European Meeting on Molecular Diagnostics
11th edition

October 09 - 11, 2019
Noordwijk, Netherlands

Luton and Dunstable University Hospital
NHS Foundation Trust

An investigation of the compatibility of a new molecular transport medium (Sigma MM™) with a PCR analytical platform

Kamran Khan
Luton and Dunstable University Hospital, Luton, United Kingdom

Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a major nosocomial pathogen that causes severe morbidity and mortality worldwide (Cookson *et al.* 2011). In 2017/2018, a mandatory population-based bacteraemia surveillance scheme reported a total of 12,784 MRSA bacteraemia and methicillin-susceptible *S. aureus* (MSSA) cases in England alone (PHE 2018). Alongside this, CRE (Carbapenem-resistant *Enterobacteriaceae*) are an emerging threat to public health producing high levels of resistance to antibiotics. These CRE bacteria contain resistant genes, known as; KPC, OXA-48, NDM, VIM AND IMP-1 which can be detected via PCR. MRSA and CRE specimens represent a significant proportion of routine microbiological specimens and are very important for the management of critically ill patients. In recent years the introduction of self-contained cartridge systems for the rapid identification of target pathogens using nucleic acid amplification has transformed the ability of clinical microbiology laboratories to provide accurate and timely diagnostic data, allowing correct treatment to be commenced immediately, and also preventing unnecessary treatment. When culture is not required, it can be convenient and safer to transport the specimen in a medium which effectively inactivates the pathogen without disrupting the DNA or RNA so that analysis is still possible. If such a system is used, however, it is essential to know that the medium will be compatible with such an analysis.

For this study we investigated the ability of Sigma MM™, a liquid transport medium developed by Medical Wire and Equipment (MWE), to recover analytical quality DNA from specimens spiked with MRSA and CRE specimens, while demonstrating that the biological pathogen had been inactivated and lysed. The PCR analysis was performed using Cepheid GeneXpert ® PCR analyser and Cepheid Xpert MRSA and CARBA-R kits. The results for both PCR and culture were compared with those for identical specimens inoculated into Sigma-Transwab ® (liquid Amies medium), or Sigma MM™ as appropriate.

Methods

MRSA

Samples were spiked with known positive MRSA strain NCTC 12493. A concentration of 0.5 McFarland bacterial was prepared in sterile saline and diluted 10^{-1},10^{-2}, and 10^{-3}. Aliquots of 100µl for each dilution were inoculated directly into the tubes of 6 Sigma Transwabs® and 6 Sigma MM™, giving a total of 18 inoculated devices. A further 2 devices of each type were inoculated with 100µl of sterile saline as negative controls.

CRE

The same method was used for the CRE testing with strains *Klebsiella pneumoniae* NCTC13438 (KPC), *Pseudomonas aeruginosa* NCTC13437 (VIM), *Klebsiella pneumoniae* NCTC13443 (NDM), *E.coli* NCTC13476 (IMP-1) and *Klebsiella pneumoniae* NCTC13442 (OXA-48) 100µl of each bacterial suspension being inoculated into 2 Sigma Transwabs® and 2 Sigma MM™, giving a total of 60 inoculated devices. After a holding time of 24 hours all devices were tested by inoculating into the test kits for GeneXpert, and by plating onto MRSA Chromogenic agar plates, and CRE chromogenic agar as appropriate. Specimens for GeneXpert were processed according to the manufacturer's instructions. All agar plates were incubated at 37^{O}C for 18 hours.

This study was supported by MWE
mwe medical wire
www.mwe.co.uk

References
1. Cookson, B., Bonten, M.J.M., MacKenzie, F.M., Skov, R.L., Verbrugh, H.A., Tacconelli, E. (2011) Meticillin-resistant Staphylococcus aureus (MRSA): screening and decolonisation. *International Journal of Antimicrobial Agents*. **37**:195-201. doi:10.1016/j.ijantimicag.2010.10.023.
2. Public Health England, (2018) "Annual epidemiological commentary: MRSA, MSSA and E. coli bacteraemia and C. difficile infection data, up to and including financial year April 2017 to March 2018," Public Health England, London.
3. Edmond, M.B., Ober, J.F. and Bearman, G. (2008) "Active surveillance cultures are not required to control MRSA infections in the critical care setting", *Journal of Infection Control*, **36** (6), 461-463

Results

Table 1. CRE findings on Cepheid GeneXpert ® PCR using Sigma Transwab ®

Dilution	Samples tested for each organism	Target	Incubation culture growth results after 18 hours	Target	Incubation culture growth results after 18 hours	Target	Incubation culture growth results after 18 hours	Target	Incubation culture growth results after 18 hours	Target	Incubation culture growth results after 18 hours
		IMP-1		VIM		OXA-48		KPC		NDM	
10-1	2	Detected	Moderate Growth	Detected	Moderate Growth	Detected	Moderate Growth	Detected	Moderate Growth	Detected	Moderate Growth
10-2	2	Detected	Moderate Growth	Detected	Moderate Growth	Detected	Moderate Growth	Detected	Moderate Growth	Detected	Moderate Growth
10-3	2	Detected	Scanty Growth	Detected	Scanty Growth	Detected	Scanty Growth	Detected	Scanty Growth	Detected	Scanty Growth

Table 2. CRE findings on Cepheid GeneXpert ® PCR using Sigma MM™

Dilution	Samples tested for each organism	Target	Incubation culture growth results after 18 hours	Target	Incubation culture growth results after 18 hours	Target	Incubation culture growth results after 18 hours	Target	Incubation culture growth results after 18 hours	Target	Incubation culture growth results after 18 hours
		IMP-1		VIM		OXA-48		KPC		NDM	
10-1	2	Detected	No Growth	Detected	No Growth	Detected	No Growth	Detected	No Growth	Detected	No Growth
10-2	2	Detected	No Growth	Detected	No Growth	Detected	No Growth	Detected	No Growth	Detected	No Growth
10-3	2	Detected	No Growth	Detected	No Growth	Detected	No Growth	Detected	No Growth	Detected	No Growth

Table 3. MRSA findings on Cepheid GeneXpert ® PCR using Sigma Transwab®

Dilution	Samples Tested	Target	Incubation culture growth results after 18 hours
		MRSA	
10-1	6	Detected	Moderate Growth
10-2	6	Detected	Moderate Growth
10-3	6	Detected	Scanty Growth

Table 4. MRSA findings on Cepheid GeneXpert ® PCR using Sigma MM™

Dilution	Samples Tested	Target	Incubation culture growth results after 18 hours
		MRSA	
10-1	6	Detected	No growth
10-2	6	Detected	No Growth
10-3	6	Detected	No growth

All negative controls gave negative PCR results and no growth on culture

Discussion and Conclusion

With reports of multi-drug resistant organisms continuing to increase and therapeutic options decreasing, infection control methods are of increasing importance. The launch of Sigma MM™, a commercial specimen inactivation/transport medium enables downstream molecular processing of MRSA sample. This screening strategy uses a testing modality with a rapid turnaround time (less than one hour) whilst inactivating the biological pathogen but preserving its DNA and RNA, therefore increases the capacity of laboratory technicians to execute more demanding tasks.

This study has shown that both the Sigma MM™ devices and the culture based Sigma Transwab® devices are compatible with the GeneXpert PCR analyser for both MRSA and CRE. There was no interference with the chemistry. The Sigma MM™ devices were also completely effective at killing the MRSA and CRE at all concentrations, rendering the specimens non-infective.