INACTIVATION OF SARS-COV-2 IN MWE'S ∑–MM™ INACTIVATION MEDIUM

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 circumstances with limited biological containment facilities. A plethora of unregulated collection and transportation devices has come to market, many claiming to inactivate coronavirus, but with little supporting evidence. It seems to be assumed that all that is required is a claim that the transport medium is "guanidine based" is sufficient, but various studies have demonstrated that claims to inactivation properties are often exaggerated, and that many samples are likely to retain infective virus.

∑–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 most PCR platforms, so was capable of rendering specimens safe, but preserving the viral RNA for accurate diagnostic reporting.

A study was designed in cooperation with the Medical Research Council & University of Glasgow's Centre for Virus Research to measure the inactivation of SARS-CoV-2 achieved in specimens collected using ∑–MM™. According to BS EN 14476 [1], there should be a titre reduction of more than 4 $\log_{10}$ for effective virucidal activity, so this has been used as a benchmark in this study, as in a similar study by Public Health England [2].

MATERIALS

Assays were performed in Vero E6 MESO cell line, which is a subclone of Vero E6 cell line based on susceptibility to SARS-CoV-2.

SARS-CoV-2-CVR-Gla-1 strain used in this study was originally Isolated from a patient sputum sample, and it contains D614G mutation in Spike gene (GISAID accession: EPI_ISL_461705).

Inactivation buffer: ∑–MM™, Ref MWMM, Lot.20M16, Exp 2021/12

METHODS

SARS-CoV-2-Gla-1 virus isolate was mixed with the ∑–MM™ Medium at predetermined ratios and times (Table 1). Each condition has been performed in a technical triplicate. 100 µl or 500 µl of SARS-CoV-2 was added to 1.5 ml of MM Sigma medium and incubated for 1 min & 5 min. Untreated virus sample was used as the control, where the Sigma buffer was replaced with PBS. After inactivation the cytotoxic component of the medium was removed using the PEG precipitation method.

Briefly, PEG 8000 was added 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 each time. Samples were resuspended in 500 µl DMEM supplemented with 2% FBS. Survival of the virus in the samples was assessed by plaque assay on Vero E6 MESO cell line. Titre reduction was calculated by subtracting the mean logarithmic virus titre for ∑–MM™ buffer-treated and PEG-pelleted sample from the logarithmic virus titre for the PBS-treated input virus, with standard errors of the mean calculated.

	Buffer		Virus Amount	inactivation time
	Buffer	Buffer Volume		
1	∑–MM™	1.5ml	100 µl	1 min
2	∑–MM™	1.5ml	100 µl	5 min
3	∑–MM™	1.5ml	500 µl	1 min
4	∑–MM™	1.5ml	500 µl	5 min
5	PBS	1.5ml	100 µl	30 min
6	PBS	1.5ml	500 µl	30 min

Table 1. Inactivation conditions used in this study.

RESULTS

∑–MM™ Molecular Medium 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 2)

	Buffer		Virus Amount	inactivation time	Virus detectable in titration [A,B]	Titre Reduction [C,D] (log10 [±SE])
	Buffer	Buffer Volume				
1	∑–MM™	1.5ml	100 µl	1 min	0/3	5.89 [± 0.0]
2	∑–MM™	1.5ml	100 µl	5 min	0/3	5.89 [± 0.0]
3	∑–MM™	1.5ml	500 µl	1 min	0/3	5.78 [± 0.0]
4	∑–MM™	1.5ml	500 µl	5 min	0/3	5.78 [± 0.0]
5	PBS	1.5ml	100 µl	30 min	2/2	1.01 [±0.89][E]
6	PBS	1.5ml	500 µl	30 min	2/2	1.11 [±0.15][E]

Table 2. Virus inactivation results.

[A] Number of positive samples per number of replicates

[B] The limit of detection in plaque assay was 1 pfu/ml

[C] Titre reduction calculated in comparison to PBS-treated input virus (5.89 log10 for 100ul virus samples, and 5.78 log10 for 500ul virus samples)

[D] Standard Error (SE) = Standard deviation / square root of total number of samples,

[E] Titre reduction calculated in comparison to input virus

CONCLUSION

BS EN 14476[1,3], 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[4], 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[2,5]. Another standard, ASTM E1052-20[6] 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 inocula represent dilutions below and above the ASTM requirement, so the results can also be interpreted as meeting this standard, although further specific dilutions should be assayed.

Regardless of these results, it is essential that safe handling procedures continue to be observed for this emerging pathogen until the epidemiology is more fully understood.

REFERENCES

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