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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	Triton™ X-100 (synonyms: ***t*-Octylphenoxypolyethoxyethanol; polyethylene glycol *tert*-octylphenyl ether)**
Concentration	0.1% (v/v) 0.5% (v/v)

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	
Contact times	Experiment 1: 30 minutes Experiment 2: <2 minutes; 10 minutes; 30 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. **Test 3:** Treated, unpurified samples were extracted and subjected to SARS-CoV-2 specific real-time PCR, in triplicate for each treatment condition. This test reports Ct values for each treatment condition and indicates the effect on SARS-CoV-2 RNA stability. This test measures levels of viral RNA only and does not reflect virus infectivity.

Table of results (experiment 1)			
Maximum detectable virus reduction in test (log_{10} TCID50/ml)			5.1
	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	5.8	-	Virus detected (all replicates)
0.1% Triton X-100	≤0.9	≥4.9	Virus detected (all replicates)
0.5% Triton X-100	≤0.7	≥5.1	No virus detected

Table of results (experiment 2)				
Maximum detectable virus reduction in test (log_{10} TCID50/ml)			6.2	
	Test 1: Virus titration post-treatment		**Test 2: Passage of samples in cell culture**	**Test 3: Effect on viral RNA**
	Mean virus titre (log_{10} TCID50/ml)	Titre reduction (log_{10} TCID50/ml)	Virus detected/Virus not detected	Mean Ct (standard deviation)
PBS-treated	6.9	-	Virus detected (all replicates)	10.8 (0.1)
0.5% Triton X-100 (<2 minutes)	≤1.0	≥5.9	Virus detected (all replicates)	10.9 (0.4)
0.5% Triton X-100 (10 minute)	≤0.7	≥6.2	No virus detected*	12.2 (0.2)
0.5% Triton X-100 (30 minute)	≤0.7	≥6.2	No virus detected*	14.4 (0.1)

* Cell culture passages in this experiment were not continued past passage 2

Interpretation

Test 1: Both 0.1% and 0.5% Triton X-100 treatment for 30 minutes result in a considerable reduction of virus titre (≥4.9 and ≥5.1 $\log_{10}$ respectively). 0.5% Triton X-100 treatment with shorter contact times showed rapid knockdown of virus (≥5.9 $\log_{10}$ reduction with less than 2 minutes treatment and ≥6.2 $\log_{10}$ reduction, the maximum detectable in the test, after 10 minutes treatment).

Test 2: No infectious virus was detected in samples treated with 0.5% Triton X-100 for 30 minutes after four serial passages in cell culture. Similarly, no virus was detected from samples treated with 0.5% Triton X-100 for 10 minutes after two serial passages in cell culture. Infectious virus was recoverable from samples following 30 minute treatment with 0.1% Triton™ X-100, or from samples treated <2 minutes with 0.5% Triton X-100, indicating that virus inactivation with these conditions was incomplete.

Demonstrating complete inactivation is dependent on the starting titre of virus used for testing, and it is highly likely that complete inactivation could be achieved under all the above Triton X-100 conditions 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.

Test 3: We observed a contact time-dependent increase in Ct value in SARS-CoV-2 specific real-time PCR in the presence of 0.5% Triton X-100, indicating a detrimental effect on RNA stability with increasing treatment times. Use of Triton X-100 for inactivation of samples for molecular testing may therefore affect assay sensitivity. The combination of Triton X-100 and a guanidinium-containing buffer may increase RNA stability as these conditions have been shown to be compatible with RT-PCR and whole genome sequencing at concentrations up to 1% Triton X-100[1].

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.

[1]Lewandowski, K., Bell, A., Miles, R., Carne, S., Wooldridge, D., Manso, C., Hennessy, N., Bailey, D., Pullan, S. T., Gharbia, S. & Vipond, R. 2017. The Effect of Nucleic Acid Extraction Platforms and Sample Storage on the Integrity of Viral RNA for Use in Whole Genome Sequencing. *The Journal of Molecular Diagnostics,* 19**,** 303-312.

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: Header and disclaimer edited; date issued to PHE's COVID Incident Virology Cell added; key guidance points added to interpretation; additional data added, and interpretation edited
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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