Capture and Concentration of Influenza A from Viral Transport Media with Nanotrap® Virus Particles

Improving RNA extraction for a more sensitive qRT-PCR assay

Key Advantages

> Circumvent the low input sample volume restrictions of most extraction methods by capturing influenza A from larger volumes.

> Capture and concentrate influenza virus from transport media with Nanotrap particles for greater viral RNA extraction yield and improved qRT-PCR assay sensitivity.

Introduction

Influenza is responsible for seasonal epidemics each year in the United States that result in an average of 200,000 hospitalizations and tens of thousands of deaths. Accurate and early diagnosis of influenza viral infections are critical for effective treatment and limiting the spread of disease.

Nucleic acid-based tests demonstrate high sensitivity and specificity as compared to antigen-based tests; however, limitations on input sample volume can still present sensitivity challenges in complex sample types. Using a simple and efficient pre-concentration workflow in combination with existing molecular test formats can improve detection at earlier time points following infection.

Nanotrap Virus Particles can capture, concentrate, and preserve viral pathogens from biological samples and improve downstream diagnostic assays such as enzyme-linked immunosorbent assays (ELISA), next generation sequencing assays (NGS), lateral flow assays (LFA), plaque infectivity assays, and quantitative reverse-transcription polymerase chain reaction assays (qRT-PCR).[1-6]

Here we demonstrate the impact of Nanotrap particle sample processing on influenza virus RNA extraction from viral transport media with QIAGEN's QIAamp® Viral RNA Mini kit, followed by qRT-PCR detection.

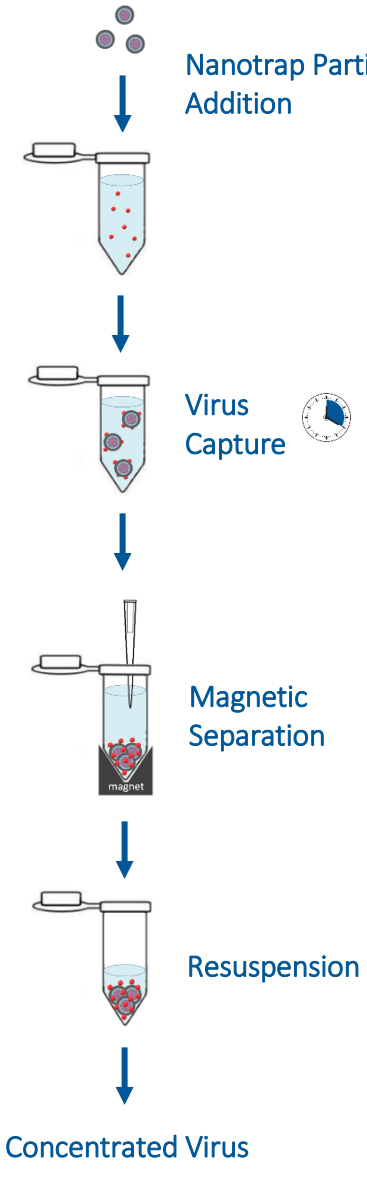

Figure 1. Nanotrap Virus Particle Processing Workflow for improved RT-PCR detection

Materials and Methods

Sample Preparation

Remel™ Xpect™ Flu A Control Swabs (Thermo Scientific) were used to inoculate 1.5 mL of fresh viral transport media (Puritan Universal Transport Media) according to the manufacturer's instructions. Spiked viral transport media was then diluted to appropriate concentrations for testing.

Nanotrap Particle Processing

Two hundred microliters of magnetic Nanotrap Virus particles were added to each sample and incubated at room temperature for 20 minutes with shaking. Following this incubation, the samples were placed in a magnetic rack for 2 minutes to pellet the virus-bound particles. The supernatant was removed and the virus-bound particle pellets were resuspended in 140 µL of 1X PBS for RNA extraction.

RNA Extraction

Viral RNA was extracted from samples processed with Nanotrap particles and from unprocessed samples using the QIAamp® Viral RNA Mini Kit (QIAGEN). For the unprocessed samples, 140 µL of spiked viral transport media was used for RNA extraction following the manufacturer's instructions with no deviations.

For each Nanotrap particle sample, 560 µL of QIAGEN AVL Buffer was added to the resuspended Nanotrap particle pellets, according to the manufacturer's instructions. The samples were incubated for 10 minutes at room temperature with shaking followed by magnetic separation. The supernatant was then carefully removed and transferred to a clean RNase/DNAse free microcentrifuge tube. Five hundred and sixty microliters of 100% ethanol was added to the samples and briefly vortexed. The samples were then added to the QIAamp Mini columns, centrifuged, and the flow-through discarded. Five hundred microliters of Buffer AW1 was added to the columns followed by centrifugation. The QIAamp Mini columns were transferred to clean 2 mL collection tubes and 500 µL of Buffer AW2 was added. The columns were centrifuged again and then transferred to a clean 2 mL microcentrifuge tube. To ensure residual ethanol was removed, an additional centrifugation was performed at maximum speed, and columns were then transferred to a clean 1.5 mL collection tube. For elution, 60 µL of Buffer AVE was added to the columns and incubated for 1 minute at room temperature, followed by centrifugation.

qRT-PCR Analysis

The viral RNA extracted from the samples processed with Nanotrap particles and the unprocessed (QIAamp extraction only) control samples was measured using the Genesig® Human Influenza A Virus Subtype H1 qRT-PCR kit (Primerdesign). The kit reagents, PCR controls, and standard curve 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 followed by 5 µL of RNA template. The plate was sealed with an optical adhesive film and loaded into a Roche LightCycler 96 and run using the amplification settings specified for use with oasig™ lyophilised OneStep RT-qPCR MasterMix.

Results

Sample Volume

Each QIAamp mini column has a maximum input volume of 140 µL. For larger sample volumes, multiple spin columns must be used which adds many additional steps and more time for RNA extraction, ultimately delaying detection and diagnosis. Nanotrap particles eliminate this input sample volume restriction by pre-concentrating virus from larger volumes. At 140 µL of sample, Nanotrap processing upstream of QIAamp RNA extraction produced a 2 – fold improvement on the detected flu virus concentration. Increasing the starting volume of sample processed to 2 mL had a more significant effect, providing a 14 – fold enrichment over QIAamp RNA extraction alone (Figure 2).

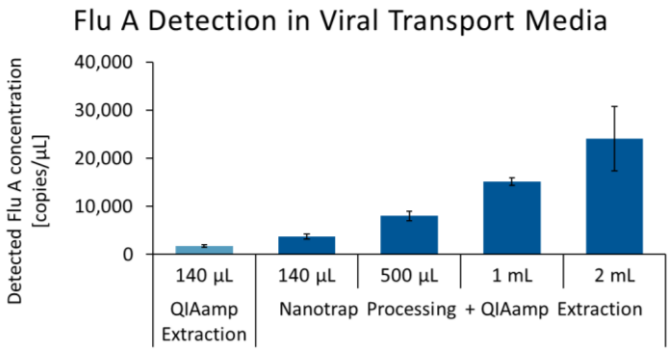

Figure 2. Impact of increased sample volume on flu A detection with Nanotrap processing. Viral transport media was inoculated using Flu A swabs as described, then diluted 1:250. Nanotrap particles were used to process volumes ranging from 140 µL up to 2 mL. RNA was extracted from 140 µL with the QIAamp Viral RNA mini kit as a reference control. Five technical replicates were evaluated via qRT-PCR for each sample.

qRT-PCR Sensitivity

Pre-concentrating virus with Nanotrap Virus Particles results in higher viral RNA concentrations, resulting in a more sensitive qRT-PCR assay. Capturing and concentrating virus from 2 mL of spiked virus transport media with Nanotrap particles upstream of RNA extraction and RT-PCR, enabled detection of flu virus at 2 copies/µL and reliable quantitation at 5.5 copies/µL, an improvement over the QIAamp extraction method alone (Figure 3).

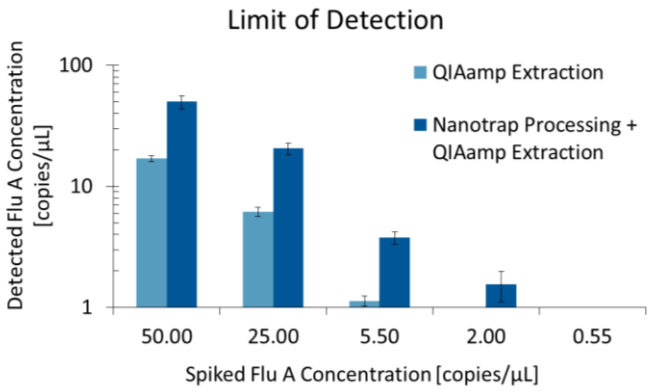

Figure 3. Flu qRT-PCR assay sensitivity with and without upstream Nanotrap processing. Viral transport media was inoculated using Flu A swabs as described and used to spiked fresh transport media across five concentration points. Nanotrap particles were used to process 2 mL aliquots of each spiked concentration before RNA extraction. RNA was extracted from 140 µL of unprocessed spiked transport media at each concentration point with the QIAamp Viral RNA mini kit. All samples were analyzed via qRT-PCR in technical triplicates.

Conclusions

Ceres' Nanotrap Virus Particles are an easy-to-use and effective solution for improving commercial flu virus RNA extraction and qRT-PCR detection methods through the up-stream capture and concentration of flu virus from larger volumes of viral transport media. The methods described in this application note are amenable to automated workflows.

Nanotrap particles have been shown to preserve pathogens at elevated temperatures (data not shown). This presents another opportunity to further streamline sample preparation workflows by incorporating Nanotrap particles in the viral transport media prior to sample collection.

References

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