Nanotrap Particles Capture and Concentrate Hepatitis A from Produce Wash Water and Wastewater

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Products:
Nanotrap® Microbiome A Particles
Nanotrap® Enhancement Reagent 1

Introduction

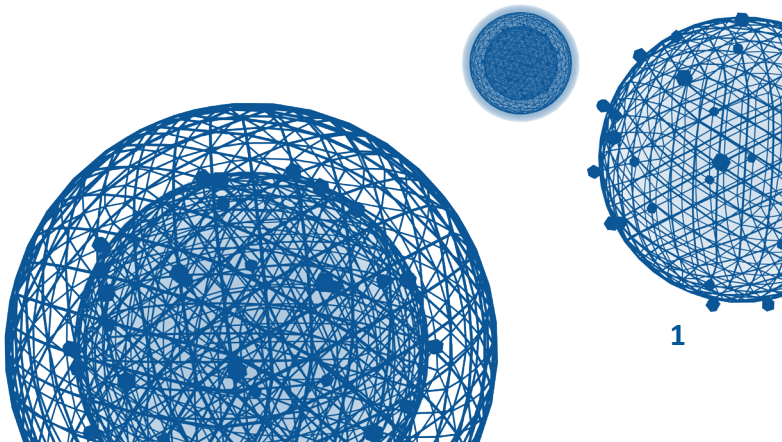
Hepatitis A is an acute inflammatory liver disease which results from infection by the hepatitis A virus (HAV). It is faeco-orally spread, and its severity is age dependent.[1] Recently, cases of acute hepatitis of unknown origin among young children have been reported from 12 countries around the world.[2] Routine food safety testing can be used to effectively identify HAV present on foods, while wastewater surveillance for HAV can provide an indication of infection levels and affected geographical locations.

Municipal wastewater harbors a variety of pathogenic viruses.[3] Extensive research has been conducted on the persistence of human enteric viruses, which are transmitted via the fecal-oral route, in wastewater and in the aquatic environment.[4] Wastewater surveillance is appealing because a single sample can 'test' a large population, it can enable detection from both asymptomatic and symptomatic individuals, and it can provide an indication of changing trends in population infection levels about 7 days ahead of clinical testing.[5,6] CDC and HHS, in collaboration with agencies throughout the federal government, implemented the National Wastewater Surveillance System (NWSS) in response to the COVID-19 pandemic as a resource to help public health officials and communities utilize wastewater data to guide community level response and decision making.[5,6]

Key Benefits

- **Nanotrap® Microbiome A Particles enable simple, automated methods for bio surveillance of a broad spectrum of infectious diseases including hepatitis A.**
- **The automated Nanotrap Particle method is compatible with several large volume sample types including produce wash water and wastewater.**
- **The automated Nanotrap Particle method offers equivalent or better hepatitis A viral detection from wastewater samples, as compared to an HA filtration method.**

There is an interest in using the infrastructure in place to detect and monitor other common and potentially deadly pathogens. Nanotrap® Microbiome A Particles have demonstrated sensitive, rapid, and easy-to-use automated and manual methods for wastewater SARS-CoV-2 testing.[7] In this application note, we show that Nanotrap Microbiome A Particles can also capture HAV from wastewater samples and from simulated agricultural produce wash samples.



Materials

Product	Company	SKU
Nanotrap® Microbiome A Particles	Ceres Nanosciences	44202
Nanotrap® Enhancement Reagent 1 (ER1)	Ceres Nanosciences	10111
Hepatitis A virus strain HM175/18f	ATCC	VR-1402
NucleoMag® DNA/RNA Water Kit	MACHEREY-NAGEL	744220.1
QuantStudio™ 5 Real-Time PCR instrument or equivalent	Thermo Fisher Scientific	--

Product	Company	SKU
KingFisher™ Apex with 96 Deep-well Head	Thermo Fisher Scientific	5400930
KingFisher™ 24 Deep-well Plate, Barcoded	Thermo Fisher Scientific	95040470B
KingFisher™ 24 Deep-well Tip Comb & Plate, Barcoded	Thermo Fisher Scientific	97002610B
KingFisher™ 96 Deep-well Plate, Barcoded	Thermo Fisher Scientific	95040450B
KingFisher™ 96 Deep-well Tip Comb, Barcoded	Thermo Fisher Scientific	97002534B
KingFisher™ 96 Plate (200 µL), Barcoded	Thermo Fisher Scientific	97002540B
GN Metricel™ MCE membrane disc filters - 47 mm, grid (100/pkg)	Cytiva	63020
TaqPath™ 1-Step RT-qPCR Master Mix, CG	Thermo Fisher Scientific	A15299
2.0 N Hydrochloric Acid (2.0 N HCl)	--	--
Local Produce wash water samples (mushroom and strawberry)	--	--
Seventeen wastewater samples from different collection sites in the United States	--	--

Organisms	Company	SKU
Chlamydia trachomatis	ATCC	VR-571B
Legionella anisa	ATCC	35292

Primers and Probes

Custom Unmodified DNA Oligo and MGB probes: Eurofins Genomics; Primer Probe information

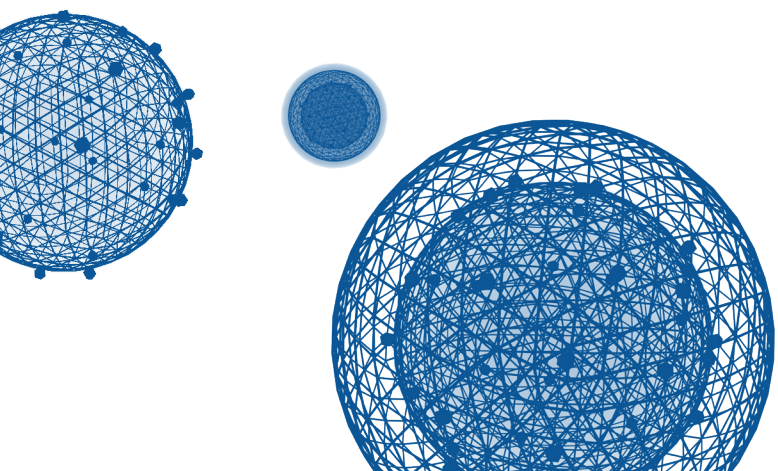
Type	Sequence (5'-3')
Forward	CTCTTTGATCTTCCACAAGRGGT
Reverse	GCCGCTGTTACCCTATCCAA
Probe	FAM-AGGCTACGGGTAAAC-MGB-EQ

Methods

Sample Preparation

Two produce wash samples were prepared by rinsing mushrooms and strawberries with tap water with no detergent. Post-wash fluid was collected in sample collection bottles. Both types of produce wash samples were spiked with HAV at 50 cp/mL, 100 cp/mL, and 500 cp/mL and mixed thoroughly by inverting multiple times.

Two wastewater samples sourced from two different locations in the United States were spiked with HAV at 50 cp/mL, 100 cp/mL, and 500 cp/mL and mixed thoroughly by inverting multiple times. Fifteen wastewater samples were obtained from two geographical areas (multiple collection sites) at different time points in the USA. Samples were screened for HAV with no additional alteration.



HAV Concentration Using Nanotrap® Particles

Nanotrap MIcrobiome A Particles and Nanotrap® Enhancement Reagent (ER1) were used to capture and concentrate HAV from wastewater samples using an automated protocol.[8] Briefly, a 10 mL wastewater sample was split between two 24 deep-well plates (4.875mL into each of two wells). 50 µL of ER1 was added directly to each wastewater sample well. 75 µL of Nanotrap Particles were added to each well immediately after the ER1. The 24 deep-well sample plates were loaded onto a KingFisher™ Apex System for virus capture and concentration. No filtration or centrifugation of the wastewater samples was required prior to the addition of the Nanotrap Particles.

HAV Concentration Using HA Filtration

As a comparison method, membrane disc filters were used to capture and concentrate HAV. In this method, pH of wastewater samples was adjusted to pH 3.5 – pH 4, using 2.0 N HCl. Fifty milliliter wastewater samples were then filtered through the membrane disc filters via a glass funnel and base. After vacuum filtration (10 - 90 minutes), the filter membrane was processed as described in the NucleoMag® DNA/RNA water kit user manual.

RNA Extraction

Viruses captured by the Nanotrap Particles or HA filter were lysed in the MWA1 Lysis Buffer (included in the NucleoMag DNA/RNA water kit). RNA extraction was completed using the magnetic bead based NucleoMag DNA/RNA water kit on the KingFisher Apex system.[8]

RT-PCR Analysis

RT-PCR was prepared with Thermo Fisher TaqPath™ 1-Step RT-qPCR Master Mix, CG. Custom primers and probe sequences for this assay were obtained from Pearson et al. publication.[9] The primer and probe sequences used are indicated in the Materials section.

Each reaction contained 500 nM forward and reverse primers and 300 nM probe. For each sample, a total of 20 µL reaction mix was prepared with 15 µL of reagents and 5 µL of sample and was added to an optical 96-well reaction plate. The plate was sealed with an optical adhesive film, loaded into a QuantStudio 5 PCR instrument, and run using the amplification settings specified in the PCR protocol.[10] Results were analyzed with the Design & Analysis Software, version 2.6.0 (Thermo Fisher Scientific).

Results

HAV were successfully detected in contrived produce wash samples, contrived wastewater samples, and real wastewater samples.

Detection of HAV in Produce Wash Water Samples

Nanotrap Microbiome A Particles can capture and concentrate HAV from produce wash water samples **(Figure 1)**.

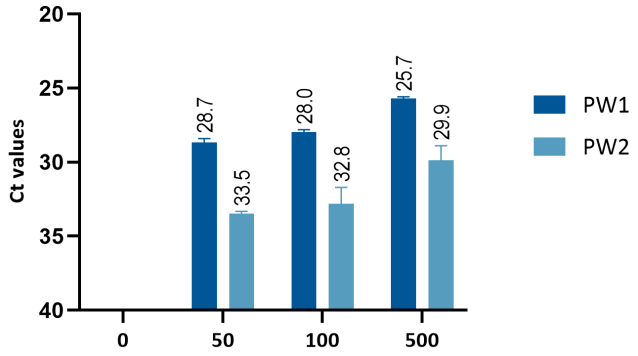
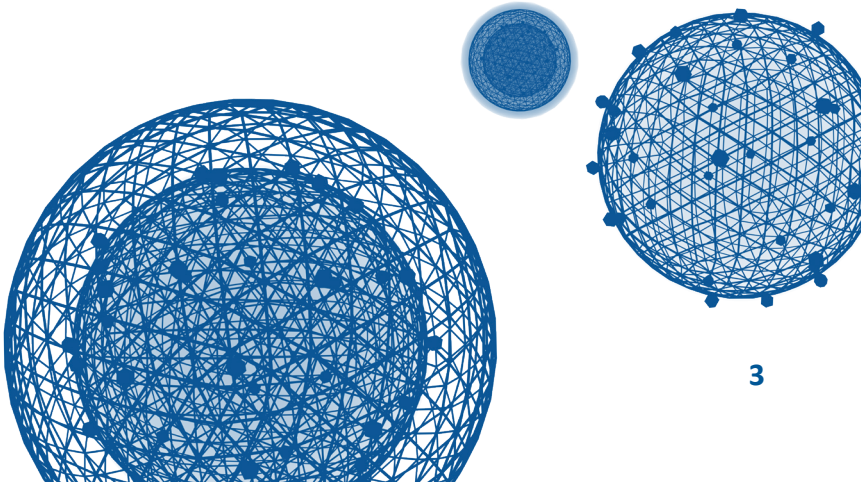


Figure 1

Nanotrap Microbiome A Particles enable HAV detection in produce wash water samples. Two produce wash water samples were spiked with HAV. The samples were processed using the



automated Nanotrap method on the KingFisher Apex system. MACHEREY-NAGEL NucleoMag DNA/RNA Water kit was used for RNA extraction.

No signal detection in 0 cp/mL samples demonstrates the specificity of the primer and probe sets to this as-say. HAV was detected at 50 cp/mL, 100 cp/mL, and 500 cp/mL in both produce wash samples. This demon-strates the Nanotrap Particle method can be used for HAV detection from produce wash samples.

Detection of HAV in Wastewater Samples

Nanotrap Microbiome A Particles can capture and concentrate HAV from contrived wastewater samples **(Figure 2)**.

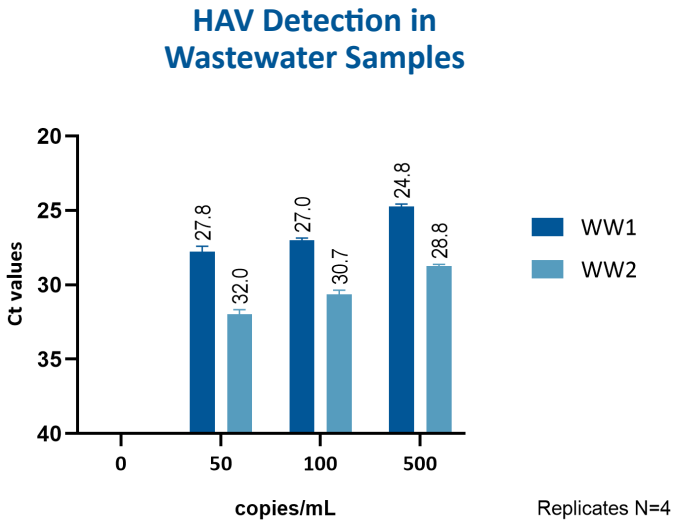


Figure 2

Nanotrap Microbiome A Particles enable HAV detection in wastewater samples. Two wastewater samples were spiked using HAV. The samples were processed using the automated Nanotrap method on the Kingfisher Apex system. MACHEREY-NAGEL Nucleo-Mag DNA/RNA Water kit was used for RNA extraction.

HAV was also detected at 50 cp/mL, 100 cp/mL, and 500 cp/mL in both the wastewater samples. This demonstrates that the Nanotrap Particle method can be used for HAV detection from wastewater samples.

Nanotrap Particles vs. HA Filtration

The Nanotrap Particle method (processing a 10 mL wastewater sample) was compared against a HA filtration method (processing a 50 mL wastewater sample). In this experiment, two wastewater samples were spiked with HAV at 500 cp/mL and processed using the two different methods.

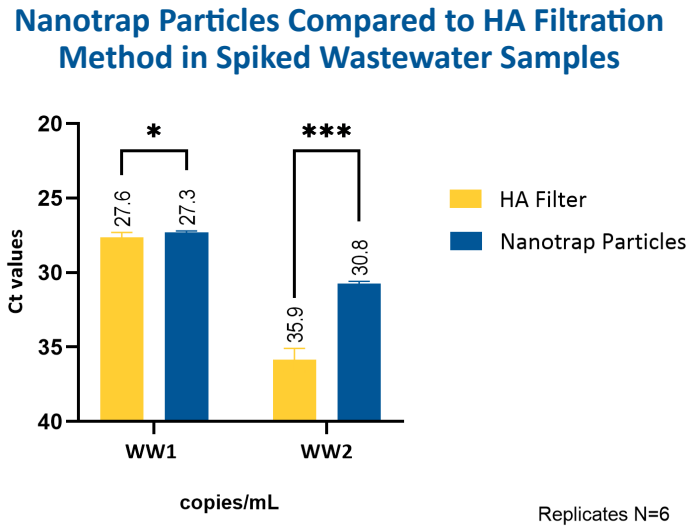


Figure 3

Nanotrap Microbiome A Particles outperformed the HA Filtration method for HAV capture and concentration in two spiked wastewater samples. Asterisk indicates the multiple t tests analysis results (* $P \leq 0.05$; *** $P \leq 0.0001$).

Despite the fact that the Nanotrap Particle method is processing 5 times less wastewater than the HA filtration method, the Nanotrap Particle method demonstrated equivalent or better viral RNA detection in these two samples.

Hepatitis A Virus Detection in Wastewater Samples

Sample	Result	Ct Value
Location 1	Negative	–
Location 2	Negative	A15299
Location 3	**Positive**	**30.5**
Location 4	Negative	–
Location 5	Negative	–
Location 6	Negative	–
Location 7	**Positive**	**28.6**
Location 8	Negative	–
Location 9	Negative	–
Location 10	Negative	–
Location 11	Negative	–
Location 12	Negative	–
Location 13	Negative	–
Location 14	Negative	–
Location 15	Negative	–

Table 1

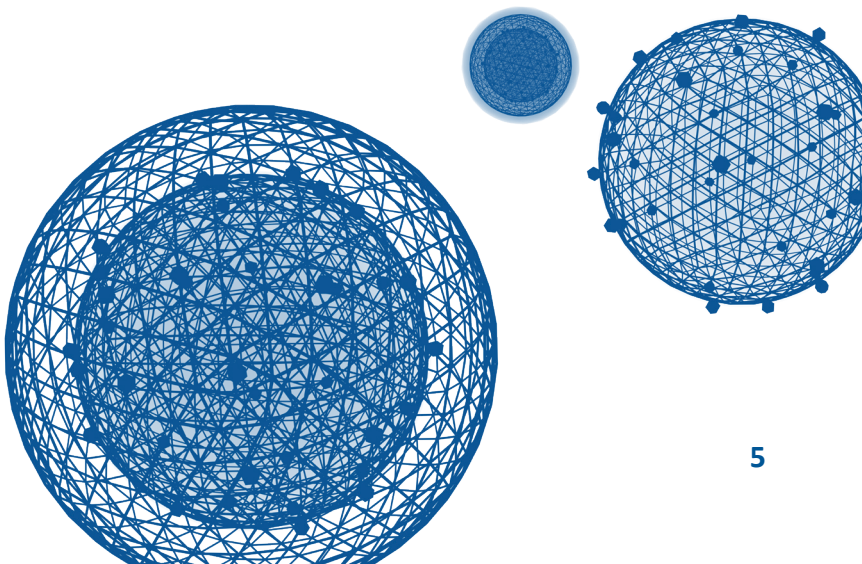
The Nanotrap wastewater method was further assessed by screening fifteen wastewater samples sourced from different locations. All samples were processed using the KingFisher Apex system. MACHEREY-NAGEL Nucleo-Mag DNA/RNA Water kit was used for RNA extraction. Two wastewater samples tested positive for HAV.

Conclusions

The Nanotrap Particle method enables hepatitus A Virus detection from wastewater and produce wash water samples at low concentration (50 cp/mL). A similar trend in Ct values was observed across individual produce wash and wastewater samples at different concentrations.

Nanotrap Microbiome A Particles can provide a fast, simple, and automated workflow for virus concentration from wastewater samples prior to nucleic acid purification. This was demonstrated by comparing the automated Nanotrap wastewater method (10 mL sample) to the manually processed HA filtration method (50 mL sample).

This study shows that Nanotrap Microbiome A Particles can be used as an effective tool for surveillance of multiple viruses from wastewater and environmental water samples.



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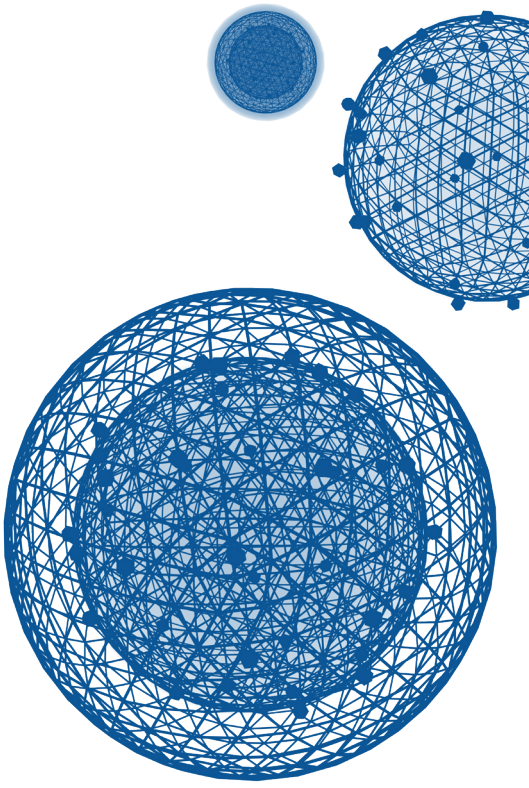

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