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

P. Andersen[1], D. Goldfarb[1], J. Warsh[1], D. Munns[1], E. Porter[1], B. Lepene[1], R. Barbero[1]
[1]Ceres Nanosciences, Inc., Manassas, VA, USA

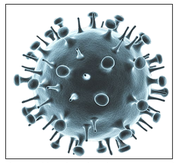
Sunday - 25
ASM Clinical Virology Symposium
May 5-8, 2019 Savannah, Georgia

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.

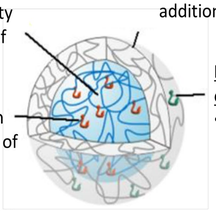

https://molecular.diasorin.com/

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.

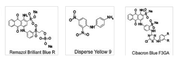
Core:
- Highly porous hydrogel facilitates rapid analyte exchange
- Functionalized with affinity bait to capture analytes of interest

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



Shell (optional):
- Second layer of hydrogel performs size exclusion.
- Can be chemically modified for additional functionalization.

Molecular Functionalization of Shell (optional):
- Selected to provide additional functionality, such as to repel charged analytes



Nanotrap® particles for viral enrichment

Nanotrap® particles concentrate viral pathogens in biological fluids for improved detection by downstream diagnostic assays such as enzyme-linked immunosorbent assay (ELISA), next generation sequencing (NGS), lateral flow assays (LFA), plaque infectivity assays, and quantitative reverse-transcription polymerase chain reaction (qRT-PCR).

Table 1. Nanotrap® Particles for Flu A Viral Capture

NT Catalog No.	Product Description
CN1030	Reactive Red 120 Core Hydrogel Particles
CN3080	Magnetic Reactive Red 120 Core Hydrogel Particles

Experimental Overview

Objectives

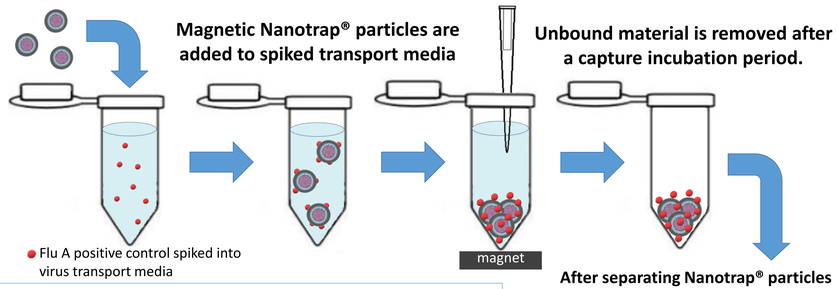
1. To demonstrate that magnetic Nanotrap® particles can significantly improve sensitivity of a flu A molecular assay by enriching flu virus from a larger sample.
2. To demonstrate the use of Nanotrap® particles to pre-concentrate Flu A from large volume viral transport media samples instead of a viral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



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.

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

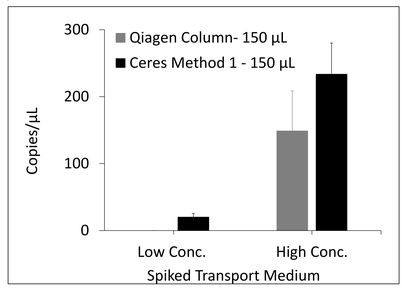


Figure 1. QIAGEN viral RNA mini Kit and Ceres Method 1 for H1N1 virus RT-qPCR applications

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.

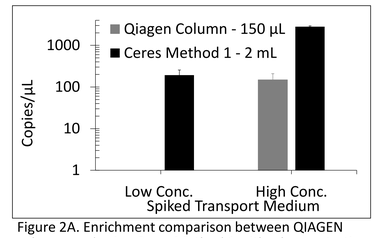


Figure 2A. Enrichment comparison between QIAGEN columns and using Ceres Method 1 with a larger starting sample volume (2 mL).

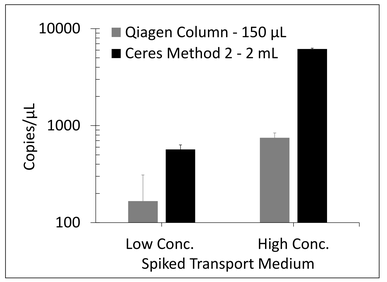


Figure 2B. Method comparison between QIAGEN columns alone and Ceres Method 2 with increased sample volume (2 mL).

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.

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.

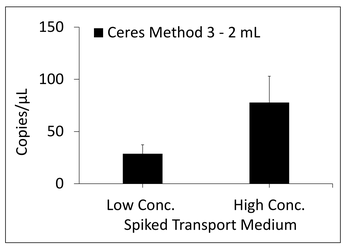


Figure 3. Ceres Method 3 performance across high and low concentrations of spiked transport medium.

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.