Public Health England

SARS-CoV-2 inactivation testing: interim report

Report identifier	HCM/CoV2/029/v2
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Undertaken by High Containment Microbiology, NIS Laboratories, National Infection Service, Public Health England N.B. This is an interim report and may be updated as further results are obtained	

Product/treatment details	
Product/treatment	MAST MELT Medium B
Manufacturer	Mast Group
Product code	(B)
Available information on product composition, as supplied	1-10% Triton X-100 5% EDTA
Manufacturer's recommended ratio of sample to product	Swab to be placed directly in tube containing 0.5ml buffer

Sample details	
Sample type tested	Tissue culture fluid containing 5% (v/v) foetal calf serum
Virus strain tested	SARS-CoV-2 England 2
Ratio of spiked virus stock to sample matrix	Not applicable; tissue culture fluid used undiluted

Experimental conditions	
Ratio of sample to product tested	1 volume sample to 10 volumes product
Contact time/s	5 minutes; 10 minutes; 15 minutes
Temperature of incubation	Room temperature

Brief description of tests performed	Triplicate samples were treated with test buffer for indicated contact time/s or mock-treated in triplicate with an equivalent volume of PBS. All samples were then subjected to a purification step to remove cytotoxic buffer components. PBS-treated samples were subjected to the same purification procedure in parallel. **Test 1:** Purified samples were immediately titrated on Vero E6 cells to establish virus titre. This test is quantitative and reports the titre of virus in each treatment condition in TCID50 per ml. Reduction in virus titre following treatment is given as the difference between the mean $\log_{10}$ TCID50/ml for treated conditions and the PBS control. **Test 2:** In parallel, purified samples were seeded onto Vero E6 monolayers to amplify any remaining virus over the course of up to four serial passages. Virus amplification over each passage was detected by visual (microscopic) examination of monolayers for cytopathic effect, and confirmed by SARS-CoV-2-specific real-time PCR. This test is qualitative and reports either the presence or absence of virus amplification. This test may detect levels of virus that are below the detection limit of the titration assay (test 1) due to a greater sample plating volume and the opportunity for any virus present to amplify over serial passages.

Table of results			
Maximum detectable virus reduction in test 1 (log_{10} TCID50/ml)			7.0
	Test 1: Virus titration post-treatment		**Test 2: Passage of samples in cell culture**
	Mean virus titre (log_{10} TCID50/ml)	Titre reduction (log_{10} TCID50/ml)	Virus detected/ Virus not detected
PBS-treated	7.7	-	Virus detected (all replicates)
Test buffer-treated (5 minute)	≤0.7	≥7.0	Virus not detected
Test buffer-treated (10 minutes)	≤0.7	≥7.0	Virus detected (1 replicate)
Test buffer-treated (15 minutes)	≤0.7	≥7.0	Virus detected (1 replicate)

Interpretation

Treatment with MELT Medium B gave ≥7.0 log_{10} reduction in infectious virus titre at all treatment times tested. The maximum detectable virus reduction in this test was 7.0 log_{10} TCID50/ml.

Residual virus was detected in one replicate from each treatment time (either in test 1 or test 2), indicating that inactivation by this product was not complete.

Demonstrating complete inactivation is dependent on the starting titre of virus used for testing, and it is likely that complete inactivation could be achieved if samples contained lower levels of infectious virus than those tested here. Conversely, sample treatments that inactivate virus effectively in our testing may fail to inactivate samples containing higher levels of virus than those evaluated in this study.

This test has been performed on tissue culture fluid containing 5% (v/v) foetal calf serum. The effectiveness of this treatment against SARS-CoV-2 may vary when used to inactivate clinical samples or other types of sample matrix. Any results of inactivation testing using other sample matrices will be released as they become available.

Inactivation reagents should not be assumed to be 100% effective against SARS-CoV-2.

Suitability of products and treatments for inactivation of other pathogens has not been evaluated in this study.

All COVID-19 laboratory testing workflows must be subjected to suitable and sufficient risk assessment, with consideration given to any inactivation step. Risk assessments should be reviewed regularly as new information on the inactivation of SARS-CoV-2 becomes available.

The impact of chosen inactivation method on the sensitivity of subsequent SARS-CoV-2 detection should also be assessed locally.

Summary of revisions

Version 1: New document
Version 2: Addition of test 2 data; interpretation updated

Queries regarding this report or HCM inactivation testing should be directed to HCMgroup@phe.gov.uk

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