Evaluation of Microbial Viability and Nucleic Acid Integrity After Exposure to New Molecular Transport Medium (Sigma MM™)

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INTRODUCTION

Sigma MM™ is a new molecular medium described as revolutionary due to its ability to kill bacteria and viruses whilst releasing intact nucleic acids for molecular diagnostics. As sample collection often occurs outside the laboratory and transport of the specimen requires safe and robust handling, ensuring the integrity of the sample is preserved and maintained is of utmost importance.

The aim of this study was to evaluate the new molecular transport medium (Sigma MM™) for its ability to kill microorganisms whilst preserving and maintaining DNA integrity.

METHODS

RAPID INACTIVATION ASSAY

Organisms used - *Escherichia coli, Enterococcus faecalis, Staphylococcus aureus, Methicillin-resistant Staphylococcus aureus* (MRSA), *Pseudomonas aeruginosa, Salmonella typhimurium, Streptococcus pyogenes, Klebsiella pneumoniae, Candida auris, Candida albicans, Haemophilus influenzae.*

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Cultures grown on appropriate agar and under appropriate conditions then used to inoculate phosphate buffered saline (PBS) using the 0.5 McFarland standard as reference.

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100 µL of culture used to inoculate Sigma MM™. Sample was incubated at room temp for 1 min and 2 min after which a 100 µL aliquot was removed and plated. Aliquot was also removed at 1 minute and used to inoculate 10 mL of TSB which was incubated at room temperature for 10 days and observed for turbidity indicating growth.

NUCLEIC ACID INTEGRITY ASSAY

Overnight bacterial culture centrifuged at 5000 x *g* for 10 min

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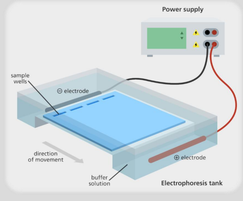
Pellet resuspended in Sigma MM™ and incubated at room temp for 10 min

DNA extraction using QIAGEN DNEasy Blood and Tissue Kit

DNA analysis using Nandrop One and agarose gel electrophoresis



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RESULTS AND DISCUSSION

RAPID INACTIVATION ASSAY

The Sigma MM™ molecular medium was effective at killing all the organisms (Gram-positive, Gram-negative and yeast) within 2 minutes of exposure. Also no turbidity was observed in the microtitre wells after a 10 day holding period (Table 1).

Table 1. Rapid inactivation of bacteria and fungi (within 1 minute) following incubation with Sigma MM™ molecular medium. Data obtained from 3 independent experiments.

Microorganisms	Untreated control CFU/ml	Treated (1 min) CFU/ml	Treated (2 min) CFU/ml	10 day Holding period
Escherichia coli	1.31E+08	0	0	No turbidity
Enterococcus faecalis	9.71E+07	0	0	No turbidity
Staphylococcus aureus	8.51E+07	0	0	No turbidity
MRSA	1.40E+08	0	0	No turbidity
Pseudomonas aeruginosa	7.98E+07	0	0	No turbidity
Salmonella typhimurium	1.56E+08	0	0	No turbidity
Streptococcus pyogenes	1.69E+08	0	0	No turbidity
Klebsiella pneumoniae	6.54E+07	0	0	No turbidity
Candida auris	2.00E+07	0	0	No turbidity
Candida albicans	1.57E+07	0	0	No turbidity
Haemophilus influenzae	6.94E+07	0	0	No turbidity

FIGURE 1 – Agarose gel electrophoresis of DNA extracted from bacteria using the QIAGEN DNeasy Blood and Tissue Kit. Lane L, 1 kbp DNA Ladder; lane 1-3, *E. coli* replicates; lane 4-6, MRSA replicates.

DNA extraction after incubation of the test organisms (*E. coli* and *S. aureus*) in Sigma MM™ was successful. The DNA extract was of good quality and high purity demonstrated by A_{260}/A_{280} ratios between 1.8 – 2.0. Using gel electrophoresis, we were successful at visualising DNA extracted from *E. coli,* though poor yield of MRSA resulted in faint DNA bands (Figure 1).

CONCLUSION

This study demonstrated that the Sigma MM™ molecular medium is able to rapidly inactivate bacteria and yeast whilst preserving bacterial DNA. Following an extended exposure time. Gel electrophoresis was suitable in visualising intact DNA of *E. coli* however low yields observed indicate the importance of PCR to amplify DNA. Further studies are ongoing to assess DNA integrity of other organisms over an extended holding period using PCR based methods.