Rapid SARS-CoV-2 RNA Extraction from Universal Transport Medium Pooled Sample Mimics

Key Advantages

- Nanotrap® Magnetic Virus Particles enable RNA extraction of viral RNA from 4 mL UTM sample volumes in less than 20 minutes, representing a sample extraction method that is roughly 2X faster than column-based methods.
- Up to a 4-fold Ct value improvement for large volume pools across a range of viral titers, enabling detection of low viral load samples against a background of 7 negative samples in pooled sample mimics.

Introduction

As demand increases for testing for SARS-CoV-2, technologies that enable pooled testing for the virus will play a critical role in the efforts to remobilize the economy. Recent analyses from public health experts estimate that pooled testing could lower testing costs by up to three-quarters and could increase testing efficiency by as much as 400%, but concerns remain about reduced test sensitivity associated with pooling.[1-3]

Nanotrap® Virus Particles capture and concentrate multiple strains of influenza, RSV, and coronavirus, thus enhancing detection of those viruses, even in co-infection scenarios.[4-7] Here, we show that Nanotrap® Magnetic Virus Particles can capture and concentrate heat-inactivated SARS-CoV-2 from large volume universal transport medium samples that simulate patient sample pools. These results suggest that using Nanotrap® particles can alleviate sensitivity concerns associated with pooled sample testing.

Materials and Methods

Sample Pooling

Heat-inactivated SARS-CoV-2 was obtained from BEI Resources (NR-52286) and spiked into Puritan Universal Transport Medium (UTM) at various titers to make contrived samples. Five hundred microliters of one positive sample was then added to and mixed with 3.5 mL of negative UTM. This represents a pool in which eight samples (each 500 µL) are tested together with one sample being "infected" and the others being uninfected.

Nanotrap® Particle RNA Extraction

Three hundred microliters of Nanotrap® Magnetic Virus Particles (SKU 44202) were added to each 4 mL pool of UTM. Each sample was briefly vortexed and left to incubate at room temperature for 10 minutes. Samples were placed on a magnetic separation rack for 2 minutes to allow the particles to pellet. The supernatant was removed and discarded. The particle pellets were washed with 1X PBS. Following washing, the samples were again placed on the magnetic separation rack for 1 minute to allow the particles to pellet, and the wash solution was discarded. The particle pellet was resuspended in 50 µL of Triton X-100 (0.5% in PCR grade water), and the sample was heated at 95°C for 5 minutes to release viral nucleic acid. After the heating step, the sample was placed on a magnetic rack for 1 minute to allow the particles to pellet. The supernatant, which contained the viral nucleic acid, was collected in a clean microcentrifuge tube for downstream testing. The total processing time was less than 20 minutes.

Column RNA Extraction

Viral RNA was extracted from samples using the QIAamp® Viral RNA Mini Kit (QIAGEN). One hundred forty microliters of sample was processed following the manufacturer's instructions with no deviations. Briefly, 560 µL of QIAGEN Virus Lysis Buffer AVL was added to each sample, which was incubated for 10 minutes at room temperature. The supernatant was removed and transferred to a clean RNAse/DNAse-free microcentrifuge tube and 560 µL of 100% ethanol was added, followed by a brief vortex. The samples were added to the QIAamp Mini columns, centrifuged, and the flow-through was discarded. Two washes, one with Buffer AW1 and a second with Buffer AW2 (500 µL each), were performed with columns being placed in new collection tubes between washes. To ensure residual ethanol

was removed, additional centrifugation was performed at maximum speed, and columns were transferred to a clean 1.5 mL collection tube. For elution, 50 µL of Buffer AVE was added to the columns and incubated for 1 minute at room temperature, followed by centrifugation, eluting the RNA into a clean microcentrifuge tube for downstream testing.

Real Time RT-PCR Analysis

The extracted viral RNA was measured using Integrated DNA Technologies (IDT) 2019-nCoV RT-PCR CDC assay probes. The kit reagents and RT-PCR controls were prepared according to the manufacturer's instructions. For each reaction, 15 µL of the prepared reaction mix was added to an optical 96-well reaction plate and mixed with 5 µL of RNA template. The plate was sealed with an optical adhesive film, loaded into a Roche LightCycler® 96, and run using the amplification settings specified by IDT.

Results

Nanotrap® particles offer a quick and easy method to extract RNA from UTM sample volumes up to 4 mL. This method significantly improves the signal in the downstream RT-PCR assay, as compared to column-based RNA extraction methods of the same samples, as seen in **Figure 1**. Furthermore, the Nanotrap® particle method enabled detection of virus in an individual sample that was spiked at 100 copies / mL that was subsequently mixed with 7 other negative samples. Using the column-based RNA extraction method, there was no detectable virus in either the individual or the pooled samples. Average Ct values for all samples are in **Table 1**.

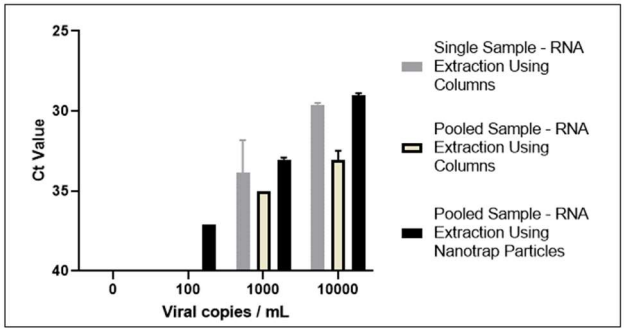

Figure 1. Nanotrap® particles improve SARS-CoV-2 detection in pooled sample mimics. One milliliter of UTM was spiked with 10^2, 10^3, or 10^4 copies/mL of heat-inactivated SARS-CoV-2. For each viral titer, 500 µL of spiked sample were added to 3.5 mL of unspiked UTM. Viral RNA was extracted from the 4 mL sample using Nanotrap® Particles or from a 150 µL aliquot using a column-based method. For comparison, viral RNA was extracted from a 150 µL aliquot of the single positive samples (prior to dilution in the pools) using the column-based method. For all methods viral RNA was detected with the IDT 2019-nCoV CDC Assay.

Conclusions

Viral Copies / mL Added to a Single Sample	Single Sample Ct Value – RNA Extraction Using Columns	Pooled Sample Ct Value – RNA Extraction Using Columns	Pooled Sample Ct Value – RNA Extraction Using Nanotrap® Particles
0	BLD	BLD	BLD
100	BLD	BLD	37.1
1,000	33.8	35.0	33.0
10,000	29.6	33.1	29.1

Table 1. Nanotrap® particle processing improves assay performance. One milliliter of UTM was spiked with a series of SARS-CoV-2 titers. For each titer, 500 µL of spiked sample was pooled with 3.5 mL of unspiked sample, and viral RNA was extracted using a Nanotrap® particle method or a column-based method. For comparison, viral RNA was extracted from a 150 µL aliquot of the single positive samples (prior to dilution in the pools) using the column-based method. Undetected Ct values are considered below the limit of detection (BLD).

Nanotrap® Magnetic Virus Particles enable enhanced SARS-CoV-2 detection in pooled patient sample UTM mimics up to 4 mL. Nanotrap® particles significantly reduced sample processing time and improved detection of virus at higher viral titers, ameliorating the loss of sensitivity that is associated with this testing strategy with no additional workflow complexity.

References

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