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SARS-CoV-2 inactivation testing: interim report

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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	GHCl 40% (w/v) Transport Medium
Manufacturer	Oxoid/Thermo Fisher Scientific
Product code	EB1351A
Composition of product, as supplied	28.3% Guanidine hydrochloride 2.1% Triton X-100 0.5% Tris 0.7% EDTA
Manufacturer's recommended ratio of sample to product	Not known

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 times	30 minutes

Temperature of incubation	Room temperature
Brief description of tests performed	**Test 1 and 2:** 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. Purified samples were immediately titrated on Vero E6 cells to establish virus titre (**test 1**). 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. In parallel, purified samples were seeded onto Vero E6 monolayers to amplify any remaining virus over the course of up to four serial passages (**test 2**). 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. **Test 3:** 100-fold dilutions of SARS-CoV-2 were spiked into test buffer aliquots and incubated at room temperature. After 0, 4 or 8 days, triplicate samples for each dilution were extracted and subjected to SARS-CoV2-specific real-time PCR. This test indicates stability of SARS-CoV-2 RNA in the test buffer by comparing Ct values for each virus dilution over time. This test measures levels of viral RNA only and does not reflect virus infectivity.

Table of results (test 1 and test 2)			
Maximum detectable virus reduction in test (log_{10} TCID50/ml)			4.6*
	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	6.3	-	Virus detected (all replicates)
Test buffer-treated	≤1.8*	≥4.6*	Virus detected (all replicates)

*Virus titre in undiluted sample could not be determined due to residual buffer toxicity

Table of results (Test 3)				
	Mean Ct value of samples spiked with increasing dilutions of SARS-CoV-2 (standard deviation)			
	Undiluted	**$1:10^2$**	**$1:10^4$**	**$1:10^6$**
0 day treatment	12.3 (0.7)	20.0 (0.3)	27.5 (0.1)	37.4 (4.4)
4 day treatment	12.8 (0.7)	20.9 (0.2)	28.1 (0.5)	33.8 (2.9)
8 day treatment	14.5 (0.2)	21.5 (0.3)	28.5 (0.2)	35.0 (0.16)

Interpretation

Test 1: Treatment with GHCl 40% (w/v) Transport Medium resulted in a ≥4.6 log10 reduction in infectious titre, the maximum detectable titre reduction in this test. Levels of infectious virus remaining in undiluted treated samples could not be evaluated due to residual buffer cytotoxicity remaining following sample purification.

Test 2: Infectious virus was recoverable from all treated sample replicates demonstrating incomplete inactivation.

Demonstrating complete inactivation is dependent on the starting titre of virus used for testing, and it is possible 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.

These tests have 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.

Test 3: We observed a small increase in Ct value (1-1.5 Ct) in SARS-CoV-2 specific real-time PCR following extended incubation times in this buffer, potentially indicating a small detrimental effect on RNA stability with increasing treatment times.

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.

Disclaimer

Summary of revisions
Version 1: New document Version 2: Addition of new data for Test 2 and Test 3 Version 3: Reformatted for publication

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

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