Public Health England

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	Heat treatment
Equipment used	Heating block

Sample details	
Sample type tested	Tissue culture fluid containing 4 % (v/v) foetal calf serum
Ratio of spiked virus stock to sample matrix	Not applicable; tissue culture fluid used undiluted
Sample volume	1ml

Experimental conditions	
Temperature and treatment times (time at temperature)	56^{o}C: 15, 30, 60 minutes 80^{o}C: 15, 30, 60, 90 minutes 95^{o}C: 1, 5 minutes
Virus strain tested	SARS-CoV-2 England 2
Cell line used for testing	Vero E6

Brief description of tests performed	Triplicate samples were heat-treated for the indicated contact times then immediately held on ice. Untreated (0 minute) control samples were kept on ice and subsequently processed in parallel with the heat-treated samples. **Test 1:** 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 plaque forming units (PFU) per ml. Reduction in virus titre following treatment is given as the difference between the $\log_{10}$ pfu/ml for treated conditions and the untreated 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 of results					
		Test 1: Titration post-treatment		**Test 2: Passage of samples in cell culture**	
		Mean virus titre (log_{10} pfu/ml)	Mean titre reduction (log_{10} pfu/ml)	Virus detected/ Virus not detected	Mean Ct value of baseline samples in SARS-CoV-2 PCR
56°C	0m	5.8	-	Virus detected (all replicates)	15.3
	15m	3.1	2.7	Virus detected (all replicates)	15.4
	30m	0.9	4.9	Virus detected (all replicates)	15.3
	60m	3.7	2.1	Virus detected (all replicates)	15.2
80°C	0m	5.7	-	Virus detected (all replicates)	16.5
	15m	2.2	3.5	Virus detected (≥1 replicate)	17.8
	30m	1.3	4.4	Virus detected (all replicates)	20.2
80°C (longer treatment times)	0m	5.6	-	Virus detected (all replicates)	16.0
	30m	1.5	4.1	Virus detected (≥1 replicate)	20.5
	60m	≤0.5	≥5.1	Virus detected (≥1 replicate)	25.5
	90m	≤0.5	≥5.1	Virus not detected	27.7
95°C	0m	5.7	-	Virus detected (all replicates)	16.5
	1m	≤0.5	≥5.2	Virus not detected	21.6
	5m	≤0.5	≥5.2	Virus not detected	22.0

Interpretation

Data for heat treatment at 56°C shown above are representative of results obtained in independent experiments by two laboratories. Considerable variation between sample replicates could be observed following treatment at this temperature, and no treatment time tested at 56°C reliably gave ≥4 $\log_{10}$ reduction in titre. Infectious virus was recoverable from all 56°C-treated samples in Test 2.

At 80ºC, treatment times of 30 minutes reduced the average titre by ≥4 $\log_{10}$ PFU/ml, but variation (up to 2 $\log_{10}$) between replicates was observed at this temperature. After 60 minutes a low level of viable virus was detected following by passage in cell culture. After 90 minutes at 80ºC, viable virus was not detected by either test.

No virus could be detected in either test from samples following heating to 95ºC for 1 or 5 minutes.

Baseline Ct values obtained by SARS-CoV-2 specific real-time PCR in Test 2 showed that the integrity of viral RNA was adversely affected by heating to 80ºC and 95ºC.

This test has been performed on tissue culture fluid containing 4% (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.

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.

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Summary of revisions	
Version 1:	New document
Version 2:	Reformatted for publication
Version 3:	Updated to include further passage data

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

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