Purpose

This application note quantifies the stability of nCoV-19 RNA (EURM-19 reference standard) in a variety of Viral Inactivation Buffers (referred to as VIBs in this note) over an extended time period with samples stored at both room temperature (21 °C) and an elevated temperature of 35 °C to mimic potential extremes in the supply chain.

A range of VIBs are tested. 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 has 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, replacing Triton X-100 with Tween 80 in line with phasing out of Triton X-100 at end of 2020. An alternative SDI inactivating buffer is also tested, based on a different inactivating mechanism with a lower overall hazard assessment. This has also been tested by PHE and shown to inactive nCoV-19 to the limit of detection of their assays.

The SDI mix contains sodium iodide as a chaotrope, allowing direct to extraction processing. This has been proved to be compatible with standard commercial RNA extraction kits. It has been validated against exemplars of the Qiagen™ QIAamp™ Viral RNA Mini Kit for spin columns and the Applied Biosystems™ MagMAX™ Viral RNA isolation kit for magnetic-bead based extractions.

Results

Extractions were completed in two batches with duplicate samples for each run, with duplicates of each for QPCR detection. The Ct values determined for each extraction are shown in Figure 1, with the samples stored at 21°C in the left panel and at 35°C in the right panel. These figures show the control replicates have a Ct of around 22 and the extracted RNA in the 26+ range. This 4 Ct loss is primarily due to the 10-fold loss from using 5ul in assays from 50 ul eluted from the purification. Panel A shows that at 21°C all the buffers show good RNA stabilisation across the time course. Panel B at 35°C shows greater variation, although some of this seems to be from procedural issues, as there is good recovery at the 48 hour time point from all the buffers.

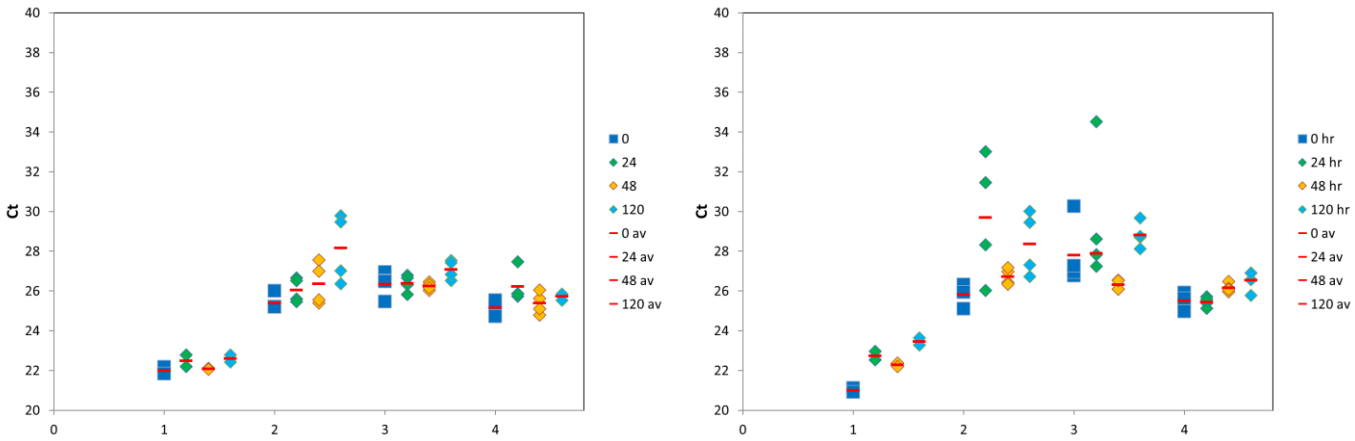

Figure 1: Left panel shows stability of samples stored at 21°C, right panel at 35°C. Samples are 1: Viral RNA control, 2: Inactivir™ Guanidine-based, 3: Gen2 REACH-compliant Guanidine-based**,** 4: SDI

Conclusions

Figure 1, shows is little degradation of RNA in any of the samples in VIBs stored at 21°C across the timecourse. At the elevated 35°C, modelling worst case storage, the VIBs evidence a 1 - 2 Ct drop. This is unlikely to produce false negatives as a low proportion of 'real world' positive samples are in the 34-37 Ct range at the limit of sensitivity, and published work by La Scola indicates these samples generally do not contain culturable virus[1].

Methods

Extractions were based on purification from pseudo-samples replicating the environment of sampled viral RNA, containing both human and bacterial cells, as would be expected in a swab or saliva sample. Samples were made in duplicate of 25 ul of a 1/1000 dilution of Covid-19 reference material EURM-019[2] 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.

Samples were purified using a 'home made' kit based on a published extraction procedure outlined by the Baker lab and used by Addenbrookes Hospital, Cambridge, Uk[3]. 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 either 21°C or 35°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[4] was used with a FAM / BHQ-1 quencher combination for the probe (Sigma Aldrich). Samples were amplified using PCRBiosystem 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.

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[1] LaScola et al. Eur J Clin Microbiol Infect Dis. 2020; 39(6): 1059–1061 doi: 10.1007/s10096-020-03913-9
[2] https://crm.jrc.ec.europa.eu/p/EURM-019
[3] bioRxiv preprint https://doi.org/10.1101/2020.04.14.041319
[4] https://www.cdc.gov/coronavirus/2019-ncov/lab/rt-pcr-panel-primer-probes.html

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