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**Research Article**

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# Virucidal Efficacy of Guanidine-Free Inactivants and Rapid Test Buffers against SARS-CoV-2: Implications for Risk Assessment of Diagnostic Procedures

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## Abstract

A pathogen inactivation step during collection or processing of clinical samples has the potential to reduce infectious risks associated with diagnostic procedures. It is essential that these inactivation methods are demonstrated to be effective, particularly for non-traditional inactivation reagents or for commercial products where the chemical composition is undisclosed. This study assessed inactivation effectiveness of twenty-four next-generation (guanidine-free) nucleic acid extraction lysis buffers and twelve rapid antigen test buffers against SARS-CoV-2, the causative agent of COVID-19. These data have significant safety implications for SARS-CoV-2 diagnostic testing and support the design and evidence-based risk assessment of these procedures.

## Introduction

The international response to the COVID-19 pandemic has required diagnostic testing of a vast number of patient samples worldwide for the detection of SARS-CoV-2[1]. SARS-CoV-2 is classified as a Hazard Group 3 (HG3) pathogen in the UK by the Advisory Committee for Dangerous Pathogens due to its potential to cause severe respiratory disease; propagative work for any purpose must be carried out at containment level 3 (CL3)[2], which can severely restrict testing capabilities. Due to the exceptional circumstances presented by COVID-19, non-propagative diagnostic testing may be carried out at a lower level of containment with heightened control measures and suitable, sufficient risk assessment of procedures[2,3]. In the UK, infectious material must be inactivated by a validated method before being handled outside a microbiological safety cabinet (MSC)[2]; selected inactivation methods must be both effective at reducing pathogen infectivity and compatible with downstream sample processing.

Development of rapid tests designed for near-patient or point-of-care (POC) use, including rapid RT-PCR, loop-mediated isothermal amplification (LAMP) and lateral flow immunoassay (LFIA) antigen tests, has permitted SARS-CoV-2 testing to move outside traditional laboratory settings into the community. As for all diagnostic procedures, performing these tests at POC or near-POC requires a suitable and sufficient risk assessment and implementation of appropriate control measures[2-4]: it is essential that the rapid testing procedure, which may involve swab stirring/mixing into an elution buffer with the potential for associated generation of aerosols, does not place individuals performing these tests at an increased risk of SARS-CoV-2 exposure. It is therefore highly desirable that rapid testing procedures include a step

that reduces the titre of infectious virus present e.g. by eluting samples into an effective lysis buffer.

The chaotropic agents guanidine thiocyanate (GTC) and guanidine hydrochloride (GHCl) are commonly used to inactivate viruses prior to nucleic acid testing[5] and guanidine-based lysis buffers are effective at reducing SARS-CoV-2 titre[6,7]. However, reagents containing GTC and GHCl are a chemical hazard in testing laboratories due to their toxicity and incompatibility with sodium hypochlorite-based disinfectants[8]. Their hazardous nature also limits use of GTC and GHCl buffers in home-sampling kits because of the potential for misuse by members of the public. There are now many commercial non-hazardous alternatives to guanidine-based lysis buffers; these would prove valuable additions to COVID-19 testing procedures if effective at inactivating SARS-CoV-2. Currently, there is limited information available on the efficacy of SARS-CoV-2 inactivation by either guanidine-free lysis buffers or by rapid test buffers. This study provides data on inactivation of SARS-CoV-2 by twenty-four guanidine-free molecular extraction reagents and specimen transport media, and twelve LFIA antigen test buffers.

## Methods

All handling of infectious SARS-CoV-2 was performed inside Class III MSCs within a CL3 facility. SARS-CoV-2 (England/02/2020) was propagated in Vero E6 cells (Vero C1008; ATCC CRL-1586) as previously described[6]. Virus was used in inactivation tests at passage 2 or 3 (with virus titres of approximately $1x10^7$ PFU per ml). Approximately 10-fold concentrated SARS-CoV-2 virus stocks (approximately $1x10^8$

PFU per ml) were produced by centrifuging SARS-CoV-2 supernatant through Amicon Ultra-15 (100K) filters (Millipore) according to the manufacturer's instructions.

Guanidine-free lysis buffers and LFIA tests kit buffers evaluated and the active ingredients of these products where disclosed are listed in Table 1. Inactivation testing was performed essentially as previously described[6]. In brief, SARS-CoV-2 was incubated with nucleic acid extraction buffers at a ratio and contact time indicated by the manufacturer's instructions, where these were available. LFIA buffers were tested after a 1-minute treatment time, to mimic likely contact times in real-life testing scenarios. 5- and 10-minute treatment times were additionally evaluated to determine the effect of longer incubation times. For most products, treated samples were passed through a filtration matrix (Detergent Removal Resin [Pierce], Sephadex LH-20 [GE Healthcare], Sephacryl S-400HR [Sigma-Aldrich] or BioBeads SM2 [Biorad]) to remove cytotoxic chemicals prior to virus titration to improve the limit of detection (LOD)[6]. The optimum purification method for each product was assessed as previously described[6], and is listed in Table 1. After sample clean-up, samples were 10-fold serially diluted in PBS and plated on to 96-well plates containing $2.5x10^4$ Vero E6 cells. Plates were incubated for 6-7 days at 37°C/5%$CO_2$, then fixed and stained with crystal violet to visualise cytopathic effect (CPE). The 50% tissue culture infectious dose ($TCID_{50}$) was calculated according to the Spearman-Karber method[9]. As a control for virus recovery, SARS-CoV-2 was mock-treated with PBS in parallel, at the same ratio as the reagent being tested. PBS-treated samples were subjected to the same filtration and titration methods as product-treated samples. All tests were carried out in triplicate and titre reduction was calculated by subtracting the mean $log_{10}$ titre of tested samples from the mean $log_{10}$ titre of PBS controls. A cytotoxicity control

was purified (if applicable) and titrated alongside other test samples, and used to determine the LOD for each test; this control consisted of PBS in place of virus, treated with an equivalent volume of product as test samples. 95% confidence intervals for titre reductions were calculated as mean $\log_{10}$ titre reduction ±1.96 standard error[9]. Products were tested against an unconcentrated SARS-CoV-2 stock as standard. Concentrated virus preparations were used to increase the maximum possible titre reduction for products (indicated in Table 1) that displayed high levels of cytotoxicity and/or that were previously found to reduce the titre of unconcentrated virus to below the test LOD.

## Results

The guanidine-free lysis buffers listed in Table 1 were tested for their ability to inactivate SARS-CoV-2. These reagents are variously marketed as appropriate for lysis of different sample types, including nasopharyngeal/oropharyngeal swabs, sputum and saliva, prior to nucleic acid extraction or as inactivating specimen transport media. A >4.0 $\log_{10}$ reduction in SARS-CoV-2 titre, required by the British European standard BS EN 14476 for quantitative virucidal suspension tests[9], was demonstrated for most guanidine-free nucleic acid extraction buffers tested (Table 1 and Figure 1A), including: virusPHIX+, virusPHIX-LV and virusPHIX-P9; VitaPCR Sample Collection Buffer; MELT Medium B, V3 and V4; D-Biotech Virus Preservation Solution; ID NOW COVID-19 Elution Buffer (after a 5-minute treatment); OMNIgene Oral; PROmate Sample Preparation Buffer containing either Triton X-100 or Triton X-100 reduced; Zeesan Saliva RNA Sample Collector Kit buffer; Zeesan Viral RNA Collection Kit buffer (after 30 minutes); IsoHelix GeneFix and Buccalfix buffers; GeneMe FRANKD (after 5 minutes) and SAVD buffers.

Of the molecular extraction reagents tested, we failed to demonstrate >4.0 $log_{10}$ reduction in SARS-CoV-2 titre for Salicovgel-1 (1.0 $log_{10}$ after 1 hour), Salicovgel-2 (3.1 and ≥3.4 $log_{10}$ reduction after 1 and 3 hours, respectively), MicroLYSIS-RNA (1.0 $log_{10}$ reduction after the recommended 5 minute contact time and 1.9 $log_{10}$ reduction after 20 minutes), virusPHIX-CU (1.0 $log_{10}$ reduction after 30 minutes) and Arcis Coronavirus Extraction Reagent 2506 (≥3.3 $log_{10}$ reduction after 10 minutes) (Table 1 and Figure 1A). Testing of the Arcis product using a 10-fold concentrated SARS-CoV-2 preparation to improve the maximum detectable titre reduction demonstrated only a 0.7 $log_{10}$ reduction after 10 minute treatment suggesting this buffer may be less effective with high concentrations of virus or protein (Table 1 and Figure 1A).

Only three of the twelve LFIA buffers tested reduced SARS-CoV-2 titre by greater than 4 $log_{10}$ (Table 2 and Figure 1B): the BD Veritor Extraction Reagent (≥4.5 $log_{10}$ reduction after a 1-minute treatment); the ScheBo SARS-CoV-2 Quick Antigen extraction buffer (5.3 $log_{10}$ and ≥6.0 $log_{10}$ reduction after 1 minute and 5 minutes, respectively); and the Mologic Rapid Antigen Test Sample Buffer (≥5.4 $log_{10}$ reduction after 1 minute). Several LFIA buffers reduced SARS-CoV-2 titre more modestly. Standard Q Extraction Buffer from the SD Biosensor COVID-19 Ag LFIA gave a reduction of 2.9 $log_{10}$ after 10 minutes. Maximum demonstrable titre reductions for Healgen and Standard Q Saliva LFIA buffers were 1.6 $log_{10}$ and 1.8 $log_{10}$, respectively, after 10 minutes. ESPLINE, Panbio, LumiraDx, Anhui Deepblue, Innova and Vstrip LFIA buffers had a negligible effect on infectious SARS-CoV-2 , even after a 10-minute contact time (Table 2 and Figure 1B).

## Discussion

Incorporation of novel products into existing diagnostic workflows requires them to be effective at inactivating pathogens; this study has provided inactivation efficacy data for twenty-four new products against SARS-CoV-2. Many new nucleic acid extraction reagents are guanidine-free and their inclusion in diagnostic procedures in place of hazardous guanidine-based reagents has the potential to eliminate the chemical hazards associated with guanidine use. Furthermore, many new products are supplied as non-hazardous: non-hazardous inactivating specimen transport media have the potential to increase the safety of sample transport and the speed of testing processes while not posing a chemical hazard to users at the sampling stage. SARS-CoV-2 inactivation effectiveness of twelve buffers provided with LFIA tests was additionally evaluated in this study, since these are being increasingly employed as COVID-19 testing capabilities are scaled up to include home and workplace testing.

Of the products that were demonstrated to inactivate SARS-CoV-2 effectively (i.e. >4 $\log_{10}$ reduction in titre following treatment), four nucleic acid extraction buffers (MELT medium B, D-Biotech Virus Preservation Solution, Abbott ID NOW Elution Buffer, PROmate Sample Preparation Buffer, and two LFIA buffers (BD Veritor and Schebo) are known to contain the non-denaturing detergent Triton X-100 as an active ingredient; we and others have previously shown that the Triton X-100 is effective at reducing SARS-CoV-2 titres by >5-6 $\log_{10}$[6,10]. Commercial products containing Triton X-100 reduced (PROmate and MELT V3), Triton CG-110 (MELT V4), SDS (OMNIgene Oral), trifluoroacetamide/trimethylglycine (virusPHIX+, -LV and -P9), sodium hydroxide (VitaPCR), sodium lauroyl sarcosinate (Zeesan Saliva RNA Collector Kit buffer) were also effective at reducing SARS-CoV-2 infectivity. Several guanidine-free

products (Geneme FRANKD and SAVD buffers, GeneFix and BuccalFix) and one LFIA buffer (Mologic) were effective but information on chemical composition for these buffers has not been disclosed by the manufacturers. Enzymatic inactivation may be an alternative to chemical inactivation, and the inactivation of viruses has been previously demonstrated using proteolytic enzymes[11]. Virucidal activity of the enzyme-based inactivants Salicovgel-1 and Salicovgel-2 were assessed in this study; while a >4 log10 titre reduction could not be demonstrated by either of these products, Salicovgel-2 reduced SARS-CoV-2 titre by ≥3 $\log_{10}$ after 1-hour treatment, indicating potential for enzymatic products as SARS-CoV-2 inactivants. Data presented in this study will aid testing laboratories that are required to replace Triton X-100 with Triton X-100 alternatives, due to the inclusion of Triton X-100 on the European Authorisation list (Annex XIV) of the Registration, Evaluation, Authorisation and Restrictions of Chemicals (REACH) for phasing out of use[12,13]. In addition, several of the products shown to be effective inactivators of SARS-CoV-2 are marketed as non-hazardous specimen transport media, indicating that these may have potential for use in home-sampling kits.

LFIA tests require mixing of a test swab or specimen with the buffer followed by use of a pipette or dropper to load a test cassette; the LFIA testing procedure may therefore lead to the generation of infectious aerosols. Furthermore, the infectious titre of samples tested directly after patient sampling (as is the case for POC tests) is likely to be higher than for those that are transported from sampling sites to testing laboratories before further handling. Inactivation efficacy of POC test buffers is a key consideration for risk assessment of POC testing processes: only three of the twelve LFIA buffers tested in this study were effective at inactivating SARS-CoV-2. Several LFIA buffers

tested were completely ineffective against SARS-CoV-2 and of those that did reduce virus titre, most gave modest reductions in comparison with other extraction buffers. Data presented here indicate that testing centres should not rely upon LFIA buffers to completely inactivate infectious samples and that additional control measures should be implemented to ensure the protection of test operators.

Data on the effectiveness of any inactivation step is crucial for designing and risk assessing testing procedures. Findings presented here are relevant for SARS-CoV-2 diagnostic laboratories, testing centres and mass testing programmes worldwide, providing data to support evidence-based risk assessment of testing procedures.

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## Author Contributions Statement

KD, UA, HB, CB, SRW, NG, RS, TBK, NH, MJH, MJ and MJK conducted inactivation experiments; KD, PS and MJK analysed data; KD and MJK prepared the manuscript; PAC, CBB, NW, ADG and MJK conceived and designed the work. All authors reviewed the manuscript.

## Additional Information

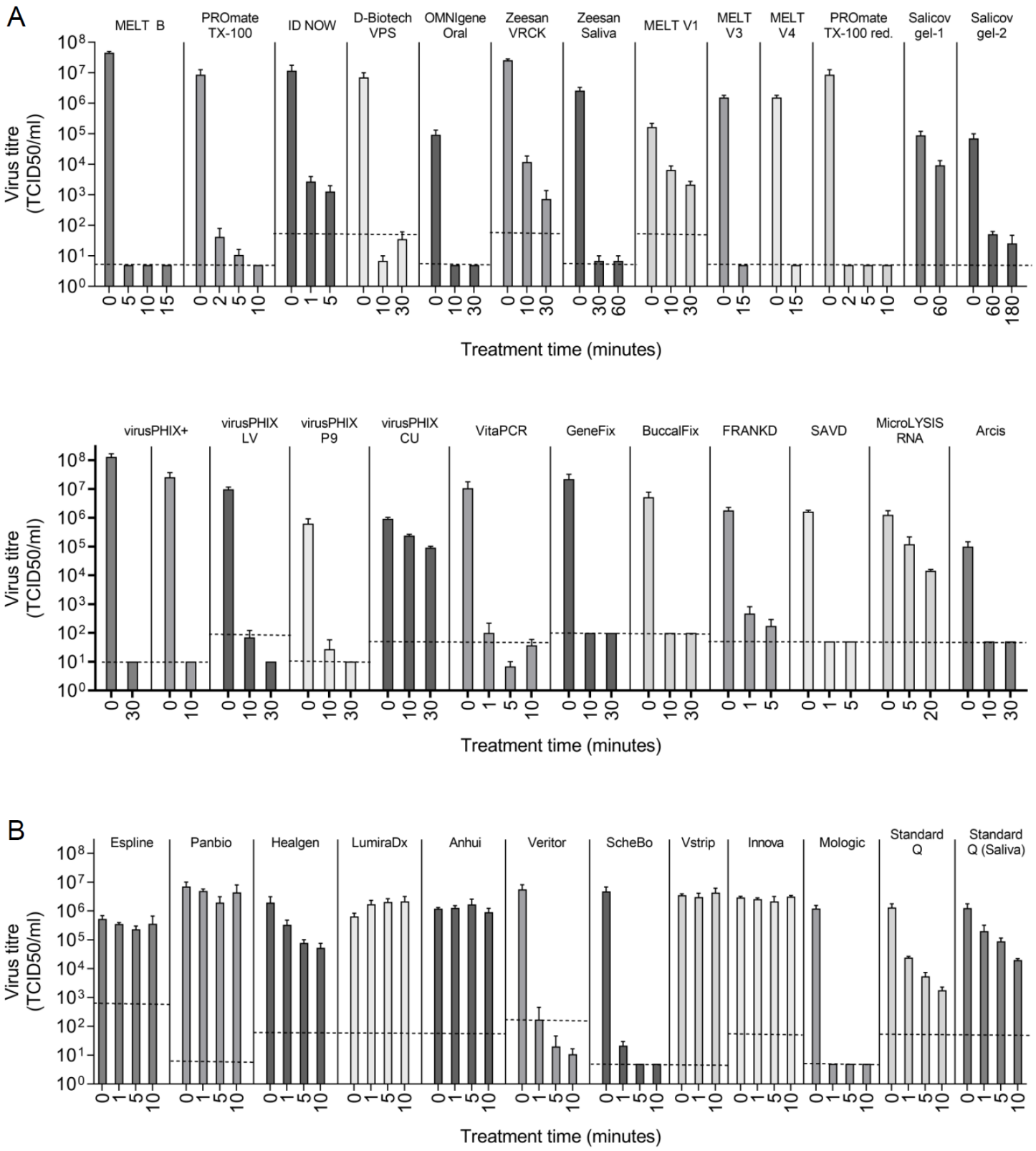
The authors declare no competing interests.

## Figure Legend

**Figure 1: SARS-CoV-2 titre reductions following treatment with guanidine-free molecular extraction reagents and LFIA buffers**

SARS-CoV-2 suspension was treated with guanidine-free molecular extraction reagents (A) or LFIA buffers (B), using the contact times and concentrations stated in Table 1 and Table 2, respectively. SARS-CoV-2 was mock-treated with an equivalent volume of PBS in parallel. Samples were purified by methods indicated in Table 1 and Table 2 to remove product-associated cytotoxicity, then titrated by $TCID_{50}$ on Vero E6 cells to determine virus titre. All treatments and mock-treatments were performed in triplicate; bars show the mean of sample triplicates and error bars the standard deviation of triplicates. The limit of detection for each test was determined using the cytotoxicity control for each test, and is indicated on the graph for each test by a dashed line. Where cytotoxicity of sample replicates and/or treatment times for a product differed, the highest LOD for the entire test is displayed. Variation in virus titres for PBS-treated samples between product tests was due to differences in the titre of virus stock used, the ratio of sample to product or PBS used for treatment, and differing virus recovery following sample filtration with different methods[6]. N.B. The test of Arcis Extraction Reagent 2506 against unconcentrated virus only is shown. VRCK: Virus RNA Collection Kit; VPS: Virus Preservation System; Triton X-100 red.: Triton X-100 reduced.

**Figure 1**


A
MELT B
PROmate TX-100
ID NOW
D-Biotech VPS
OMNIgene Oral
Zeesan VRCK
Zeesan Saliva
MELT V1
MELT V3
MELT V4
PROmate TX-100 red.
Salicov gel-1
Salicov gel-2
virusPHIX+
virusPHIX LV
virusPHIX P9
virusPHIX CU
VitaPCR
GeneFix
BuccalFix
FRANKD
SAVD
MicroLYSIS RNA
Arcis
Virus titre (TCID50/ml)
Treatment time (minutes)
B
Espline
Panbio
Healgen
LumiraDx
Anhui
Veritor
ScheBo
Vstrip
Innova
Mologic
Standard Q
Standard Q (Saliva)
Virus titre (TCID50/ml)
Treatment time (minutes)

## Table 1: SARS-CoV-2 inactivation by guanidine-free nucleic acid extraction reagents

| Product name (manufacturer) | Active ingredient/s, if known* | Purification resin‡ | Reagent: virus ratio | Contact time (minutes) | Titre reduction, $log_{10}$ $TCID_{50}$/ml [±95% CI] † | Virus detectable in $TCID_{50}$ (virus detected in n/N replicates) |
|---|---|---|---|---|---|---|
| virusPHIX+ (RNAssist) | 20-90% trifluoroacetamide<br>20-90% trimethylglycine | PDRR | 3:1 | 10<br>30 | ≥6.4 [6.2-6.7]<br>≥7.1 [6.9-2.4] | No[3]<br>No[3] |
| virusPHIX-LV (RNAssist) | 20-50% trifluoroacetamide<br>20-50% trimethylglycine | PDRR | 10:1# | 10<br>30 | ≥5.1 [4.8-5.5]<br>≥6.0 [5.7-6.3] | No[4]<br>No[3] |
| virusPHIX-P9 (RNAssist) | 2-5% trifluoroacetamide<br>1-3% trimethylglycine | LH20 | 3:1 | 10<br>30 | ≥4.4 [4.1-4.6]<br>≥4.8 [4.5-5.1] | Yes (1/3)<br>No[3] |
| virusPHIX-CU (RNAssist) | Urea, choline chloride | None | 3:1 | 10<br>30 | 0.6 [0.1-1.0]<br>1.0 [0.6-1.4] | Yes (3/3)<br>Yes (3/3) |
| VitaPCR™ SARS-CoV-2 Sample Collection Buffer (Credo Diagnostics) | 0.2% sodium hydroxide | PDRR | 10:1# | 1<br>5 | ≥5.0 [4.7-5.3]<br>6.2 [5.9-6.5] | Yes (3/3)<br>Yes (3/3) |
| MELT Medium B (Mast Group) | 1-10% Triton X-100 | PDRR | 10:1# | 5 | ≥7.0 [6.6-7.3] | Yes (1/3) |
| MELT V1 (Mast Group) | Ethylene oxide propylene oxide copolymer mono (nonylphenyl) ether | PDRR | 10:1 | 10<br>30 | 1.4 [1.0-1.8]<br>1.9 [1.5-2.2] | Yes (3/3)[1]<br>Yes (3/3) |
| MELT V3 (Mast Group) | Triton X-100 reduced | SM2 | 10:1 | 15 | ≥5.5 [5.2-5.8] | No[1] |
| MELT V4 (Mast Group) | Triton CG-110 | SM2 | 10:1 | 15 | ≥5.5 [5.2-5.8] | No[1] |
| Virus Preservation Solution (D-Biotech) | Citric acid<br><0.1% Triton X-100 | PDRR | 10:1# | 10<br>30 | ≥6.0 [5.7-6.3]<br>≥5.3 [5.0-5.6] | Yes (2/3)<br>No[2] |
| ID NOW COVID-19 Elution Buffer (Abbott) | 0.1% Triton X-100 | PDRR | 25:1# | 1<br>5 | 3.6 [3.2-4.0]<br>4.0 [3.6-4.4] | Yes (3/3)<br>Yes (3/3) |
| OMNIgene Oral (DNA Genotek) | 1-5% SDS | S400HR | 1:1 | 10 | ≥4.3 [4.0-4.6] | No[1] |
| COVID-19 PROmate Sample Preparation Buffer with standard Triton X-100 (Novacyt) | 0.5% Triton X-100 | SM2 | 10:1# | 2<br>5<br>10 | 5.3 [5.0-5.7]<br>5.9 [5.6-6.2]<br>≥6.2 [5.9-6.6] | Yes (3/3)<br>Yes (3/3)<br>Yes (1/3) |
| COVID-19 PROmate Sample Preparation Buffer with Triton X-100 reduced (Novacyt) | 0.5% Triton X-100 reduced | SM2 | 10:1# | 2 | ≥6.2 [5.9-6.6] | Yes (2/3) |
| Saliva RNA Sample Collector Kit (Zeesan) | 5-15% sodium lauroyl sarcosinate | PDRR | 2:1 | 30 | ≥5.6 [5.3-5.9] | Yes (2/3) |
| Virus RNA Collection Kit (Zeesan) | 5-10% sodium lauroyl sarcosinate | SM2 | 2:1 | 10<br>30 | 3.3 [2.9-3.7]<br>4.5 [4.3-4.8] | Yes (3/3)<br>Yes (3/3) |
| Salicovgel-1 (LGC) | Enzymatic | S400HR | 10:1 | 60 | 1.0 [0.6-1.4] | Yes (3/3) |
| Salicovgel-2 (LGC) | Enzymatic | S400 HR | 10:1 | 60<br>180 | 3.1 [2.9-3.4]<br>≥ 3.4 [3.2-3.7] | Yes (3/3)<br>Yes (2/3) |
| GeneFix Saliva RNA Collector (IsoHelix) | Not available | S400HR | 1:1 | 10 | ≥5.3 [5.0-5.6] | No[4] |
| BuccalFix (IsoHelix) | Not available | S400HR | 5:1# | 10 | ≥4.7 [4.4-5.0] | No[4] |
| MicroLYSIS-RNA (Clent Life Science) | Not available | S400HR | 1:1 | 5<br>20 | 1.0 [0.6-1.4]<br>1.9 [1.6-2.3] | Yes (3/3)<br>Yes (3/3) |
| Coronavirus Extraction Reagent 2506 (Arcis Biotechnology) | Not available | None | 2:1 | 10 | ≥3.3 [3.0-3.6] | No[5] |
| | | | 2:1# | 10<br>30 | 0.7 [0.3-1.2]<br>2.6 [2.3-3.0] | Yes (3/3)<br>Yes (3/3) |
| FRANKD Buffer (GeneMe) | Not available | PDRR | 10:1 | 1<br>5 | 3.6 [3.3-3.9]<br>4.0 [3.7-4.3] | Yes (3/3)<br>Yes (3/3) |
| SAVD Buffer (GeneMe) | Not available | PDRR | 4:1 | 1 | ≥4.5 [4.2-4.8] | No[5] |

See overleaf for table footnotes

Where identical results were obtained at multiple contact times, only the shortest contact time is shown

*As indicated on product safety data sheets or other product literature

†Values are given as ≥ when at least one replicate was below the limit of detection

#Tested using concentrated virus stock

‡PDRR: Pierce Detergent Removal Resin; SM2: Biorad SM2 BioBeads; S400HR: Sephacryl S-400HR; LH20: Sephadex LH-20

STM: Specimen transport media

Limit of detection: [1]0.7 $\log_{10}$ $TCID_{50}$/mL; [2]0.8 $\log_{10}$ $TCID_{50}$/mL; [3]1.0 $\log_{10}$ $TCID_{50}$/mL; [4]2.0 $\log_{10}$ $TCID_{50}$/mL; [5]1.7 $\log_{10}$ $TCID_{50}$/mL

## Table 2: SARS-CoV-2 inactivation by rapid antigen test buffers

| Product name (manufacturer) | Active ingredient/s, if known* | Purification resin‡ | Reagent: virus ratio | Contact time (minutes) | Titre reduction, $log_{10}$ $TCID_{50}$/ml [±95% CI)† | Virus detectable in $TCID_{50}$ (virus detected in n/N replicates) |
|---|---|---|---|---|---|---|
| ESPLINE® SARS-CoV-2 Extraction Solution (Fujirebio) | ≥0.25-≤0.5% cetrimonium chloride | SM2 | 2.3:1 | 1 | 0.2 [-0.3-0.6] | Yes (3/3) |
| | | | | 5 | 0.4 [-0.1-0.8] | Yes (3/3) |
| | | | | 10 | 0.2 [-0.2-0.6] | Yes (3/3) |
| Panbio COVID-19 Ag Rapid Test Device Buffer (Abbott) | 0.49% Tween-20 | PDRR | 3:1 | 1 | 0.2 [-0.3-0.6] | Yes (3/3) |
| | | | | 5 | 0.6 [0.1-1.0] | Yes (3/3) |
| | | | | 10 | 0.2 [-0.2-0.7] | Yes (3/3) |
| Healgen Coronavirus Ag Rapid Test Cassette Swab Buffer (Zhejiang Orient Gene) | Not known | PDRR | 3:1 | 1 | 0.8 [0.4-1.2] | Yes (3/3) |
| | | | | 5 | 1.4 [1.0-1.8] | Yes (3/3) |
| | | | | 10 | 1.6 [1.2-2.0] | Yes (3/3) |
| LumiraDx SARS-CoV-2 Ag Test Extraction Buffer (LumiraDx) | 0.5% unspecified detergent 1.00% Tween 20 | PDRR | 7.5:1 | 1 | -0.4 [-0.9-0.0] | Yes (3/3) |
| | | | | 5 | -0.5 [-0.9- -0.1] | Yes (3/3) |
| | | | | 10 | -0.5 [-1.0- 0.0] | Yes (3/3) |
| COVID-19 (SARS-CoV-2) Antigen Test Kit Extraction Reagent (Anhui Deepblue Medical Technology) | Not known | None | 3:1 | 1 | 0.0 [-0.4-0.3] | Yes (3/3) |
| | | | | 5 | 0.0 [-0.6-03] | Yes (3/3) |
| | | | | 10 | 0.1 [-0.3-0.5] | Yes (3/3) |
| Veritor Extraction Reagent (BD) | 0.1-<1% Triton X-100 | PDRR | 3.25:1 | 1 | ≥4.5 [4.2-4.8] | No[3] |
| | | | | 5 | ≥5.4 [5.2-5.7] | No[2] |
| | | | | 10 | ≥5.7 [5.4-6.0] | No[1] |
| SARS-CoV-2 Quick™ Antigen Extraction Buffer (ScheBo) | < 1% Triton X-100 | PDRR | 2.5:1 | 1 | 5.3 [5.1-5.6] | Yes (3/3) |
| | | | | 5 | ≥6.0 [5.7-6.3] | Yes (2/3) |
| | | | | 10 | ≥6.0 [5.7-6.3] | No[4] |
| Vstrip COVID-19 Antigen Rapid Test Extraction Buffer (Panion & BF Biotech) | <1% Tergitol <1.2% Methanol | PDRR | 10:1 | 1 | 0.1 [-0.3-0.4] | Yes (3/3) |
| | | | | 10 | 0.0 [-0.5-0.3] | Yes (3/3) |
| SARS-CoV-2 Antigen Qualitative Test Extraction Solution (Innova) | Not known | None | 2:1 | 1 | 0.1 [-0.3-0.5] | Yes (3/3) |
| | | | | 5 | 0.1 [-0.2-0.5] | Yes (3/3) |
| | | | | 10 | 0.0 [-0.5-0.4] | Yes (3/3) |
| COVID-19 Rapid Antigen Test Sample Buffer (Mologic) | 0.4% unspecified surfactant | PDRR | 3.5:1 | 1 | ≥5.4 [5.1-5.7] | No[1] |
| Extraction Buffer from Standard Q COVID-19 Ag Test Kit (SD Biosensor) | Not known | PDRR | 2:1 | 1 | 1.7 [1.3-2.1] | Yes (3/3) |
| | | | | 5 | 2.4 [2.0-2.8] | Yes (3/3) |
| | | | | 10 | 2.9 [2.4-3.3] | Yes (3/3) |
| Extraction Buffer from Standard Q COVID-19 Ag Saliva Test Kit (SD Biosensor) | Not known | PDRR | 3.5:1 | 1 | 0.8 [0.4-1.2] | Yes (3/3) |
| | | | | 5 | 1.1 [0.7-1.6] | Yes (3/3) |
| | | | | 10 | 1.8 [1.4-2.2] | Yes (3/3) |

*As indicated on product safety data sheets or other product literature

†Values are given as ≥ when at least one replicate was below the limit of detection

‡PDRR: Pierce Detergent Removal Resin; SM2: Biorad SM2 BioBeads;

Limit of detection: [1]1.0 $log_{10}$ $TCID_{50}$/mL; [2]1.3 $log_{10}$ $TCID_{50}$/mL; [3]2.2 $log_{10}$ $TCID_{50}$/mL; [4]0.7 $log_{10}$ $TCID_{50}$/mL; [5]1.7 $log_{10}$ $TCID_{50}$/mL