Viral Concentration and Viral RNA Purification from Wastewater on the KingFisher™ Flex Purification System

Promega Corporation, 2800 Woods Hollow Road, Madison, WI 53711, USA

Kits:

- Maxwell® HT Environmental TNA Kit, Custom (Cat.# AX9190)
- GoTaq® Enviro Wastewater SARS-CoV-2 RT-qPCR Systems (Cat.# AM2100, AM2110, AM2120, AM2130)

Materials Required:

- Nanotrap® Magnetic Virus Particles, 30ml (Ceres, SKU# 44202-30)
- Nanotrap® Enhancement Reagent 2, 30ml (Ceres, SKU# 10112-30)
- KingFisher™ 24 deep-well plate, sterile (Thermo Fisher Scientific, Cat.# 95040480)
- KingFisher™ 24 deep-well tip comb and plate, sterile (Thermo Fisher Scientific, Cat.# 97002620)
- KingFisher™ 96 deep-well plate, sterile (Thermo Fisher Scientific, Cat.# 95040460)
- KingFisher™ 96 tip comb for deep-well magnets (Thermo Fisher Scientific, Cat.# 97002534)
- KingFisher™ Flex Purification System (Thermo Fisher Scientific, Cat.# 5400630)

Abstract

Monitoring SARS-CoV-2 viral RNA in wastewater can be used to detect and predict COVID-19 disease outbreaks. Here, we describe an automated high-throughput protocol for viral concentration and viral RNA purification from wastewater for SARS-CoV-2 detection.

Introduction

Early in the COVID-19 pandemic, studies showed that SARS-CoV-2 viral RNA could be detected in the feces of infected individuals. Detection of SARS-CoV-2 in wastewater can indicate the presence or prevalence of COVID-19 within a community. Routine testing of large numbers of wastewater samples is important to obtain sufficient and timely data on viral spread or emergence of new variants.

To address the need for high-throughput wastewater surveillance workflows, we developed the Maxwell® HT Environmental TNA Kit, which allows automated high-throughput purification of viral RNA. The following protocol uses Nanotrap® Magnetic Virus Particles for automated concentration of SARS-CoV-2 and PMMoV from wastewater samples, then purification of viral RNA using the Maxwell® HT Environmental TNA Kit on a KingFisher™ Flex Purification System.

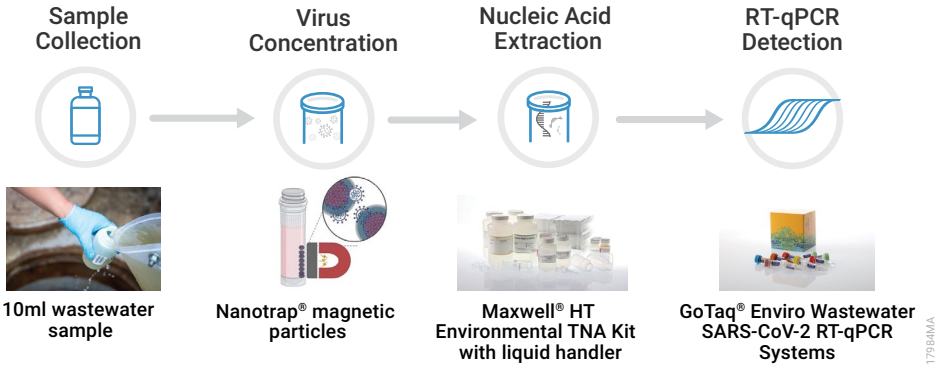

Figure 1. Overview of high-throughput workflow for SARS-CoV-2 wastewater surveillance.

Methods

Concentration of Virus with Nanotrap® Magnetic Virus Particles

1. Prepare Sample Plate 1 (KingFisher™ 24 well plate):
   a. Add 4.875ml of wastewater samples to each well.
   b. Add 50µl of Nanotrap® Enhancement Reagent 2 (ER2) to each well.
   c. Add 75µl of Nanotrap® Magnetic Virus Particles to each well.
2. Prepare Sample Plate 2 (KingFisher™ 24 well plate):
   a. Add 4.875ml of wastewater samples to each well.
   b. Add 50µl of Nanotrap® ER2 to each well.
   c. Add 75µl of Nanotrap® Magnetic Virus Particles to each well.
3. Prepare Elution Plate (KingFisher™ 24 well plate): Add 300µl of Cell Lysis Buffer (CLD) to each well.
4. Prepare Tip Plate: Place KingFisher™ 24 tip comb into an empty KingFisher™ 24 well plate.
5. Start KingFisher™ method "Ceres_Concentration_24well_10ml.bdz" and follow prompts for plate placement (total run time is 56 minutes).
6. Remove plates from the KingFisher™ System and continue with RNA purification.

Purification of Viral RNA from Concentrated Sample

7. Prepare Wash 1 Plate (KingFisher™ 96 well plate): Add 100µl of 50% ethanol and 900µl of wash buffer to each well.
8. Prepare Wash 2 Plate (KingFisher™ 96 well plate): Add 100µl of 50% ethanol and 900µl of wash buffer to each well.
9. Prepare Ethanol Wash Plate (KingFisher™ 96 well plate): Add 450µl of 50% ethanol to each well.
10. Prepare Elution Plate (KingFisher™ 96 well plate): Add 50µl of 25mM Tris-HCl (pH8.0) to each well.
11. Prepare Tip Plate: Place KingFisher™ 96 tip comb into an empty KingFisher™ 96 well plate.
12. Prepare Lysis and Bind Plate (KingFisher™ 96 well plate):
    a. Transfer 300µl of each sample eluate from the concentration step to individual wells of a KingFisher™ 96 well plate.
    b. Add 50µl of Alkaline Protease (APA) to each well containing a sample.
    c. Add 400µl of Isopropanol (100%) to each well containing a sample.
    d. Add 35µl of Resin to each well containing sample.

    Note: Mix resin thoroughly (shake/vortex) to resuspend before addition.
13. Start KingFisher™ method "Promega_Maxwell_HT_Enviro_Wastewater.bdz" and follow prompts for plate placement (total run time is 40 minutes).

Results and Conclusion

Viral RNA was successfully purified from wastewater on the KingFisher™ Flex Purification System using Nanotrap® Magnetic Virus Particles for concentration and the Maxwell® HT Environmental TNA Kit for viral RNA purification. Our results show that this method can be a high-throughput solution for SARS-CoV-2 wastewater surveillance.

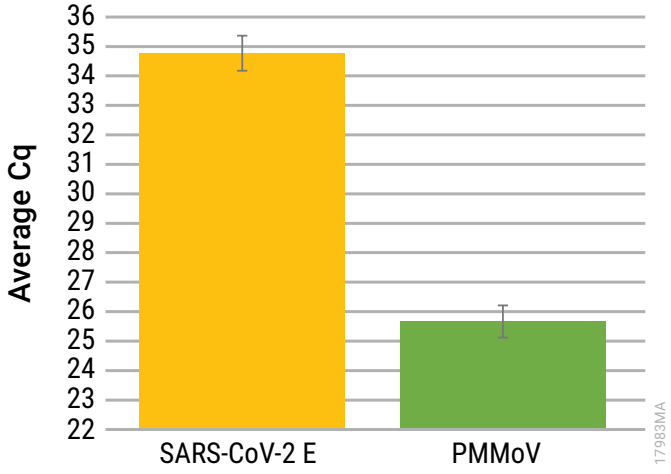

Figure 2. SARS-CoV-2 and PMMoV detection in wastewater. 10ml of wastewater was processed on the KingFisher™ Flex using Nanotrap® Magnetic Virus Particles for virus capture and concentration prior to RNA purification. Viral RNA was purified from the concentrated wastewater on the KingFisher™ Flex using the Maxwell® HT Environmental TNA Kit, Custom. Following purification, 5µl of each extract was assayed for SARS-CoV-2 RNA (envelope target) with the Wastewater SARS-CoV-2 RT qPCR System N1/N2/E Kit (Cat.# AM2100). Reactions were cycled on a Bio-Rad CFX96 according to the product specifications. Average Cq and standard deviation for SARS-CoV-2 (envelope gene) and PMMoV (Pepper Mild Mottle Virus, the most abundant viral RNA found in human feces) is shown for N=8 replicates.