EFFICACY OF AN INACTIVATION MEDIUM FOR MOLECULAR SAMPLE PROCESSING OF SARS-COV-2

INTRODUCTION

The extensive testing and surveillance response to the SARS-CoV-2 pandemic has highlighted the need to render specimens safe for transportation, and for testing in facilities with limited containment facilities.

∑–Molecular Medium™ (∑–MM™) has been on the market for a number of years and has consistently been shown to effectively eliminate infectious microorganisms from specimens, including mycobacteria, bacteria and viruses.

During the pandemic it was demonstrated to be compatible with PCR diagnostics, capable of rendering specimens safe, while preserving the viral RNA for accurate diagnostic reporting.

A study was designed in cooperation with the Medical Research Council and University of Glasgow's Centre for Virus Research to measure the inactivation of SARS-CoV-2 achieved in specimens collected using ∑–MM™.

METHODS

MATERIALS

- Assays were performed in Vero E6 MESO cell line.
- Cell line based on susceptibility to SARS-CoV-2. SARS-CoV-2-CVR-Gla-1 strain used.
- Contains D614G mutation in Spike gene (GISAID accession: EPI_ISL_461705.
- Inactivation buffer: ∑–MM™, Ref MWMM, Lot.20M16, Exp 2021/12.

METHOD

- SARS-CoV-2-Gla-1 virus isolate was mixed with the ∑–MM™ Medium at predetermined ratios and times (Table 1).
- Triplicate aliquots of 100 µl or 500 µl of SARS-CoV-2 was added to 1.5 ml of ∑–MM™ medium and incubated for 1 min & 5 min.
- Untreated virus sample was used as the control, where the ∑–MM™ was replaced with PBS.
- Inactivation medium was removed using the PEG precipitation method by adding PEG 8000 to the final concentration of 30% to the inactivated virus solution.
- After overnight incubation at 4°C the virus was pelleted by centrifugation for 1h at 1500 rpm.
- Then the pellets were washed twice by addition of PBS and centrifugation for 10 min at 1500 rpm.
- Samples were resuspended in 500 µl DMEM supplemented with 2% FBS.

RESULTS

∑–MM™ was tested for the ability to inactivate SARS- CoV-2-Gla-1 isolate. Two virus-to-buffer ratios (1.5 to 0.5 and 1.5 to 0.1), and two inactivation times (1 min and 5 min) were used. As a control PBS was used to replace the buffer. After treatment, the cytotoxic component was removed using PEG precipitation. Following washing and resuspension of the pellet, all the samples in their entirety were titred by plaque assay on Vero E6 MESO cells starting with a neat dilution. Input virus stock was also titred to assess recovery of virus following PEG precipitation. Titre reduction was calculated by subtracting the mean logarithmic virus titre for ∑–MM™ buffer-treated and purified sample from the logarithmic virus titre for the PBS-treated input virus, with standard errors of the mean calculated. We observed with the PBS-treated sample that there was a loss of approximately 1 Log10 pfu/ml of virus during the PEG precipitation. Following treatment with ∑–MM™ for all the samples, reduction in titre was over 6 log10 when compared to input virus, or over 5 log10 when compared to recovered virus. (Table).

	Buffer		Virus Amount	Inactivation Time	Virus detectable in titration[A,B]	Titre Reduction[C,D] (log_{10} [±SE])
	Buffer	Buffer Volume				
1	∑–MM™	1.5 ml	100 µl	1 min	0/3	5.89 [±0.0]
2	∑–MM™	1.5 ml	100 µl	5 min	0/3	5.89 [±0.0]
3	∑–MM™	1.5 ml	500 µl	1 min	0/3	5.78 [±0.0]
4	∑–MM™	1.5 ml	500 µl	5 min	0/3	5.78 [±0.0]
5	PBS	1.5 ml	100 µl	30 min	2/2	1.01 [±0.89][E]
6	PBS	1.5 ml	500 µl	30 min	2/2	1.11 [±0.15][E]

Table 1. Virus inactivation results

CONCLUSIONS

BS EN 144761,3, requires that there should be a titre reduction of more than 4 log10 for virucidal suspension tests. ∑–MM™ consistently exceeded this requirement for both the time points and concentrations used in the study. In fact, within 1 minute of inoculation there was no detectable virus at all in 6 out of 6 samples tested.

Given that the test concentrations were higher than would be the case for clinical specimens, the study demonstrates that ∑–MM™ can be used as a safe transport system for SARS-CoV-2 specimens, offering rapid inactivation. The results are consistent with other studies using different inactivation reagents and methods.

Another standard, ASTM E1052-206 requires that one part of virus suspension is added to nine parts of the test substance before holding at the desired temperature for the required contact time, and then assayed for viable virus in an appropriate host system. In the study the 100ul and 500ul aliquot represent dilutions above and below the ASTM requirement, so the results can also be interpreted as meeting this standard, although further specific dilutions should be assayed.

EAST KENT HOSPITAL CASE STUDY

Recently East Kent Hospitals revolutionised their SARS-CoV-2 workflow with the introduction of MWE's ∑–MM™ and were able to demonstrate that for every 100,000 samples, they could save 233 working days after implementing the ∑–MM™ into the workflow. This removed the previous labour intensive inactivation process and resulted in a staff saving of 1.1 FTE at Agenda For Change Band 6 which equates to a yearly financial saving of over £49,000.

Table showing time saved and financial saving when MWE's ∑–MM™ was implemented:

For 100,000 specimens each year	Minutes	Days	FTE	£ Saving
Pre-∑–MM™	200,667	446		
Post-∑–MM™	95,750	213		
Time Saved	104,917	233		
FTE Saving			1.1	
Financial Saving				49,041

Table 2. Cost savings analysis

Tel: +44 (0)1225 810 361 | Web: mwe.co.uk
Corsham, Wiltshire, England SN13 9RT