Inactivir™ Viral Inactivation Buffers stabilise DNA for sampling

Purpose

For the purposes of home sampling or even within near POC testing, the stability of the molecular target for analysis is critical to assay sensitivity. This may be necessitated by the site of collection, where the ability to analyse samples immediately may be limited[1].

This application note quantifies the stability of a DNA target (a DNA version of portions of the SARS nCoV2 virus) in Viral Inactivation Buffers VIB-003 Inactivir™ SD LITE and VIB-004 Inactivir™ SD COMPLETE (referred to as VIBs in this note) over a 72 hour time-period with samples stored at room temperature (21 °C). This covers a time period up to that which could be expected for home sampling and postal return of samples. Sample stability is tested using pseudo-samples containing known quantities of bacteria and human cells to represent the major components expected in swab samples.

The SD Inactivir™ Viral Inactivation Buffers tested are chemically classified as non-hazardous. They have also been tested by PHE and shown to inactivate the SARS nCoV2 virus to the limit of detection of their assays.

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. We have used an in-house purification method using EZ-10™ columns for the data presented in this report.

Results

Extractions were completed from 3 sets of duplicate samples. Each sample had the extracted DNA quantified in duplicates using QPCR detection with CDC N2 primers. The Ct values determined for each extraction are shown in Figure 1, with averages for the extractions shown by the red bars.

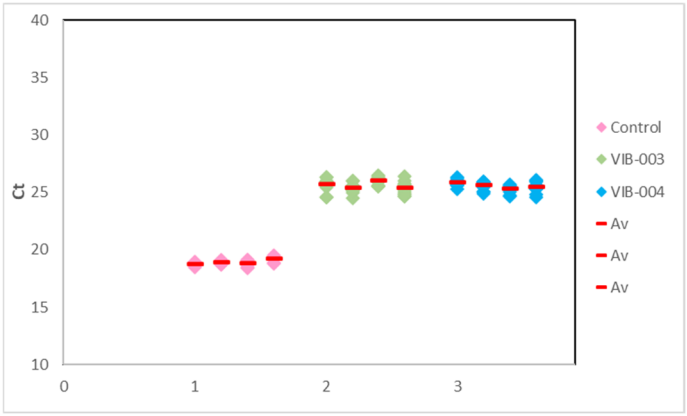

Fig1 Ct values of samples stored at 21°C. Samples are 1: Viral DNA control, 2: VIB-003 Inactivir™ SD LITE, 3: VIB-004 Inactivir™ SD COMPLETE**.** Data for each are shown at 0, 24, 48 and 120 hours (left to right).

[1] Blow et al *Journal of virological methods*, (2008) https://doi.org/10.1016/j.jviromet.2008.02.003

These figures show the DNA control replicates have a Ct of around 19 and shows less than 1 Ct run to run variation. The extracted RNA from the pseudo samples (Fig 1) is in the range of Ct 25-26. This 6 Ct loss is primarily due to the 10-fold dilution caused by using 5 µl in assays from 50 ul eluted from the purification. There is no significant variation in the Ct values from the recovered DNA over the 72 hour period. This shows no degradation of the samples across the 72-hour storage period.

Conclusions

The data shows excellent preservation of DNA within a pseudo sample made with a reference viral DNA target across a 72-hour time course at 21 °C. This demonstrates that these Inactivir™ Viral Inactivation Buffers are highly effective at preserving DNA in samples for subsequent analysis.

Methods

Pseudo-samples were made to replicate the environment of typical sampled viral DNA. These contained both human and bacterial cells, as would be expected in a swab or saliva sample, along with a DNA reference standard made from a plasmid containing SARS nCoV-2 sequence. This is a synthetic DNA encoding parts of the Covid genome routinely used for detection using the published CDC N2 primer set that has been extensively used for viral RNA detection. This allowed DNA stability to be assessed using the same procedures as have been widely established and validated previously. Samples were made in duplicate of 25 µl of our DNA reference material plus 50 µl of Hela cells (~$10^{\wedge 6}$ ml^{-1}) in Phosphate Buffered Saline (PBS) and 20 µl of E. coli (~1 OD stock). To this sample 1 ml of the VIB under test was added.

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[2]. 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. From the stock 200 µl samples were taken and extracted with the previously validated 'home made' protocol, as follows. 200 µl of VIB inactivated sample was mixed with 100 µl 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 µl 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 µl 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, 50 µl of ultrapure water was added and after 2 min the sample was spun and the purified DNA collected.

For detection of the DNA the CDC published N2 primer / probe combination[3] was used with a FAM / BHQ-1 quencher combination for the probe (Sigma Aldrich). Samples were amplified using PCR Biosystem qPCRBIO

[2] bioRxiv preprint https://doi.org/10.1101/2020.04.14.041319

[3] https://www.cdc.gov/coronavirus/2019-ncov/lab/rt-pcr-panel-primer-probes.html

Probe 1-Step Go master mix. For each tube 5 µl of sample was added to a total 20 µl reaction volume with the addition of N2 primers to 250 nM and N2 probe to 125 nM. Samples were amplified using a Corbett Research Rotor Gene 6000. The protocol was 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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