Inactivir™ Viral Inactivation Buffers stabilise RNA for extended periods

Purpose

It is often necessary or advantageous to be able to store samples containing DNA or RNA to be analysed for extended periods of time. This may be necessitated by the site of collection, where the ability to analyse samples immediately may be limited[1], or where it is advantageous to store a time course of samples to then be analysed simultaneously more conveniently and without the worry of run-to-run variation in the generated data[2].

This application note quantifies the stability of nCoV-19 RNA (EURM-19 reference standard) in a variety of chaotrope-based Viral Inactivation Buffers (referred to as VIBs in this note) over an extended time period with samples stored at both room temperature (21 °C) for up to 5 days (120 hours) and for a period of up to 10 weeks when stored at -20 °C.

A range of VIBs are used. The base standard is the 'first generation' Inactivir™ Viral Inactivation Buffer, a guanidine / Triton X-100 based buffer developed by LSG and validated by PHE. This is analogous to the buffer developed and validated by Buron et al for inactivation and testing of Ebola[3]. The Inactivir™ buffers have been tested by PHE and accepted as both inactivating nCoV-19 and stabilising the released RNA for future analysis. This buffer is compared to a set of 'second generation' buffers. This includes a REACH-compliant guanidine-based buffer, phasing out the use of Triton X-100 at end of 2020. An alternative SDI inactivating buffer is also tested, based on an alternative chaotrope, which is compatible with subsequent bleach cleaning agents and has a lower overall hazard assessment than guanidine. This has also been tested by PHE and shown to inactivate nCoV-19 to the limit of detection of their assays.

All the Inactivir™ buffers contain chaotropes that allow direct to extraction processing without further addition of a binding buffer. This increases the amount of sample that can be processed if this first step is excluded from the extraction protocol, increasing sensitivity and saving time. The buffers are compatible with standard commercial RNA extraction kits. They have been validated against exemplars of the Qiagen™ QIAamp™ Viral RNA Mini Kit for silica spin columns and the Applied Biosystems™ MagMAX™ Viral RNA isolation kit for magnetic-bead based extractions.

[1] Blow et al *Journal of virological methods*, (2008) https://doi.org/10.1016/j.jviromet.2008.02.003
[2] Han et al. *Lancet Infect Dis.*, (2021) https://doi.org/10.1016/S1473-3099(20)30439-4
[3] Burton et al. *Journal of virological methods*, (2017) https://doi.org/10.1016/j.jviromet.2017.09.020

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Results

Extractions were completed with duplicate samples for each run with duplicates for QPCR detection. The Ct values determined for each extraction are shown in Figure 1, with the pseudo samples stored at 21°C in the left panel and patient samples stored at -20 °C in the right panel.

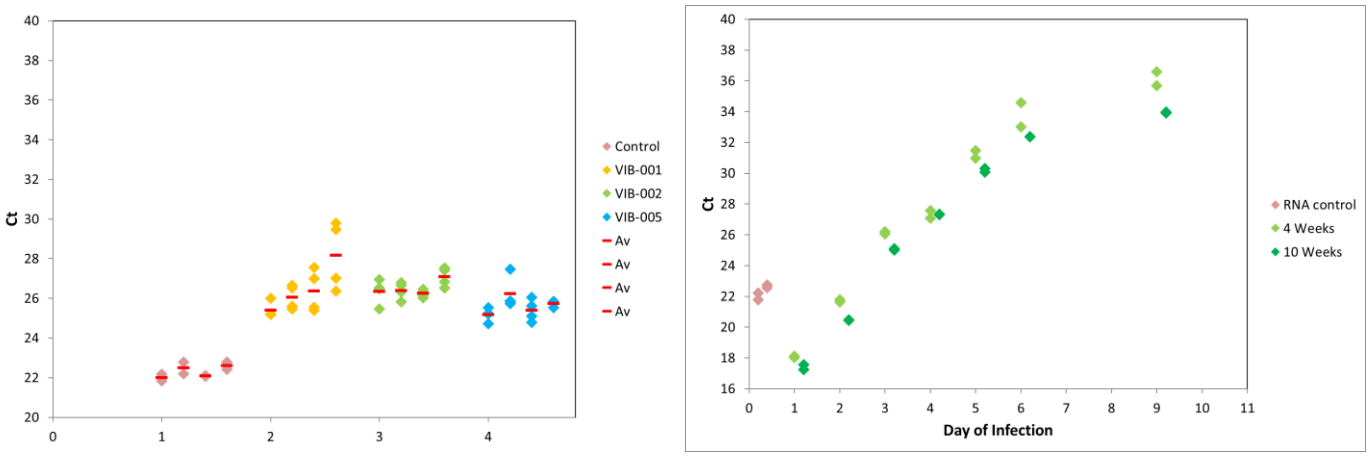

Fig1 Left panel (A) shows Ct values of samples stored at 21°C. Samples are 1: Viral RNA control, 2: VIB-001 Inactivir™ Guanidine-based, 3: VIB-002 REACH-compliant Guanidine-based**,** 4: VIB-005 SDI**.** Data for each are shown at 0, 24, 48 and 120 hours (left to right). Right panel (B) shows Ct values of Covid patient swab samples taken over the time course of infection directly into VIB-002 and then stored for 4 weeks or 10 weeks at -20 °C before analysis.

These figures show the RNA control replicates have a Ct of around 22, which conforms with the expectation of the EURM-19 standard and shows less than 1 Ct run to run variation. The extracted RNA from the pseudo samples (Fig 1A) is in the 26+ range. This 4 Ct loss is primarily due to the 10 fold dilution due to using 5ul in assays from 50 ul eluted from the purification. This shows that at 21°C all the buffers show good RNA stabilisation across the at room temperature across the 120 hour storage period, with only VIB-001 showing a small decrease after 120 hours.

Figure 1B shows a set of patient samples taken across an infection time course and stored for either 4 or 10 weeks. These show remarkable consistency, with less than 1 Ct average difference between the sample when stored for 4 or 10 weeks. The range of values covers the full range expected for a potentially infectious person across a Covid infection. The sample set after 10 weeks storage shows consistently slightly lower Ct values, evidencing a small but consistent variation which is presumed to be in the RNA extraction step, as the RNA control samples are virtually identical.

Conclusions

The data shows very high preservation of viral RNA from both pseudo samples made with reference RNA at 21 °C across a 120 hour time course and also from patient samples stored frozen at -20 °C for up to 10 weeks. This demonstrates that these chaotrope containing Inactivir™ Viral Inactivation Buffers are highly effective at preserving RNA in samples for subsequent analysis.

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Methods

Pseudo-samples were made to replicate the environment of typical sampled viral RNA. These contained both human and bacterial cells, as would be expected in a swab or saliva sample, along with EURM-19 reference standard Covid RNA. This is a synthetic single-strand RNA encoding parts of the Covid genome routinely used for detection[4]. Samples were made in duplicate of 25 ul of a 1/1000 dilution of Covid-19 reference material EURM-019 plus 50 ul of Hela cells (~$10^{\wedge 6}$ ml^{-1}) in Phosphate Buffered Saline (PBS) and 20 ul of E. coli (~1 OD stock). To this sample 1 ml of the VIB under test was added.

The patient samples were standard nasopharyngeal swabs taken using nylon flocked swabs and immediately placed into tubes containing 0.5 ml Inactivir™ VIB-002. These were then frozen at -20 °C. Samples were defrosted for analysis after 4 weeks and immediately refrozen and stored before resampling at 10 weeks.

Samples were purified using a 'homemade' kit based on a published extraction procedure outlined by the Baker lab and used by Addenbrookes Hospital, Cambridge, Uk[5]. This has previously been validated to produce similar results to extractions from the commercial Qiagen™ QIAamp™ Viral RNA Mini Kit and the Applied Biosystems™ MagMAX™ Viral RNA isolation kit.

Samples were taken immediately after inactivation, and subsequently at each timepoint after storage at 21°C or -20 °C. From the stock 200 ul samples were taken and extracted with the previously validated 'home made' protocol, as follows. 200 ul of VIB inactivated sample was mixed with 100 ul of absolute ethanol. This was added to the EZ-10 column and spun at 8k rpm for 90 sec. The flow through was discarded and 350 ul of 3M Sodium Acetate (pH 5.2) wash buffer was added and spun again with the flow through discarded. The column was washed twice with 500 ul 70% ethanol, with spun and discard. The column was then spun to dry at 12.5 k rpm for 2 min. Finally, the column was placed in a fresh tube, 50ul of ultrapure water was added and after 2 min the sample was spun and the purified RNA collected.

For detection of the RNA the CDC published N2 primer / probe combination[6] was used with a FAM / BHQ-1 quencher combination for the probe (Sigma Aldrich). Samples were amplified using PCR Biosystem qPCRBIO Probe 1-Step Go master mix. For each tube 5 ul of sample was added to a total 20 ul reaction volume with the addition of N1 primers to 250 nM and N1 probe to 125 nM. Samples were amplified using a Corbett Research Rotor Gene 6000. The protocol was 50 C for 10 minutes for reverse transcription, followed by 95 C for 2 mins and 40 cycles of 95 C 5 sec, 60C 20 sec with read on the FAM channel

All Inactivir® products are registered as a medical device with MHRA

[4] https://crm.jrc.ec.europa.eu/p/EURM-019
[5] bioRxiv preprint https://doi.org/10.1101/2020.04.14.041319
[6] https://www.cdc.gov/coronavirus/2019-ncov/lab/rt-pcr-panel-primer-probes.html

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