mastdiscs® ID

Mast Group

MAST®ICT (D74)

Frequently Asked Questions and Answers

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. They are inhibited by clavulanate (various degree of inhibition), tazobactam and boronic acids and usually hydrolyse cephalosporins or penicillin more effectively than carbapenems. This class of enzymes includes KPC (*Klebsiella pneumoniae* carbapenemases), IMI, SME, NMC-A and GES enzymes. They are commonly produced by members of the Enterobacteriaceae but have also been detected in *Acinetobacter baumannii* and *Pseudomonas aeruginosa*.

Class B enzymes are known as Metallo β-lactamases (MBL) and can hydrolyse carbapenems efficiently but not aztreonam. They require zinc as a metal co-factor for their catalytic activity and are inhibited by chelating agents such as EDTA. MBLs include the IMP VIM and NDM families and SPM-1, and have been detected in *P. aeruginosa*, members of the Enterobacteriaceae family and *A.baumannii*.

Class D enzymes have an active serine residue and hydrolyze carbapenems weakly and are inhibited weakly by clavulanate. Class D enzymes belong to the OXA family and are most commonly produced by *Acinetobacter* spp. but have also been identified in *P.aeruginosa, E. coli* and *K. pneumoniae* strains.

What is a MBL?

Metallo β-lactamases belong to Class B β-lactam enzymes; despite significant sequence diversity at the amino acid level they share three distinct functional properties. They are capable of hydrolysing carbapenems inhibited by chelating agents such as EDTA. They are unable to inactivate aztreonam as B1 MBLs bind monobactams with a very low affinity and the positioning of the drug in the MBL active site does not favour hydrolysis. MBLs can be chromosomally mediated or encoded by transferable genes. Due to molecular sequencing chromosomal mediated genes have been increasingly found however these tend to be present in obscure non-clinical bacteria.

What is a KPC?

KPC or *Klebsiella pneumonia* carbapenemases belong to Ambler Class A. An active-site serine residue at position 70 (according to the Ambler numbering system) is required for hydrolysis. They are characterised by having reduced susceptibility to imipenem and are inhibited by clavulanate, tazobactam and boronic acids. KPCs are capable of hydrolysing a broad range of β-lactams including penicillins, aztreonam, carbapenems and cephalosporins.

KPC's can be differenced from the other member of the 2f enzymes group (Ambler class A) which accounts for a notable proportion of the carbapenemases utilising serine at thire active site, by two characteristics. The sequence for KPC enzymes are found on transferable plasmids, and they can hydrolyse aminothiazoleoxime cephalosporins e.g. cefotaxime. Due to being located on transferable plasmids, KPCs have the greatest potential to spread and *K.pneumoniae* is known for its ability to transfer and accumulate resistance determinants.

What is an OXA-48-like carbapenemase?

OXA-48-like carbapenemases belong to Ambler Class D and contain an active site serine. These have a wider range of activity on substrates than AmpC enzymes. OXA-48-like enzymes hydrolyse aminopenicillins, ureidopenicillins and carbapenems at low levels, but do not significantly hydrolyse broad-spectrum cephalosporins. OXA-48-like carbapenemases are inherently difficult to detect due to their low-level resistance and the lack of specific inhibitors.

Why is it important to detect them?

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. Enterobacteriaceae are carried in the bowel flora and are therefore highly transmissible when patients have diarrhoea or high dependency on healthcare professionals. Carbapenem resistant Enterobacteriaceae (CRE) often carry genes that confer resistance to other antimicrobials leading to limited therapeutic options. Pan-resistant KPC producing strains have been reported world wide and CRE have been associated with high mortality rates. Infections caused by CRE are notifiable in certain regions of the world

What is MAST®ICT (D74)?

MAST®ICT (D74)is a single test paper based device with three tabs. The central tab is impregnated with a penem, where as the outer tabs are impregnated with a mixture of AmpC inhibitor, ESβL inhibitor and porinating agents. This is used to detect the presence of carbapenemase activity in *Enterobacteriaceae*, *Pseudomonas* and *Acinetobacter* spp. D74 is based on the Indirect Carbapenemase Test and works on similar principles.

What does MAST®ICT (D74) detect?

D74 detects the presence of carbapenemase production in *Enterobacteriaceae, Pseudomonas and Acinetobacter* spp. This includes MβL or KPC and OXA resistance mechanisms but doesn't differentiate been them.

How does MAST®ICT (D74) work?

MAST®ICT is based on the principles of the Indirect Carbapenemase Test (ICT). The ICT method utilises a cell permeabilizing agent to release Carbapenemase enzyme from a Carbapenemase-Producing-Organism (CPO) to hydrolyse an indicator antibiotic in the test system. This allows a normally carbapenem susceptible reporter organism to grow where antibiotic has been hydrolysed, thus producing a distorted zone of inhibition. If the test organism produces no carbapenemase, the reporter organism will form a regular, circular zone of inhibition around the indicator tip.

Why do we need to differentiate carbapenemases from ESβLs and AmpCs?

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

Which organisms produce carbapenemase?

Enterobacteriaceae, *P.aeruginosa* and *A. baumannii* produce carbapenemases.

Would you know prior to testing if you were looking for a *Pseudomonas* or *Acinetobacter spp* and select an appropriate reporter organism?

We would assume that most customers would be using this product after bacterial identification. However, if the identification is unknown then yes, it would be necessary to test for both eventualities (i.e. *Escherichia coli* ATCC®26922 and *Klebsiella pneumoniae* ATCC®70063 reporter organisms incubated both aerobically and anaerobically, respectively).Furthermore ,the user would need to include more positive control organisms

How many devices would I need to use to cover all possible resistance mechanisms?

There are six quality control organisms listed on the IFU. However, the user is not required to test all of these. The user should select at least one representative organism to demonstrate a positive reaction and one organism to demonstrate a negative reaction. The choice of QC organism ids determined by the organism being tested (assuming species

identification has already taken place); for example, if *Pseudomonas aeruginosa* is being tested, Ps. *aeruginosa* NCTC 13437 is an appropriate positive control. Similarly, if an Enterobacteriaceae is being tested, at least one *Klebsiella pneumonia* QC strain should be tested. There are three *K.pneumoniae* QC organisms that demonstrate a positive reaction. Each strain produces a different carbapenemase. For QC purpose it is not necessary to test all three, but users may find it helpful to have a comparison for each resistance mechanism. The OXA-48 producer (*Klebsiella pneumoniae* NCTC 13442) in particular generates a weaker result.

What are the limitations of the MAST®ICT (D74)?

MAST®ICT (D74) detects the presence of MBL, KPC and OXA resistance mechanisms, but does not distinguish them or the type of enzyme acquisition e.g. VIM, IMP, NDM etc.

What countries are affected by carbapenemases?

Carbapenemases are a global issue with a high level of incidence present in several regions such as South America, USA, Asia and Europe.

What is the incidence in the UK?

A report issued by the Health Protection Agency in October 2016 showed an increase in the number of confirmed carbapenemase positive isolates in the UK, rising from three (3) in 2003 to one thousand eight hundred and ninety three (1893) in 2015.

What is the pack size?

Each pack contains 25 individually packaged devices 1 device is required per test.

What is the shelf life and storage of MAST®ICT (D74)?

MAST®ICT should be stored at 2-8°C in the containers provided until the expiry date shown on the pack label.