Public Health England

SARS-CoV-2 Inactivation Testing: Interim Report

Report identifier	HCM/CoV2/037/v2
Report date	29 September 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	Coronavirus Extraction Reagent 2506 from Arcis Virus Sample Prep Kit.
Manufacturer	Arcis
Product code	Coronavirus Extraction Reagent 2506
Available information on product composition, as supplied	No information available
Manufacturer's recommended ratio of sample to product	Extraction reagent prepared by adding 1 volume ribonuclease inhibitors to 16 volumes extraction buffer. Swab to be added to 200ul of product.

Sample details	
Sample type tested	Tissue culture fluid containing 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	Extraction reagent prepared by adding 1 volume ribonuclease inhibitors to 16 volumes extraction buffer. 1 volume sample added to 2 volumes product.
Contact time/s	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. **Test 1:** Following the indicated contact times, samples were immediately titrated on Vero E6 cells to establish virus titre. For experiments in Table 1, all samples (including PBS-treated samples) were subjected to a purification step prior to titration. For all other experiments, samples were immediately titrated without purification. Test 1 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, 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 1*			
Maximum detectable virus reduction in test 1 (log_{10} TCID50/ml)			6.0†
	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	8.6	-	Virus detected (all replicates)
Test buffer-treated (10 minutes)	8.1	0.6	Virus detected (all replicates)
Test buffer-treated (30 minutes)	5.5	3.2	Virus detected (all replicates)

†Limit of detection was 2.6 log_{10} TID50 due to buffer cytotoxicity

*All samples subjected to a purification step before titration

Table 2#				
	Tissue culture fluid (concentrated)		Tissue culture fluid (unconcentrated)	
	Mean virus titre (log_{10} TCID50/ml)	Titre reduction (log_{10} TCID50/ml)	Mean virus titre (log_{10} TCID50/ml)	Titre reduction (log_{10} TCID50/ml)
PBS-treated	6.3	-	5.0	-
Test buffer-treated (10 minutes)	5.6	0.7	≤1.7†	≥3.3†
Test buffer-treated (30 minutes)	3.6	2.6	≤1.7†	≥3.3†

†Limit of detection was 1.7 log_{10} TID50 due to buffer cytotoxicity

#Unpurified samples titrated

Interpretation

Table 1 shows results of test 1 using a concentrated preparation of virus. Treatment of this high titre virus preparation with Arcis Extraction Reagent 2506 for 10 or 30 minutes reduced virus titre by 0.6 and 3.2 $\log_{10}$, respectively in test 1 (Table 1). Considerable levels of virus were detectable after treatment in both test 1 and test 2.

Test 1 was repeated to compare efficiency of testing against a concentrated and an unconcentrated virus preparation (Table 2). Treatment of the concentrated virus preparation with Arcis Extraction Reagent 2506 gave similar titre reductions to those in Table 1. In contrast, no virus was detectable from treated samples for unconcentrated virus, representing a titre reduction of ≥3.3 $\log_{10}$ after 10 minute treatment; however, this demonstrated titre reduction is below the 4 $\log_{10}$ reduction required by BS EN 14476 for virucidal quantitative suspension tests for chemical disinfectants and antiseptics. A further repeat of test 1 gave a titre reduction of ≥3.7 $\log_{10}$ using an unconcentrated virus stock (data not shown).

These results suggest that this product may be less effective at inactivating SARS-CoV-2 in samples that with high virus titres or containing high amounts of protein.

This test has been performed on tissue culture fluid containing 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.

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. PHE is an Executive Agency of the Department of Health and Social Care. Unauthorised use of the PHE name and/or logo is prohibited.

Summary of revisions
Version 1: New document Version 2: Addition of new results; revision of description of tests and interpretation

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