



SARS-CoV-2 Inactivation Testing: Interim Report

Report identifier	HCM/CoV2/043/v4
Report date	07 December 2020
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	70% ethanol, made from ethanol absolute (99.8+%) diluted in deionised water or PBS
Manufacturer	Fisher Chemical
Product code	E/0650DF

Sample details	
Sample type tested	Tissue culture fluid containing 5% (v/v) foetal calf serum, or tissue culture fluid concentrated through a 100KDa molecular weight cut-off centrifugal filter (as indicated in results tables)
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; 1 volume sample to 1 volume product
Contact time/s	10 minutes; 30 minutes
Temperature of incubation	Room temperature

<p>Brief description of tests performed</p>	<p>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.</p> <p>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 log₁₀ TCID50/ml for treated conditions and the PBS control.</p> <p>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.</p>
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Table 1: 1 volume sample to 10 volumes product (64% ethanol final concentration)*†			
Maximum detectable virus reduction in test 1 (log ₁₀ TCID ₅₀ /ml)			6.0
	Test 1: Virus titration post-treatment		Test 2: Passage of samples in cell culture
	Mean virus titre in log ₁₀ TCID ₅₀ /ml [95% confidence interval]	Titre reduction in log ₁₀ TCID ₅₀ /ml [95% confidence interval]	Virus detected/ Virus not detected
PBS-treated	6.7	-	Virus detected (3/3 replicates)
Test buffer-treated (10 minutes)	≤0.7	≥6.0	Virus not detected (0/3 replicates positive)

*Test performed using tissue culture fluid containing 5% (v/v) foetal calf serum, concentrated through a 100KDa molecular weight cut-off centrifugal filter

†70% ethanol made by diluting ethanol absolute in deionised water

Table 2: 1 volume sample to 1 volume product (35% ethanol final concentration)**‡			
Maximum detectable virus reduction in test 1 (log ₁₀ TCID ₅₀ /ml)			6.1
	Test 1: Virus titration post-treatment		Test 2: Passage of samples in cell culture
	Mean virus titre in log ₁₀ TCID ₅₀ /ml [95% confidence interval]	Titre reduction in log ₁₀ TCID ₅₀ /ml [95% confidence interval]	Virus detected/ Virus not detected
PBS-treated	6.8 [6.6-7.1]	-	Test not performed
Test buffer-treated (10 minutes)	2.7 [2.4-3.0]	4.1 [3.7-4.6]	Test not performed
Test buffer-treated (30 minutes)	2.4 [2.1-2.6]	4.5 [4.1-4.8]	Test not performed

**Test performed using tissue culture fluid containing 5% (v/v) foetal calf serum

‡70% ethanol made by diluting ethanol absolute in PBS

Interpretation

Treatment with 70% ethanol at a ratio of 10 volumes product to 1 volume sample (63% ethanol final concentration) for 10 minutes reduced virus titre by $\geq 6.0 \log_{10}$, to below the limit of detection for the test (Table 1). Furthermore, no virus was recoverable from treated samples following four serial passages in cell culture.

When used at a ratio of 1 volume product to 1 volume sample (35% ethanol final concentration), SARS-CoV-2 titre was reduced by $4.1 \log_{10}$ and $4.5 \log_{10}$ titre reduction after 10 minutes and 30 minutes, respectively (Table 2). This titre reduction is above the $4 \log_{10}$ reduction required by BS EN 14476, but 70% ethanol used at a 1:1 ratio of sample:product is less effective than when used at 1:10. A final concentration of 35% ethanol may not completely inactivate samples containing (or expected to contain) high titres of infectious virus.

Here, effectiveness of 70% ethanol for SARS-CoV-2 inactivation has only been assessed in virus suspension tests. Performance in other types of inactivation tests (e.g. in surface tests) may differ.

Demonstrating complete inactivation is dependent on the starting titre of virus used for testing. Complete inactivation may occur if samples contained lower levels of infectious virus than those tested here, but 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 using tissue culture fluid. 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.

Disclaimer

PHE's evaluations of commercial products and treatments for inactivating SARS-CoV-2 have been carried out primarily for PHE's own internal use and the reports of such evaluations are shared solely for readers information; PHE does not in any way recommend any particular product for virus inactivation; and PHE shall not be responsible for the choice of product or treatment for virus inactivation, and it is the responsibility of the testing laboratory to ensure that any such product or treatment implemented has undergone the necessary verification and validation; and PHE shall not be liable, to the greatest extent possible under any applicable law, for any claim, loss or damage arising out of or connected with use of this and related reports and choice of virus inactivation products or treatments.

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Summary of revisions

Version 1: New document

Version 2: Additional wording added to interpretation

Version 3: Units corrected

Version 4: Addition of new data, update of interpretation and experiment details

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

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