Smart hydrogel particles improve detection of Flu A virus in transport media

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Introduction

Influenza viruses are 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 is critical.

Using simple and efficient pre-concentration workflows combined with existing molecular test formats can improve detection at earlier time points.

Nanotrap® Particle Technology

Nanotrap® particles are engineered hydrogel particles that use chemical affinity baits to capture, concentrate, and preserve low abundance analytes from biological samples.

Integrating shell (optional) facilitates rapid analyte exchange, and can be chemically modified for additional functionalization.

Affinity Bait: Selected to have very high affinity for a specific class of analyte, such as proteins, peptides, nucleic acids, exosomes, viral particles, or bacteria.

Magnetic Nanotrap® Particle-Based Methods

Magnetic Nanotrap® particles are added to spiked transport media

Unbound material is removed after a capture incubation period.

Nanotrap® particle method outperforms QIAGEN columns for extraction of Flu A RNA from spiked samples

Experimental Overview

Objectives
1. To demonstrate that magnetic Nanotrap® particles can significantly improve sensitivity of a flu A molecular assay by enriching flu virus from a larger sample.
2. To demonstrate the use of Nanotrap® particles to pre-concentrate Flu A from large volume viral transport media samples instead of a viral RNA extraction column for flu molecular assays.

Methods Comparison

QIAGEN Column-Based Method
150 µL of viral transport media spiked with Flu A positive control is mixed with RLT buffer and processed with QIAamp Viral RNA Mini Kit (QIAGEN) and then analyzed with a Genesig Flu A assay.

Magnetic Nanotrap® Particle-Based Methods

Ceres Method 1:
1. QIAGEN AVL buffer is used to lyse viral particles.
2. Nanotrap® particles are removed.
3. RNA is extracted.
4. Genesig Flu A assay is used for detection.

Ceres Method 2:
1. QIAGEN AVL buffer is used to lyse viral particles.
2. Nanotrap® particles are removed.
3. RNA is extracted with QIAamp Viral RNA Mini Kit.
4. Genesig Flu A assay is used for detection.

Ceres Method 3:
1. Viral particles are lysed.
2. Nanotrap® particles are removed.
3. RNA is extracted.
4. Genesig Flu A assay is used for detection.

After separating Nanotrap® particles and bound flu virus, three detection methods are possible:

Ceres Method 1:
1. Viral particles are lysed.
2. Nanotrap® particles are removed.
3. RNA is extracted.
4. Genesig Flu A assay is used for detection.

Ceres Method 2:
1. QIAGEN AVL buffer is used to lyse viral particles.
2. Nanotrap® particles are removed.
3. RNA is extracted with QIAamp Viral RNA Mini Kit.
4. Genesig Flu A assay is used for detection.

Ceres Method 3:
1. Viral particles are lysed.
2. RNA is extracted in the presence of Nanotrap® particles.
3. Genesig Flu A assay is used for detection.

Conclusions & Future Directions

- Nanotrap® particles can improve flu virus detection by pre-concentrating flu virus from viral transport media samples for RT-PCR detection.
- Methods are amenable to automation.
- Nanotrap® particles preserve pathogens at elevated temperatures. Incorporating Nanotrap® particles in the transport media could further improve sample processing workflows and downstream assay.

Large Volume Sample Enrichment

Ceres Method 1 enriches virus from large volumes of samples, which brings the low concentration sample into the detectable range, and results in a 19-fold increase in the detected levels of Flu A at the high concentration.

Combining Nanotrap® particles with QIAGEN columns in Ceres Method 2 further improves Flu A enrichment resulting in ≥ 8-fold and ≥ 3-fold increase in detected levels of Flu A.

Figure 2A. Enrichment comparison between QIAGEN columns and using Ceres Method 1 with a larger starting sample volume (2 mL).

Figure 2B. Method comparison between QIAGEN columns alone and Nanotrap® particles.

Figure 3. Ceres Method 3 performance across high and low concentrations of spiked transport medium.

Figure 4. Ceres Method 3 performance across high and low concentrations of spiked transport medium.

Nanotrap® Particles & Automation

Magnetic Nanotrap® particle-enabled large-volume sample enrichment in Ceres Method 3 offers streamlined work flows that can facilitate high-throughput automated sample processing on liquid handling platforms.

Table 1. Nanotrap® Particles for Flu A Virus Capture

| CN1030 | Reactive Red 120 Core Hydrogel Particles |
| CN3080 | Magnetic Reactive Red 120 Core Hydrogel Particles |

Methods Comparison

Qiagen Column - 150 µL
Ceres Method 1 - 2 mL

Low Conc. High Conc.

Low Conc. High Conc.

Figure 5. Ceres Method 3 performance across high and low concentrations of spiked transport medium.