Public Health England

# SARS-CoV-2 inactivation testing: interim report

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| **Report identifier** | HCM/CoV2/025/v1 |
| **Report date** | 16 July 2020 |
| Undertaken by High Containment Microbiology, NIS Laboratories, National Infection Service, Public Health England<br>N.B. This is an interim report and may be updated as further results are obtained | |

| **Product/treatment details** | |
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| Product/treatment | NP-40 (polyethylene glycol nonylphenyl ether) |
| Concentration | 0.1% (w/v)<br>0.5% (w/v) |

| **Sample details** | |
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| Sample type tested | Tissue culture fluid containing 5% (v/v) 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** | |
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| Contact times | 30 minutes |
| Temperature of incubation | Room temperature |

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| 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.<br><br>**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 $\log_{10}$ TCID50/ml for treated conditions and the PBS control.<br><br>**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.<br><br>**Test 3:** Treated, unpurified samples were extracted and subjected to SARS-CoV-2 specific real-time PCR, in triplicate for each treatment condition. This test reports Ct values for each treatment condition and indicates the effect on SARS-CoV-2 RNA stability. This test measures levels of viral RNA only and does not reflect virus infectivity. |

| **Table of results** | | | | |
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| Maximum detectable virus reduction in test ($log_{10}$ TCID50/ml) | | | 6.5 | |
| | **Test 1: Virus titration post-treatment** | | **Test 2: Passage of samples in cell culture** | **Test 3: Effect on viral RNA** |
| | Mean virus titre ($log_{10}$ TCID50/ml) | Titre reduction ($log_{10}$ TCID50/ml) | Virus detected/Virus not detected | Mean Ct (standard error) |
| PBS-treated | 7.2 | - | Virus detected (all replicates) | 11.8 (0.1) |
| 0.1% NP-40 | ≤0.7 | ≥6.5 | Virus not detected | 20.9 (0.2) |
| 0.5% NP-40 | ≤0.7 | ≥6.5 | Virus not detected | 22.1 (0.1) |

**Interpretation**

Test 1: Both 0.1% and 0.5% NP40 reduced infectious virus titre to below the limit of detection of the test. The maximum detectable titre reduction in this test was 6.5 $log_{10}$.

Test 2: Infectious virus was not detected following four serial passages of samples treated with 0.1% or 0.5% NP-40.

Demonstrating complete inactivation is dependent on the starting titre of virus used for testing. 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.

Test 3: We observed a large increase in Ct value in SARS-CoV-2 specific real-time PCR following treatment with NP-40 (+9 Cts for 0.1% and +10 Cts for 0.5%) compared with untreated samples, suggesting that this treatment may have limited use for sample processing prior to molecular testing unless additional measures are taken to protect RNA integrity.

These tests have been performed on tissue culture fluid containing 5% (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.

**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

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

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