Public Health England

SARS-CoV-2 Inactivation Testing: Interim Report

Report identifier	HCM/CoV2/051/v2
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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	70% acetone, made from acetone (99.5+%) diluted in saline or PBS
Manufacturer	VWR International
Product code	100034Q

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

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. 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.

Table 1:
1 volume of sample to 10 volumes product (64% acetone final concentration)#**

Maximum detectable virus reduction in test (log_{10} TCID50/ml)	6.0†

	Mean virus titre in log_{10} TCID50/ml [95% confidence interval]	Titre reduction in log_{10} TCID50/ml [95% confidence interval]
PBS-treated	6.7 [6.4-7.0]	-
Test buffer-treated (10 minutes)	≤0.7†*	≥6.0 [5.7-6.3]

†Limit of detection was 0.7 log_{10} TCID50/ml
*Not able to calculate 95% confidence interval
**Test performed using tissue culture fluid containing 5% (v/v) foetal calf serum, concentrated through a 100KDa molecular weight cut-off centrifugal filter
#70% acetone made by diluting acetone in saline

Table 2: **1 volume of sample to 1 volume product (35% acetone final concentration)** §‡		
Maximum detectable virus reduction in test (log_{10} TCID50/ml)		6.1†
	Mean virus titre in log_{10} TCID50/ml [95% confidence interval]	Titre reduction in log_{10} TCID50/ml [95% confidence interval]
PBS-treated	6.8 [6.6-7.1]	-
Test buffer-treated (10 minutes)	2.0*	4.8 [4.6-5.1]
Test buffer-treated (30 minutes)	1.8*	5.1 [4.8-5.3]

†Limit of detection was 0.7 log_{10} TCID50/ml

*Not able to calculate 95% confidence interval

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

‡70% acetone made by diluting acetone in PBS

Interpretation

Treatment with 70% acetone at a ratio of 10 volumes product to 1 volume sample (64% acetone final concentration) for 10 minutes reduced virus titre by ≥6.0 $log_{10,}$ to below the limit of detection for the test (Table 1).

When used at a ratio of 1 volume product to 1 volume sample (35% acetone final concentration), SARS-CoV-2 titre was reduced by 4.8 log_{10} and 5.1 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% acetone used at a 1:1 ratio of sample:product is less effective than when used at 1:10. A final concentration of 35% acetone may not completely inactivate samples containing (or expected to contain) high titres of infectious virus.

Here, effectiveness of 70% acetone 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
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Summary of revisions
Version 1: New document Version 2: Addition of new data, update of interpretation and experiment details

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