MAST® CARBA PAcE
Frequently Asked Questions and Answers

1. Product FAQs

What is MAST® CARBA PAcE?

MAST® CARBA PAcE is a rapid colorimetric assay designed to identify carbapenemase production in pure bacterial isolates of Enterobacterales, *Pseudomonas* and *Acinetobacter* spp.

What does MAST® CARBA PAcE detect?

MAST® CARBA PAcE detects the production of carbapenemase enzymes in Enterobacterales, *Pseudomonas* and *Acinetobacter* spp. It contains a novel chromogenic cephalosporin analogue, that is hydrolysed by carbapenemase enzymes, producing a colour change from yellow to orange/red. Organisms that do not demonstrate carbapenemase activity will not produce a colour change.

How does MAST® CARBA PAcE work?

Each kit comprises of 4 vials of freeze-dried pellets (VIAL PEL) and 4 vials of reconstitution buffer (VIAL RB). When mixed together, the reconstitution buffer rapidly dissolves the pellet, giving the final active solution. This solution is aliquoted into the tubes provided in the kit and colonies of the bacterial test isolate are added and vortexed to produce a turbid suspension. After incubation at 35±1℃ for 10 minutes the colour is observed by eye. A colour change from yellow to orange or red indicates the presence of carbapenemase enzymes.

Which organisms produce carbapenemase?

Enterobacterales, *P. aeruginosa* and *A. baumannii* produce carbapenemase enzymes and can be screened using **MAST®** CARBA PAcE.

What inoculum size/density is required to achieve good results with MAST® CARBA PAcE?

It is recommended that a 1-5 µl loopful (see Figure 1) of a fresh (≤24 hours old), pure culture is taken from a solid, non-selective culture medium. The bacterial inoculum should be vortexed for a minimum of 20 seconds to give a homogenous, turbid suspension equivalent to a 3.0 – 3.5 McFarland standard (approx. 10^9 CFU/ml) (see Figure 2). Failure to use a sufficient inoculum density or failure to ensure a homogenous suspension can lead to false negative results.

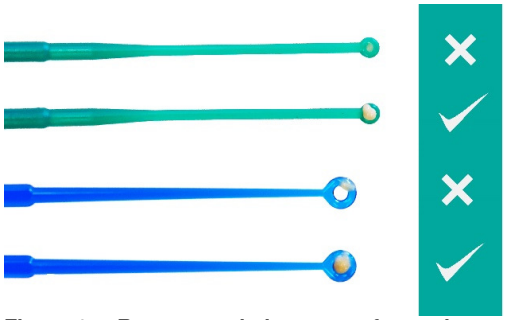

Figure 1 – Recommended amount of organism required - the green loop is a 1µl loop, and the blue loop is a 5µl loop

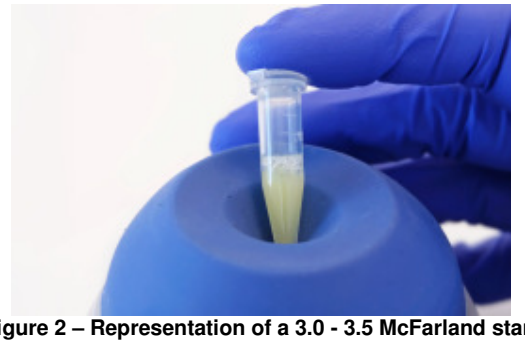

Figure 2 – Representation of a 3.0 - 3.5 McFarland standard equivalent suspension

Can I use MAST® CARBA PAcE if the test organism produces coloured pigments?

Because this is a colorimetric test, it is not recommended to use **MAST®** CARBA PAcE with organisms that produce strongly coloured pigments such as *Serratia* spp. It is advisable to check the colour of the solution immediately after inoculation; if it is not yellow then the test should be discontinued. *Pseudomonas* spp. may give a yellow/green colour, which is acceptable, and a positive result can be clearly distinguished, Figure 3 below shows two negative controls *Acinetobacter lwoffi* ATCC® 15309, and *Pseudomonas aeruginosa* ATCC® 25668 from left to right. In line with the Instructions for Use (IFU159), it is advised that internal controls must be performed to demonstrate a positive reaction and another to demonstrate a negative reaction.

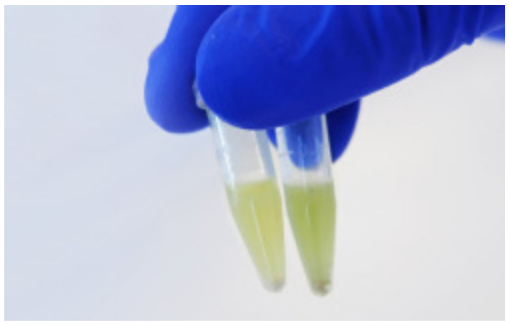

Figure 3 – Negative controls *Acinetobacter lwoffi* ATCC® 15309, and *Pseudomonas aeruginosa* ATCC® 25668 from left to right

Can I use MAST® CARBA PAcE from colonies from selective, differential or chromogenic culture media?

MAST® CARBA PAcE is suitable for testing using organisms cultured on non-selective, nutrient rich agars, including Columbia agar with and without horse blood, and chocolate agar. Results from internal studies show that organisms taken from agar media containing pH indicator for colony colour differentiation e.g. MacConkey agar, are not compatible and require subculturing on a non-selective nutritious agar before testing.

A total of six commercially available chromogenic media were tested, certain brands gave a high number of incorrect results, and only two were found to be acceptable, another trial is currently ongoing with another two commercially available chromogenic media, results of which will be updated on these FAQs once available. The current advice is to test using organisms taken from non-selective, nutritious media and testing from differential, selective or chromogenic agar is advised against.

Can I read results obtained with MAST® CARBA PAcE if the tests have been left on the bench for longer than 20 minutes after incubation?

It is not recommended that results are read more than 20 minutes after incubation as the colour may start to change, leading to false positives or negative results.

What are the limitations of MAST® CARBA PAcE?

MAST® CARBA PAcE is only able to identify carbapenemase production, and cannot differentiate between the different types of carbapenemases. If it is necessary to identify the carbapenemase produced, it is recommended that follow up testing is performed, such as **MAST**DISCS® *Combi Carba Plus* (D73C) or molecular testing.

MAST® CARBA PAcE cannot be performed using bacterial colonies taken from selective, differential or chromogenic agar. Organisms producing strongly coloured pigments are not suitable for testing using **MAST®** CARBA PAcE.

Production of certain beta-lactamases may cause false positive results, for example the K1 β-lactamase in *Klebsiella oxytoca*. Some GES-type carbapenemases might be difficult to detect.

Results obtained with this kit must be considered alongside other clinically relevant data when diagnosing an infection.

To avoid potentially erroneous results, do not mix vials from different batches.

What is the pack size?

Each pack contains 4 vials of each VIAL PEL and VIAL RB, which is sufficient to perform 48 tests.

What is the shelf life and storage of MAST® CARBA PAcE?

The shelf-life is 12 months when stored at 2 to 8 °C. Once reconstituted, the test solution must be stored at 2 to 8 °C and used within four weeks. Ensure that the reconstituted solution is clearly labelled as such and includes a date of expiry.

2. Resistance mechanism FAQs

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 Enterobacterales 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 Enterobacterales 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 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 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. Enterobacterales are carried in the bowel flora and are therefore highly transmissible when patients have diarrhoea or high dependency on healthcare professionals. Carbapenem 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 world-wide and CRE have been associated with high mortality rates. Infections caused by CRE are notifiable in certain regions of the world

Why is it important to screen for carbapenemases?

Although routine susceptibility testing can identify organisms with reduced susceptibility to carbapenems, this method lacks specificity as some carbapenemases (such as OXA-48 like enzymes) cause low level resistance which may not be detected through routine susceptibility testing. Without screening for carbapenemases, this could result in treatment failure. Screening for carbapenemases is also important from an epidemiological perspective, as carbapenemase enzymes are often encoded on highly transmissible mobile genetic elements such as plasmid. Early detection can enable the appropriate infection control measures to be implemented to limit their dissemination. Screening can differentiate carbapenemase producing organisms from organisms that are carbapenem resistant through an alternative mechanism (for example, porin loss in combination with efflux pump upregulation), the latter of which are of less epidemiological significance.