or clavulanate containing antibiotic, they can become resistant *in vivo*. Enterobacterales can also be co-producers of AmpC and ESBL enzymes.

Why is it important to detect AmpC and ESβLs?

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).

Why differentiate ESBLs from AmpCs?

It is important to actively 'seek' ESBLs and AmpCs, to minimise the reporting of false cephalosporin susceptibility. There is a possibility of underreporting AmpC incidence due to lack of reliable commercial tests, and some AmpCs look susceptible on first line screen. Cefoxitin is useful for screening for AmpCs, however not for confirming the presence of an AmpC as cefoxitin resistance can arise due to reduced permeability. Some physicians may assume that carbapenems are the drugs of choice for treating all infections due to Enterobacterales

isolates that demonstrate non-susceptibility to cefoxitin. However, in these cases the use of carbapenems may be unnecessary and may contribute to the increase of carbapenemase production. Some antibiotic combinations used to treat ESBL's can induce AmpC production e.g. piperacillin/tazobactam, so may result in failure in therapy if ESBLs and AmpC's are reported as just 'cefepodoxime resistant'. Although ESBLs can become resistant to carbapenems through porin loss, AmpC's are more likely to develop such carbapenem resistance, therefore, potentially resistance in AmpC producers is broader in spectrum than ESBL's. Treatment of ESBL's and AmpC is initially with a broad-spectrum antibiotic and rapidly deescalated once the susceptibilities are known.

What are carbapenemases?

Carbapenemases are a diverse group of enzymes (β -lactamases) that vary in their ability to hydrolyze carbapenems and other β -lactams. They are active against oxyimino-cephalosporins, cephamycins and carbapenems. Carbapenemase enzymes can be acquired via transmissible means or be chromosomally encoded. Carbapenemases belong to several Ambler classes - class A, B and D. Class A enzymes inactivate the β -lactam ring by means of a catalytically active serine residue in the enzyme active site, serine based penicillinases.

Why is it important to detect the carbapenemases?

It is important to detect carbapenemases as the majority of carbapenemase producing bacteria are extremely drug resistant and early detection is important in prevention of spread. Enterobacterales are carried in the bowel flora and are therefore highly transmissible when patients have diarrhoea or high dependency on healthcare professionals. Carbapenemase resistant Enterobacterales (CRE) often carry genes that confer resistance to other antimicrobials leading to limited therapeutic options. Pan-resistant KPC producing

strains have been reported and CRE have been associated with high mortality rates. Infections caused by CRE are notifiable in certain regions of the world.

Why do we need to differentiate carbapenemases from ESβL's and AmpC's?

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

What are the limitations of Mast D72C ESβL, AmpC and carbapenemase detection test?

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. Organisms producing a fully resistant profile i.e. no zone of inhibition on all discs could indicate demonstration of an MBL or KPC carbapenemase. A small proportion of non-carbapenemase producing Enterobacteriales may demonstrate resistance to disc F. The presence of ESβLs may be masked by carbapenemases. As the third generation cephalosporins are generally not zwitterionic, the membrane penetration ability of the D72C cephalosporin base is limited in a proportion of organisms that display high levels of impermeability or loss of porins. ESβL and AmpC producing organisms with reduced permeability may produce equivocal results.

What does the interpretation 'suspected carbapenemase or suspected ESβL/AmpC with porin loss' mean?

When D72C interprets the result as "suspected carbapenemase or suspected ESβL/AmpC with porin loss" this implies that the organism may be producing a carbapenemase, and this should be confirmed using D73C (**MASTDISCS® Combi Carba plus**).

What does the interpretation 'Equivocal' mean?

When D72C interprets the result as "Equivocal" this implies that further tests may need to be carried out; in order to determine the nature of the resistance mechanism which may involve more complex systems such as combinations of enzyme expression coupled with membrane impermeability, loss of porins or a resistance mediating enzyme that may not be within the

scope of the test. Equivocal interpretations may also be seen if the test results have been entered incorrectly and/or the testing methodology has not been properly adhered to.

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).

Distortion / flattening of the edge of the zone surrounding discs A and D?

When testing an organism with inducible AmpC activity, distortions of the zone's of inhibition surroundings discs A and/or D can sometimes be seen (this is assuming the discs have been arranged in alphabetical order). Should this occur, take the measurement of the zones from an area not affected by the distortion and record results as normal.

What is the pack size?

6 x 50 cartridges, sufficient for 50 tests.

What is the shelf life and storage of D72C discs?

Store at 2 -8 °C 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°C for 1 month

Which dispensers do they fit?

Mast D72C will fit any MAST[®] DISCMASTER dispenser.