Nanotrap® Microbiome B Particles Capture and Concentrate Bacterial Pathogens from Wastewater

Key Advantages

- Nanotrap® Microbiome B Particles rapidly capture and concentrate *Campylobacter jejuni, Escherichia coli* O157:H7, *Listeria monocytogenes*, *Clostridium difficile*, *Salmonella enterica subsp. Enterica*, and crAssphage from wastewater without the need of filtration, bead-beating, or centrifugation.
- Nanotrap Microbiome B Particles enable a simple and rapid automated or manual method for detecting pathogens, including bacteria, in wastewater samples.
- Nanotrap Microbiome B Particles are compatible with several commercially available nucleic acid extraction kits.

Introduction

Recently, wastewater surveillance has become widely recognized as a powerful tool to identify and monitor emerging pathogens in a community.[1,2] Many of the investments made to date have been focused on developing wastewater surveillance methods that can enable rapid and reliable monitoring of SARS-CoV-2, but there is growing interest in expanding the utility of these methods to other microbes.[3]

The Nanotrap particle technology enables rapid concentration of microbes from raw sewage, requiring no filtration or centrifugation and is compatible with RT-qPCR, RT-ddPCR, RT-dPCR, and sequencing based analysis methods. Labs around the world have used Nanotrap Microbiome A Particles to process many tens of thousands of wastewater samples for detection of SARS-CoV-2. Moreover, it was recently demonstrated that Nanotrap particle processing of wastewater testing can enable detection of emerging variants of concern up to 14 days earlier than clinical genomic Surveillance.[4] Nanotrap particle methods have also enabled detection of monkeypox and hepatitis A virus in wastewater.[5,6]

Here, we introduce two new products from Ceres Nanosciences: Nanotrap Microbiome B Particles and Nanotrap Enhancement Reagent 3 (ER3), along with a sensitive, rapid, and easy-to-use concentration method for wastewater-based epidemiology testing for bacterial targets. This simple and sensitive method is compatible with several magnetic bead DNA extraction kits including standard "off the shelf" kits provided by MACHEREY-NAGEL and Thermo Fisher Scientific and requires no bead-beating steps.

We show that Nanotrap Microbiome B Particles 1) capture and concentrate multiple pathogenic bacteria from wastewater samples resulting in equivalent or better detection as compared to labor-intensive manual HA Filter wastewater concentration methods, 2) are compatible with multiple automation-friendly nucleic acid extraction kits, and 3) enable detection of a wastewater control organism that is commonly utilized to normalize detection results across unique wastewater samples.

Materials

- Nanotrap Microbiome B Particles
  Ceres Nanosciences; SKU# 65202
- Nanotrap Enhancement Reagent 3 (ER3)
  Ceres Nanosciences; SKU# 10113
- *Campylobacter jejuni*: ATCC; SKU# 33560
- *Escherichia coli* O157:H7: ATCC; SKU# 43894
- *Listeria monocytogenes*: ATCC; SKU# 7644
- *Clostridium difficile*: ATCC; SKU# 9689

- *Salmonella enterica subsp. Enterica*: ATCC; SKU# 13311
- Platinum™ Taq DNA Polymerase: Thermo Fisher Scientific; SKU# 10966083
- Deoxynucleotide (dNTP) Solution Mix: New England BioLabs; SKU# N0447L
- RT-PCR Grade Water: Thermo Fisher Scientific; SKU# AM9935
- GN Metricel® MCE Membrane Disc Filters - 47 mm; Cat# 63020
- NucleoMag® DNA/RNA Water Kit: MACHEREY-NAGEL; SKU# 744220.1
- MN Bead Tubes Type B: MACHERY-NAGEL; SKU# 740812.50
- MagMAX™ Microbiome Ultra Kit: Thermo Fisher Scientific; Cat# A42354
- KingFisher™ Apex System and Material
  - 3 24-Deep Well Plates
  - 1 24-Deep Well Comb
  - 4 96-Deep Well Plates
  - 1 96-200 µL Micro Well Plate
  - 1 96-Deep Well Comb
- BIO-RAD CFX96™ or equivalent
- Wastewater samples were obtained from different collection sites in the United States
- ***Custom DNA primers/probes: IDT***
  - Primers-Standard Desalting, LabReady
  - Probes- HPLC Purification, LabReady

Salmonella enterica subsp.

Type	Sequence (5’-3’)
Forward	TCG TCA TTC CAT TAC CTA CC
Reverse	AAA CGT TGA AAA ACT GAG GA
Probe	5HEX/TCT GGT TGA /ZEN/TTT CCT GAT CGC A/3IABkFQ (250 nm PrimeTime 5' HEX/ZEN/3' IBFQ)

Campylobacter jejuni

Type	Sequence (5’-3’)
Forward	TCC AAA ATC CTC ACT TGC CATT
Reverse	TGC ACC AGT GAC TAT GAA TAA CGA
Probe	56-FAM/TTG CAA CCT/ZEN/CAC TAG CAA AAT CCA CAG CT/3IABkFQ (PrimeTime 5' 6 -FAM/ZEN/3' IBFQ)

Clostridium difficile

Type	Sequence (5’-3’)
Forward	TCT ACC ACT GAA GCA TTA C
Reverse	TAG GTA CTG TAG GTT TAT TG
Probe	56-FAM/CAC GCG GAT/ZEN/TTT GAA TCT CTT CCT CTA GTA GCG CGT G /3IABkFQ (PrimeTime 5' 6-FAM/ZEN/3' IBFQ)

Listeria monocytogenes

Type	Sequence (5’-3’)
Forward	TTG CCA GGA ATG ACT AAT CAA G
Reverse	ATT CAC TGT AAG CCA TTT CGT
Probe	56-FAM/TGC TCA AGC/ZEN/TTA CCG AAT GTA AGT GCA/ 3IABkFQ (PrimeTime 5' 6-FAM/ZEN/3' IBFQ)

Escherichia coli O157

Type	Sequence (5’-3’)
Forward	TTT GTC ACT GTC ACA GCA GAA GCC TTA CG
Reverse	CCC CAG TTC AGA GTG AGG TCC ACG TC
Probe	56-FAM-TCG TCA GGC/ZEN/ACT GTC TGA AAC TGC TCC-3IABkFQ (PrimeTime 5' 6-FAM/ZEN/3' IBFQ)

CrAssphage

Type	Sequence (5'-3')
Forward	CAG AAG TAC AAA CTC CTA AAA AAC GTA GAG
Reverse	GAT GAC CAA TAA ACA AGC CAT TAG C
Probe	56-FAM/AAT AAC GAT/ZEN/TTA CGT GAT GTA AC /3IABkFQ (PrimeTime 5' 6-FAM/ ZEN/3'IBFQ)

Methods

Sample Preparation

Multiple wastewater samples sourced from different locations were spiked with 5 common pathogenic bacteria: *C. jejuni, E. coli* O157:H7, *L. monocytogenes*, *C. difficile*, and *S. enterica subsp. Enterica*. The samples were spiked with all of the bacteria at 200 cp/mL and 500 cp/mL each. The wastewater was mixed thoroughly by inverting multiple times after spiking.

Bacteria Concentration

Automated Nanotrap Method

Nanotrap Microbiome B Particles and Nanotrap Enhancement Reagent 3 (ER3) were used to capture and concentrate bacteria from wastewater samples using an automated protocol developed for the KingFisher Apex system. Briefly, a 10 mL wastewater sample was split between two 24-deep well plates (4.875 mL into each of two wells). 50 µL of ER3 was added directly to each wastewater sample well, followed by adding 75 µL of Nanotrap Microbiome B Particles for a total of 100 µL of ER3 and 150 µL of Nanotrap Microbiome B Particles per 10 mL sample. The 24-deep well sample plates were loaded onto a KingFisher Apex platform for bacterial capture and concentration. No filtration or centrifugation of the wastewater samples was required prior to the addition of the Nanotrap Microbiome B Particles.

Manual Nanotrap Method

Nanotrap Microbiome B Particles and Nanotrap Enhancement Reagent 3 (ER3) were used to capture and concentrate bacteria from wastewater samples using a manual protocol. Briefly, a 50 mL wastewater sample was added to a 50 mL conical tube. 100 µL of ER3 was added directly to the wastewater sample, followed by the addition of 750 µL of Nanotrap Microbiome B Particles. Each sample was briefly vortexed and left to incubate at room temperature with no additional mixing for 30 minutes. After the incubation, samples were placed on a magnetic separation rack for 2 minutes to allow the Nanotrap Magnetic Microbiome B Particles to pellet. The supernatant was removed and discarded.

HA Filtration

As a comparison method, Metricel MCE Membrane Disc filters were used to capture and concentrate the bacteria. In this method, the pH of the wastewater samples was adjusted to 3.5-4 using 2.0 N HCl. Ten or fifty milliliter wastewater samples were then filtered through the 0.45-µm-pore-size, 47-mm-diameter Metricel MCE Membrane Disc filters via a glass funnel and base. After vacuum filtration (15-90 min), the filter disc was transferred into a clean 2 mL tube for bead beating using MACHEREY-NAGEL MN Bead Tubes Type B.

DNA Extraction

After bacteria concentration, DNA extraction was performed using either the NucleoMag DNA/RNA Water Kit for water and air samples (MACHEREY-NAGEL Cat# 744220.1) or the MagMAX Microbiome Ultra Kit (Thermo Fisher Cat# A42354). Captured bacteria was lysed off the Nanotrap Microbiome B Particles in 500 µL MWA1 Lysis Buffer (included in the NucleoMag DNA/RNA water kit), or 500 µL Microbiome Lysis Buffer (included in the MagMAX Microbiome Ultra Kit). Samples were processed on the KingFisher Apex system following each extraction kit manufacturer's protocol.

qPCR Analysis

PCR was performed with Thermo Fisher Scientific Platinum Taq DNA polymerase. Custom primers and probe sequences were obtained from multiple publications.[7-10] The primer and probe sequences used are indicated in

the Materials section. PCR mastermix was optimized using the concentrations indicated below:

Reagent	Stock Concentration		Final Concentration		Volume (per sample)	
Water					13.3	µL
10x PCR Buffer	100	mM	10	mM	2	µL
dNTP Mix	10	mM	0.2	mM	0.4	µL
Forward Primer	20	µM	0.3	µM	0.3	µL
Reverse Primer	20	µM	0.3	µM	0.3	µL
Probe	20	µM	0.2	µM	0.2	µL
Taq Polymerase	10	U/µL	0.15	U/µL	0.3	µL
Template (Sample)	10	X	1	X	2	µL
MgCl2	50	mM	3	mM	1.2	µL
				Total	20	µL

The plate was sealed with an optical adhesive film, loaded onto a Bio-Rad PCR instrument, and run using the amplification settings specified below:

1 cycle:
95°C, 30 sec
39 cycles:
95°C, 15 sec
60°C, 60 sec (image: FAM, HEX)

Results

Nanotrap Microbiome B Particles vs HA Filtration

The Nanotrap Microbiome B Particle concentration method was compared to the HA filtration concentration method, described in the Methods section above. Results were generated by spiking *E. coli* and *L. monocytogenes* at 500 copies/mL into two wastewater samples. 10 mL and 50 mL sample volumes were used for the experiments described below. Both concentration methods used the MACHEREY-NAGEL NucleoMag DNA/RNA Water Kit for extraction and isolation of bacterial DNA. The Nanotrap particle concentration method did not include bead beating, while the HA filtration method included a bead beating step. Equivalent recovery was observed for both 10 mL and 50 mL sample volumes for each bacteria type across the two concentration methods **[Figures 1 and 2]**.

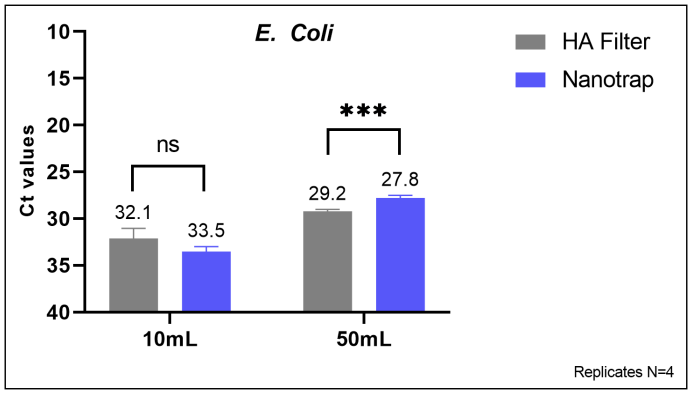

Figure 1. Nanotrap Microbiome B Particles enable simplified bacteria concentration method relative to HA filtration while delivering equivalent sensitivity. Two wastewater samples were spiked with *E. coli* at 500 cp/mL. The 10 mL samples were processed using the automated Nanotrap particle method. The 50 mL samples were processed using the manual Nanotrap particle method. All concentration methods used the MACHEREY-NAGEL NucleoMag DNA/RNA Water kit for DNA extraction. (Paired T-Test to assess significance; ns = P >.05 ; *** = P ≤ 0.001)

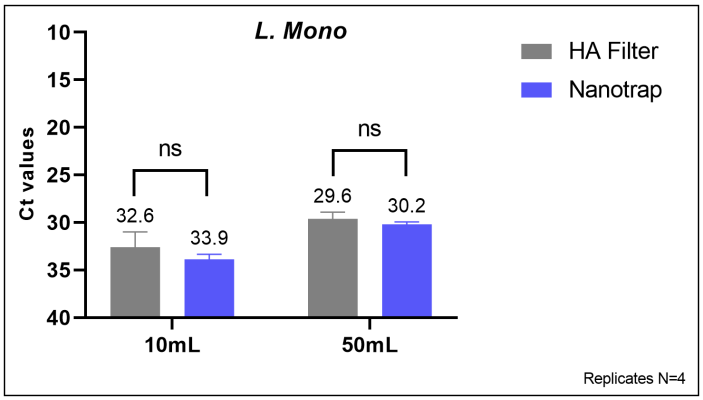

Figure 2. Nanotrap Microbiome B Particles enable a simplified *L. monocytogenes* concentration method relative to HA filtration while delivering equivalent sensitivity. Two wastewater samples were spiked with *L. monocytogenes* at 500 cp/mL. The 10 mL samples were processed using the automated Nanotrap particle method. The 50 mL samples were processed using the manual Nanotrap particle method. All concentration methods used the MACHEREY-NAGEL NucleoMag DNA/RNA Water kit for DNA extraction. (Paired T-Test to assess significance; ns = P >.05; *** = P ≤ 0.001)

Nanotrap Microbiome B Particles Extraction Kit Compatibility

Nanotrap Microbiome B Particles and ER3 were successfully used in combination with both the MACHEREY-NAGEL NucleoMag DNA/RNA extraction kit and the

Thermo Fisher Scientific MagMAX Microbiome Ultra Kit extraction kit to detect five pathogenic bacteria in contrived wastewater samples **[Figures 3 and 4]**.

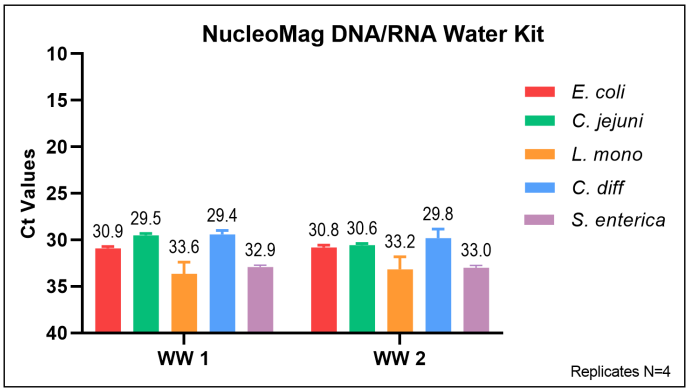

Figure 3. Nanotrap Microbiome B Particles enable detection of multiple bacteria in wastewater samples. Two wastewater samples were spiked with five different bacteria at 200 cp/mL each. The samples were processed using the automated Nanotrap method. The MACHEREY-NAGEL NucleoMag DNA/RNA Water kit was used for DNA extraction.

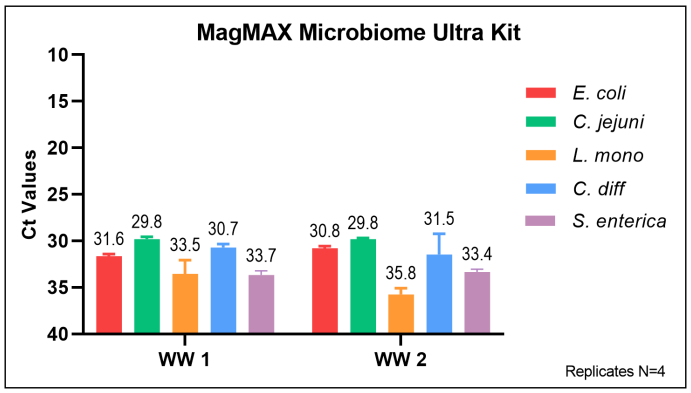

Figure 4. Nanotrap Microbiome B Particles enable bacteria detection in wastewater samples using Thermo Fisher Scientific Extraction kit. Two wastewater samples were spiked with five different bacteria at 200 cp/mL each. The samples were processed using the automated Nanotrap method. The MagMAX Microbiome Ultra Kit was used for DNA extraction.

Results were generated by spiking *C. jejuni, E. coli* O157:H7, *L. monocytogenes, C. difficile*, and *S. enterica* at 200 copies/mL into 2 negative wastewater samples. 10 mL of wastewater was processed per sample with the Nanotrap Microbiome B Particles using the automated Nanotrap particle method described in the Methods section.

There was no signal detection in 0 cp/mL samples (data data not shown), which demonstrates the specificity of the assay. All 5 bacteria were detected at 200 cp/mL in both wastewater samples. This demonstrates the Nanotrap Microbiome B Particles are compatible with multiple extraction kits and can be used to concentrate and detect multiple bacteria from wastewater samples.

Detection of a Control Organism using Nanotrap Microbiome B Particles in Wastewater

CrAssphage is a bacteriophage that infects the human gut bacterium *Bacteroides intestinali*. A common fecal virus, detection of crAssphage can be used to both demonstrate successful wastewater extraction and act as a normalization metric for wastewater amount. **[Figure 5]** shows that the Nanotrap Microbiome B Particles capture CrAssphage in real wastewater samples across two extraction kits.

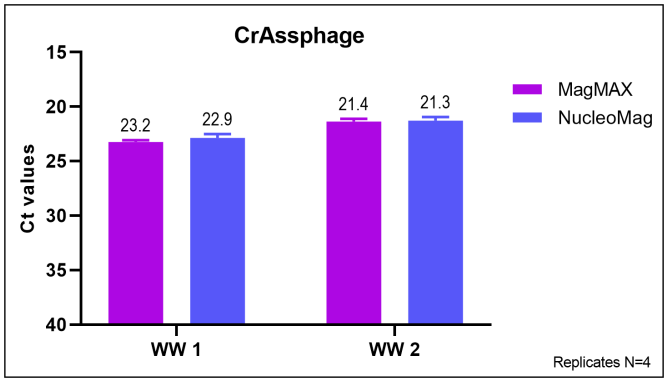

Figure 5. Nanotrap Microbiome B Particles capture CrAssphage in wastewater samples. Two wastewater samples were processed using the automated Nanotrap method. MagMAX Microbiome Ultra Kit and MACHEREY-NAGEL DNA/RNA Water Kit were used for DNA extraction.

Conclusions

By removing time consuming manual processes such as filtering, centrifuging and bead beating, the Nanotrap Microbiome B Particles enable rapid and simple bacteria detection from wastewater samples at environmentally relevant concentrations. The technology enables automated processing of wastewater samples while achieving equivalent sensitivity to more difficult and time-consuming manual methods such as HA filtration and bead beating. This study shows the Nanotrap Microbiome B Particles can be used as a fast, effective, and simple tool for surveillance of multiple pathogenic bacteria from wastewater samples.

References

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