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Concentration and Nucleic Acid Extraction of Pathogens in Wastewater

Assessment of Compatibility of Ceres Nanosciences' Nanotrap® Particles with Zymo Research *Quick*-DNA/RNA™ Water Kit for Wastewater Surveillance

E. Carrasco[1], L. Saunders[2], X. Cheng[1], and K. Locken[1]
Zymo Research Corporation[1], Ceres Nanosciences[2]

INTRODUCTION

The objective of this study is to assess the compatibility of Ceres Nanosciences' Nanotrap® Microbiome Particles and Nanotrap® Enhancement Reagents with Zymo Research's *Quick*-DNA/RNA™ Water Kit. This integrated workflow is intended to provide researchers and industry professionals with a reliable method for monitoring wastewater, facilitating timely and informed public health interventions.

METHODS

The study followed the protocol provided by Ceres Nanosciences for using Nanotrap Microbiome A and Microbiome B Particles with Enhancement Reagent 3 (ER3) to concentrate 10 mL of raw wastewater. (Figure 1) The samples were spiked with SARS-CoV-2, Influenza A, *Listeria monocytogenes*, and *Salmonella enterica* to evaluate the recovery efficiency of both viral and bacterial targets.

The concentrated samples were then processed using the *Quick*-DNA/RNA™ Water Kit, starting with resuspending the Nanotrap Particles with DNA/RNA Shield™ and incubating the samples at 56 °C for 10 minutes. The lysate was subsequently extracted of total DNA/RNA using Zymo-Spin™ technology. The eluted DNA/RNA was subsequently analyzed using digital PCR (dPCR).

METHOD OVERVIEW

- Concentration Reagents: Nanotrap Microbiome A and B Particles and Enhancement Reagent 3 (ER3)
- Nucleic Acid Extraction Kit: *Quick*-DNA/RNA™ Water Kit
- Pathogen Spike-In Targets: SARS-CoV-2, Influenza A, *Listeria monocytogenes*, *Salmonella enterica*
- Sample: 10 mL Wastewater from two different sources
- Assay: dPCR in technical duplicate

Figure 1. Nanotrap Microbiome A and B Particles.

RESULTS

The study demonstrated that Nanotrap Particles are compatible with the *Quick*-DNA/RNA™ Water Kit. Eluted DNA/RNA was analyzed using dPCR with (assay) on the QIAcuity One – 5plex Digital PCR system.

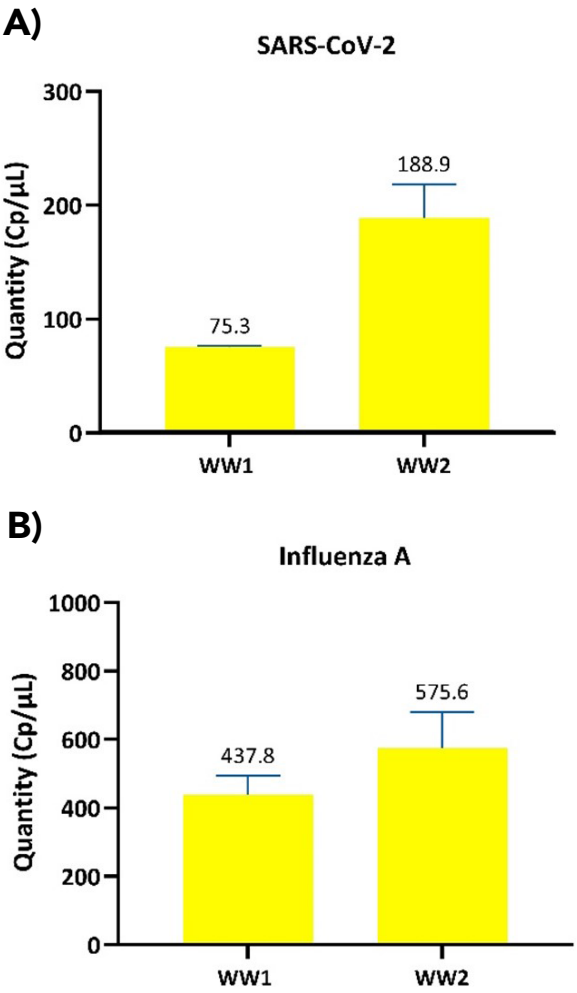

Figure 2 Viral Detection. Detection of A) SARS-CoV-2 and B) Influenza A in raw wastewater samples by dPCR that were processed using Nanotrap Microbiome Particles and the *Quick*-DNA/RNA™ Water Kit.

Viral Recovery

The combined use of Nanotrap Microbiome Particles and the *Quick*-DNA/RNA™ Water Kit successfully concentrated and extracted viral nucleic acids from wastewater samples. The recovery of SARS-CoV-2 in two wastewater samples was quantified by dPCR at 75.3 and 188.9 copies per microliter (Cp/µL), respectively. (Figure 2) For Influenza A, the recovery was 437.8 and 575.6 Cp/µL, respectively. The variation between the two samples is expected to be due to differences in the two different wastewater samples. These results indicate effective recovery of enveloped, single-stranded RNA viruses CoV-2 representing coronaviruses and Influenza A representing orthomyxoviruses).

Bacterial Recovery

Similarly, the workflow effectively concentrated and extracted bacterial nucleic acids from the wastewater samples. The recovery of *Listeria monocytogenes* was measured by dPCR at 20.9 and 56.7 Cp/µL, while *Salmonella enterica* was measured at 86.9 and 72.6 Cp/µL. (Figure 3) The variation between the two samples is expected to be due to differences in the two different wastewater samples. These results demonstrate the capability to recover both gram-positive (*Listeria monocytogenes*) and gram-negative bacteria (*Salmonella enterica*), encompassing a range of bacterial types including those that are challenging to lyse.

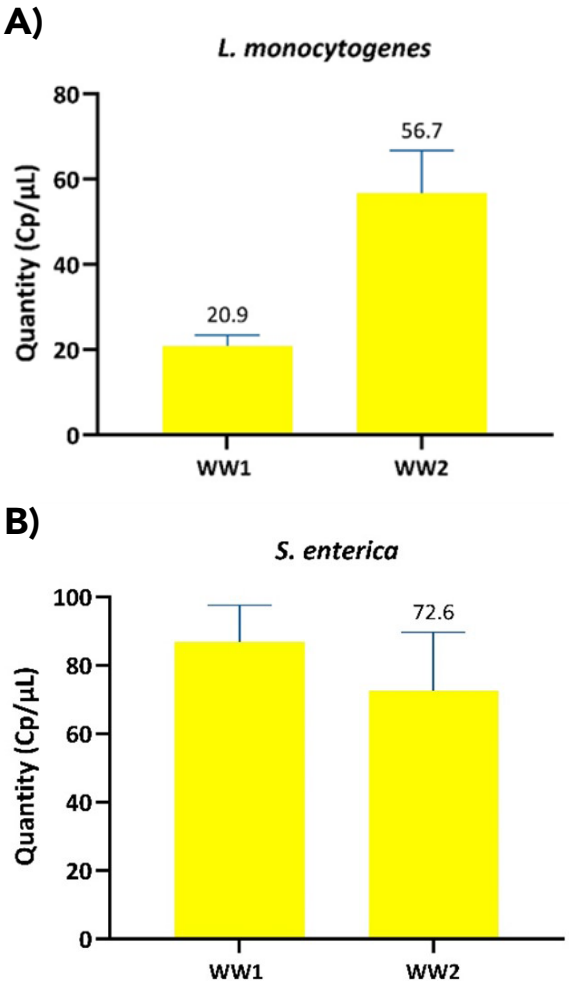

Figure 3 Bacterial Detection. PCR detection of A) L. monocytogenes and B) S. enterica in raw wastewater, utilizing Nanotrap Microbiome Particles and the Quick-DNA/RNA Water Kit for sample processing.

CONCLUSION

This study confirms that Ceres Nanosciences' Nanotrap Microbiome Particles are fully compatible with Zymo Research's *Quick*-DNA/RNA™ Water Kit, establishing an efficient process for concentrating and extracting viral and bacterial nucleic acids from wastewater. The developed workflow offers a powerful tool for wastewater surveillance, enabling accurate detection of pathogens and supporting proactive public health strategies.

LEARN MORE ABOUT THE *QUICK*-DNA/RNA™ WATER KIT

www.zymoresearch.com/pages/quick-dna-rna-water-kit