AmpC and ESBL Detection Set (D68C) Frequently asked Questions and Answers

What is an ESβL?

Extended spectrum beta-lactamases (ESβL) are bacterial enzymes which confer resistance to penicillin and extended spectrum cephalosporin antibiotics in members of the family Enterobacteriaceae. They commonly express plasmid encoded β-lactamases e.g. TEM, SHV and CTX-M, to enable them to hydrolyse 3rd generation cephalosporins including cefotaxime, ceftazidime and cefpodoxime. Most ESβLs 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 ESβL producing organisms; although therapy should be monitored to ensure resistance does not arise through porin loss. ESβLs 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 antibiotic. 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 ESβL infections, therapy should be monitored to ensure resistance does not arise through porin loss. Unlike ESβLs, 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?

Enterobacteriaceae 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 family Enterobacteriaceae - 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. Or, 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. Enterobacteriaceae can also be co-producers of AmpC and ESBL enzymes.
Why is it important to detect AmpC and ESBLs?

They hydrolyse broad spectrum antibiotics, which are the first line agents for many critically ill patients. Resistance can be shown against non-β 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).

What does D68C detect?

D68C detects ESBL positive strains, AmpC (derepressed/hyperproduced and plasmid mediated) positive strains, and co-production of AmpC and ESBL enzymes.

Why differentiate ESβL’s from AmpC?

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 Enterobacteriaceae 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 ESβL’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 ‘cefpodoxime 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 ESβL’s.
Treatment of ESβL’s and AmpC is initially with a broad spectrum antibiotic and rapidly de-escalated once the susceptibilities are known.

How will this test fit into my routine laboratory?

On the initial antibiogram, antibiotic susceptibility testing should be performed with either: both cefotaxime and ceftazidime, or cefpodoxime for the screening of ESBLs and AmpCs, and further screening with Cefoxitin for AmpCs, as isolates may not show resistance to third generation cephalosporins on the first line antibiotic panel.
Perform ESβL and AmpC confirmatory tests on isolates found to be resistant to the above antibiotics using the Mast D68C AmpC and ESβL Detection Set.
What are the limitations of Mast D68C ESβL and AmpC detection test?

To avoid potentially erroneous results do not test cartridges from different batches together-they should never be mixed. Organisms producing a fully resistant profile, i.e. no zones of inhibition on all discs could indicate the possibility of a MBL or KPC carbapenemase production, which could be masking concurrent ESβL or AmpC expression.

What does the interpretation ‘further work required’ mean?

When D68C interprets the result as ‘further work required’ this means that the zone diameters do not fit the specific criteria set. This is another interpretation, and does not mean that the isolate is ‘negative’ or ‘susceptible’. This interpretation should prompt the user to carry out further tests. This is usually in cases where the resistance mechanism is more complicated, either when the isolate is co-producing ESBL and inducible AmpC or if it has an inducible AmpC alone. D68C was not designed to detect inducible AmpCs, but ESBLs and AmpCs with cephalosporin resistance through plasmid-acquisition, hyperproduction and derepression.

The further work recommended is as follows:

- D69C (AmpC Detection Set) – this can be used to detect all types of AmpC production including inducible strains due to the novel use of an AmpC inducer contained within the test. This can detect the AmpC in the presence of an ESBL producer, which will not affect the test. However it cannot be used for ESBL detection.
- D63C (Cefepime 30ug and Cefepime 30ug/Clavulanate10ug) - The presence of an ESBL can be detected by using an AmpC stable cephalosporin e.g. (D63C) in species where an inducible AmpC may be present

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, therefore sufficient for 50 tests.

What is the shelf life and storage of D68C 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 D68C will fit any Mast disc dispenser.