



## 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	

<b>Product/treatment details</b>	
Product/treatment	Triton™ X-100
Concentration	1% (v/v)

<b>Sample details</b>	
Sample type tested	Human serum
Virus strain tested	SARS-CoV-2 England 2
Ratio of spiked virus stock to sample matrix	1 volume virus to 9 volumes serum

<b>Experimental conditions</b>	
Contact times	30 minutes; 60 minutes; 120 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 TCID<sub>50</sub> per ml. Reduction in virus titre following treatment is given as the difference between the mean log<sub>10</sub> TCID<sub>50</sub>/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.

<b>Table of results</b>			
Maximum detectable virus reduction in test (log <sub>10</sub> TCID <sub>50</sub> /ml)			5.9
	<b>Test 1: Virus titration post-treatment</b>		<b>Test 2: Passage of samples in cell culture</b>
	Mean virus titre (log <sub>10</sub> TCID <sub>50</sub> /ml)	Titre reduction (log <sub>10</sub> TCID <sub>50</sub> /ml)	Virus detected/ Virus not detected
PBS-treated	6.6	-	Virus detected (all replicates)
30 minute treatment	5.3	1.3	Virus detected (all replicates)
60 minute treatment	5.2	1.5	Virus detected (all replicates)
120 minute treatment	4.6	2.0	Virus detected (all replicates)

<b>Interpretation</b>
<p>Test 1: Treatment with 1% Triton X-100 for up to 120 minutes in a serum matrix resulted in a very modest decrease (up to 2.0 log<sub>10</sub>) in virus titre, and infectious virus was detectable in all treated sample replicates. The maximum detectable titre reduction in this test was 5.9 log<sub>10</sub> TCID<sub>50</sub>/ml.</p> <p>Test 2: Infectious virus was recoverable from all treated samples.</p> <p>We have previously demonstrated that Triton X-100 is effective at inactivating SARS-CoV-2 in a tissue culture fluid sample matrix (refer to Triton X-100 TCF report HCM/CoV2/006). We have been unable to replicate this effective inactivation in a serum matrix.</p> <p>These tests have been performed using human 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.</p> <p><b>Inactivation reagents should not be assumed to be 100% effective against SARS-CoV-2.</b></p> <p><b>Suitability of products and treatments for inactivation of other pathogens has not been evaluated in this study.</b></p>

**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.**

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#### **Summary of revisions**

Version 1: New document

Queries regarding this report or HCM inactivation testing should be directed to  
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