

Evaluation of the MAST ISOPLEX[®] CRE-ART for detection of genes encoding carbapenemases in Enterobacterales, *Pseudomonas aeruginosa* and *Acinetobacter* species.

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Introduction

- MAST ISOPLEX[®] CRE-ART kits (ref: DNA/LYO5, Lot: 417054, Expiry: 2020-02) were referred to the Freeman Hospital Microbiology department for evaluation. The kits were stored at +4°C and all tests performed between the 15th July and 7th August 2019.
- Each isolate was retrieved from storage in glycerol skimmed milk by culture on a full plate of chromID CPS Elite medium (a chromogenic agar that facilitates differentiation of species and detection of mixed bacterial populations).
- Only strains that presented as pure cultures after overnight incubation, and generated the expected chromogenic reaction were retained for evaluation.
- Suitable strains were anonymised and subcultured on Columbia blood agar for 18-20 hours at 37°C before inoculation onto Mueller-Hinton and incubation at 37°C overnight.
- A total of 248 isolates were tested in the Freeman Hospital Microbiology Department according to manufacturer instructions. The isolates included Enterobacterales (*n*=203), *Acinetobacter* species (*n*=37) and *Pseudomonas aeruginosa* (*n*=8).

Methods

- Bacterial growth from an isolated colony was suspended in 90µl of reconstitution buffer (RB3).
- 10µl of reconstitution inhibition control was added to the bacterial suspension and the contents of the tube gently mixed.
- The sample was placed on a heat block at 95°C for 5 minutes before cooling at -20°C for 5 minutes.
- A CRE-ART reaction strip was opened carefully to ensure reagents were not disturbed and 10µl of the test material was inoculated into each well of the 8-well strip.
- The LAMP reaction was then monitored in an ABI 7500 real time PCR instrument at 63°C with positive results determined within a reaction time of 30 minutes (i.e. 30 cycles).

Results Summary

	No. Tested	No. Detected (%)	Mean Ct Value	Range
Internal Control (All isolates)	248	248 (100%)	7.55	6.06 – 12.5
Carbapenem Producing Isolates	186	186 (100%)	6.8	5.01 – 10.37
Carbapenem Producing Isolates (mutation not targeted by test)	19	0	-	-
Isolates without Carbapenemase	53	2 (3.8%) FP	23.15	20.91 – 25.4

- “In our hands, CRE-ART was highly effective at detecting carbapenemase genes targeted by the assay”
- “None of the targets remained undetected”
- “Assay was very simple to perform and generated rapid results”

Discussion

- Detection of an *Acinetobacter* isolate with OXA-40 was assigned as OXA-24. This may be due to a taxonomic issue with OXA β -lactamases.
- According to Evans and Amyes – “The second group of OXA-type lactamases from *A baumannii* to be identified was the OXA-40 group. The founding member of this group, OXA-24, which was subsequently renamed OXA-40, was identified in isolates in 1997. *Evans BA, Amyes SG. OXA β -lactamases. Clin Microbiol Rev. 2014, Apr;27(2):241-63*
- The Ct values were relatively high (>20) for both false positive isolates and were negative when re-tested.
- The occurrence of weak false positives may have been due to cross contamination of reagents between wells. It was noted that several false positives were clustered in the same run.

Full Results:

	No. Tested	No. Detected (%)	Mean Ct Value	Range
Internal Control (all isolates)	248	248	7.55	6.06 – 12.5
CPE	154	154 (100%)	6.5	5.01 – 10.37
KPC	16	16 (100%)	6.7	5.53 – 7.35
VIM	13	13 (100%)	6.6	5.2 – 7.62
IMP	12	12 (100%)	8.4	7.06 – 10.37
OXA-48	61	61 (100%)	6.9	5.08 – 10.23
NDM	52	52 (100%)	5.7	5.01 – 7.91
CP – <i>P. aeruginosa</i>	4	4 (100%)	5.9	5.23 – 6.6
VIM	2	2 (100%)	5.8	5.23 – 6.48
NDM	2	2 (100%)	6.0	5.44 – 6.6
CP – <i>Acinetobacter</i> species	28	28 (100%)	7.3	6.4 – 8.3
OXA-23	23	23 (100%)	6.4	5.59 – 7.14
OXA24 / 40	2	2 (100%)	8.3	7.25 – 9.33
NDM	3	3 (100%)	7.2	6.72 – 7.53
Isolates with Carbapenemase not targeted by the assay	19	0	-	-
NMC-A (CP - Enterobacterales)	1	0	-	-
OXA-51 (CP – <i>Acinetobacter</i> spp.)	12	0	-	-
OXA-58 (CP – <i>Acinetobacter</i> spp.)	5	0	-	-
OXA-69 (CP – <i>Acinetobacter</i> spp.)	1	0	-	-
Isolates without carbapenemase	53	2 (3.8%) FP	23.15	20.91 – 25.4

* 46 carbapenemase enzymes in 36 *Acinetobacter* isolates