Mar 28, 2021

Isolation of SARS-CoV-2 RNA from Wastewater: Maxwell® RSC Environmental TNA Kit

subhanjan.mondal [1], Subhanjan Mondal[1], Nathan Feirer[1]

[1]Promega Corporation

1 Works for me dx.doi.org/10.17504/protocols.io.btqtnmwn

Coronavirus Method Development Community Promega Corp

subhanjan.mondal

SUBMIT TO PLOS ONE

ABSTRACT

Early in the COVID-19 pandemic, scientific studies demonstrated that the genetic material of SARS-CoV-2, an enveloped RNA virus, could frequently be detected in the feces of infected individuals and thereafter was also detected in wastewater. This finding mobilized the global water sector to investigate if wastewater-based epidemiology (WBE) using the genetic signal of SARS-CoV-2 could be used to track the spread of COVID-19 in communities. In the past, WBE was critical in identifying the community prevalence of poliovirus in support of the World Health Organization's (WHO) program to eradicate poliomyelitis. Detection of SARS-CoV-2 in wastewater has the potential to provide an integrated, community-level indication of the presence or prevalence of COVID-19. The process of detecting viral genetic signatures in wastewater samples involves collection of wastewater influent at specific points in the sewage system, either as a grab sample or as a 24-hour composite sample. This is followed by optional heat pasteurization and concentration of viral matter. The viral matter and/or its genetic signature is present at a low concentration in wastewater, making sample concentration a prerequisite for sensitive detection and utility in WBE. Concentration of viral matter can be performed using a variety of methods such as charged membrane filtration, centrifugal ultrafiltration and flocculation/precipitation using skimmed milk, or polyethylene glycol (PEG)/NaCl. Most of the viral concentration methods currently being used were originally developed to concentrate viral matter with the objective of culturing the virus for downstream applications, though they have been also used for direct PCR based detection. Several of the methods described above may work efficiently with several virus types, but they have proven to be inconsistent for SARS-CoV-2 RNA recovery leading to significant analytical variability. In addition, these viral concentration methods are labor intensive and time consuming.

To address these issues, we have developed a convenient method to direct capture and concentrate total nucleic acids (TNA) from a large volume (40 ml) of wastewater using silica based a GFA/silica column (PureYield Midi Column) and then eluted in 1ml. In a second step the material is purified and concentrated using the Maxwell® RSC system allowing walk-away-automation and with fast, reliable and consistent purification of total nucleic acid.

DOI

dx.doi.org/10.17504/protocols.io.btqtnmwn

PROTOCOL CITATION

subhanjan.mondal , Subhanjan Mondal, Nathan Feirer 2021. Isolation of SARS-CoV-2 RNA from Wastewater: Maxwell® RSC Environmental TNA Kit. **protocols.io** https://dx.doi.org/10.17504/protocols.io.btqtnmwn

CREATED

Mar 28, 2021

LAST MODIFIED

Mar 28, 2021

PROTOCOL INTEGER ID

48627

MATERIALS TEXT

2 x 280 ml Binding Buffer 1
1 x 50 ml Binding Buffer 2
1 x 30 ml Protease Solution
10 x 5 each PureYield™ Midi Binding Columns
2 x 150 ml Nuclease free Water
2 x 216 ml RNA Wash solution RWA
2 x 85.3 ml Inhibitor Removal Wash
1 x 48/pack RSC Plungers
48 x 1 each Maxwell Cartridge (RSCJ)
1 x 50/ pack Elution Tubes (0.5ml)

Materials and Equipment to Be Supplied by the User
·Isopropanol
·Ethanol, 95%
·Tabletop centrifuge (capable of 3,000 x g)
·Swinging bucket rotor
·50ml disposable plastic screw-cap tubes (e.g., Corning or Falcon™ brand)
·1.5ml microcentrifuge tubes
·Heat block (capable of reaching 60°C)
·Vacuum manifold (e.g., Vac-Man® Laboratory Vacuum Manifold [Promega Cat.# A7231])
·Eluator™ Vacuum Elution Device (Promega Cat.# A1071)
·Vacuum pump, single or double stage, producing pressure of approximately 650mm Hg (25.6 inches Hg, 12.57 psi, 86.7 kPa). For example: Welch Vacuum Pump (Model 2522B-01 for North America electrical, Cat.# A6720; Model 2522C-02 for Europe electrical, Cat.# A6722; Model 2522C-05 for Japan electrical, Cat.# A6724)

DISCLAIMER:

BEFORE STARTING

Inhibitor Removal Wash: Add 57ml of isopropanol to the Inhibitor Removal Wash bottle and mark on the bottle “plus isopropanol”. The reagent is stable at 15–30°C when tightly capped.

RNA Wash Solution (RWA): Add 350ml of 95% ethanol to each RNA Wash Solution (RWA) and mark the bottle “plus ethanol”. The reagent is stable at 15–30°C when tightly capped.

Materials and Equipment to Be Supplied by the User
·Isopropanol
·Ethanol, 95%
·Tabletop centrifuge (capable of 3,000 x g)

Citation: subhanjan.mondal , Subhanjan Mondal, Nathan Feirer (03/28/2021). Isolation of SARS-CoV-2 RNA from Wastewater: MaxwellÃÂ® RSC Environmental TNA Kit. https://dx.doi.org/10.17504/protocols.io.btqtnmwn

·Swinging bucket rotor
·50ml disposable plastic screw-cap tubes (e.g., Corning or Falcon™ brand)
·1.5ml microcentrifuge tubes
·Heat block (capable of reaching 60°C)
·Vacuum manifold (e.g., Vac-Man® Laboratory Vacuum Manifold [Promega Cat.# A7231])
·Eluator™ Vacuum Elution Device (Promega Cat.# A1071)
Vacuum pump, single or double stage, producing pressure of approximately 650mm Hg (25.6 inches Hg, 12.57 psi, 86.7 kPa). For example: Welch Vacuum Pump (Model 2522B-01 for North America electrical, Cat.# A6720; Model 2522C-02 for Europe electrical, Cat.# A6722; Model 2522C-05 for Japan electrical, Cat.# A6724)

Direct Capture Concentration

1 Dispense 40ml of wastewater into a 50ml conical tube. You may pasteurize wastewater by incubation at 60°C for 1 hour. Please follow your institution biosafety guidelines.

2 Add 0.5 ml of Protease Solution, mix well by inversion and incubate for 30 minutes at room temperature (approximately 22-25C).

3 Clarify sample by centrifugation at 3000xg for 10 minutes. It is important to remove solids to avoid clogging the PureYield™ Midi Binding Column.

4 Carefully decant the supernatant into two clean 50 ml conical tubes, 20mL each. Discard the 50ml conical tube containing the pellet into an appropriate biohazard waste container.

5 To each tube containing 20ml of the clarified supernatant, add 6ml of Binding Buffer 1 and 0.5ml of Binding Buffer 2

6 Mix well by inversion.

7 Add 24ml of isopropanol to each tube.

8 Mix well by inversion.

9 Remove the vacuum port cap and attach the PureYield™ Midi Column onto the vacuum manifold by pressing the nozzle gently into the vacuum port fitting.

10 Pour the mixture from each tube from step 8 into the PureYield™ Midi Binding Column (combine both tubes of the same sample if applicable), turn on the pump and apply vacuum to capture TNA on the column. The content of two tubes is added stepwise in 20ml increments to a single column.
Note: Empty the blue Vac-Man® Laboratory Vacuum Manifold as needed. Dispose of the alcohol containing waste following your institutional policies .
Note: If the manifold flow rate is <2ml/ minutes or the pressure reading on the vacuum pump is lower than 10 inches

Citation: subhanjan.mondal , Subhanjan Mondal, Nathan Feirer (03/28/2021). Isolation of SARS-CoV-2 RNA from Wastewater: MaxwellÃÂ® RSC Environmental TNA Kit. https://dx.doi.org/10.17504/protocols.io.btqtnmwn

Hg/250mm Hg, close the valves on the unused ports of the vacuum manifold. If this does not improve flow rate, there may be a leak in the tubing connections or in the Vac-Man® Laboratory Vacuum Manifold. Consider replacing your existing vacuum manifold.

11 Pass 5ml Inhibitor Removal Wash through the PureYield™ Midi Binding Column by vacuum.

12 Pass a total of 20 ml of RNA Wash Solution (RWA) through the PureYield™ Midi Binding Column by vacuum. Draw vacuum for an additional 30 seconds after all visible fluid has passed.

13 Pre-heat 2ml per sample of Nuclease–Free Water to 60°C

14 Assemble the elution device by placing a 1.5ml microcentrifuge tube in the base of the Eluator™ Vacuum Elution Device (Promega Cat.# A1071) and securing the tube cap in the open position, as shown. Insert the PureYield™ Midi Binding Column into the top of the Eluator Device, making sure the column is fully seated on the collar. Place the Eluator™ Device assembly onto a vacuum manifold.

15 Elution by vacuum. Panel A. A 1.5ml microcentrifuge tube is placed in the base of the Eluator™ Vacuum Elution Device with the microcentrifuge tube cap is locked as shown. The final Eluator™ Vacuum Elution Device assembly, including the binding column, ready for use on a vacuum manifold.

16 Add 250µl of pre-heated (60°C) Nuclease-Free Water to the PureYield™ Midi Binding Column. Apply maximum vacuum for 1 minute or until all liquid has passed through the column. Repeat the process by adding another 250µl of pre-heated Nuclease-Free Water to the PureYield™ Midi Binding Column to collect a total of 0.5ml of TNA solution.

TNA Extraction and Clean-up

17 Cartridge Preparation
a. Place the cartridge to be used in the Deck Tray(s) with well #1 (the largest well) facing away from the elution tube.
b. Press down on the cartridge to snap it into position. Carefully peel back the seal so that all plastic comes off the top of the cartridge. Ensure that all sealing tape and any residual adhesive are removed before placing cartridges in the instrument.
c. Place a plunger in well #8 of each cartridge. Well #8 is the well closest to the elution tube. 4. Place an empty elution tube in the elution tube position for each cartridge. Add at 80µl of Nuclease Free Water to the bottom of each elution tube. Notes: Use only the Elution Tubes (0.5ml) provided in the kit; other tubes may be incompatible with supported Maxwell® Instruments.

18 Sample Processing:
a. Add 150µl of Binding Buffer 1 and 50µl of Binding Buffer 2 to the 0.5ml of liquid eluted Direct Capture Concentration Step.
b. Load the entire volume of mixture to well #1 (the largest well).

19 Maxwell® Instrument Setup and Run
a. Turn on the Maxwell® Instrument and Tablet PC. Log in to the Tablet PC and start the Maxwell® software by double-touching the icon on the desktop. The instrument will power up, proceed through a self-check and home all moving parts.
b. Touch Start to begin the process of running a method.
c. Touch the PureFood GMO and Authentication method. On the 'Cartridge Setup' screen touch the cartridge positions to select/deselect the positions to be used for this extraction run. Enter any required sample tracking information, and touch the Proceed button to continue. Note: When using 48-position Maxwell® Instruments, press the Front and Back buttons to select/deselect cartridge positions on each deck tray.
d. After the door has been opened, confirm that all Extraction Checklist items have been performed. Verify that

Citation: subhanjan.mondal , Subhanjan Mondal, Nathan Feirer (03/28/2021). Isolation of SARS-CoV-2 RNA from Wastewater: MaxwellÃÂ® RSC Environmental TNA Kit. https://dx.doi.org/10.17504/protocols.io.btqtnmwn

cartridges are loaded on the instrument, preprocessed samples are added to well #1 of the cartridges, uncapped elution tubes are present with 80µl of Nuclease Free Buffer and plungers are present in well #8. Transfer the deck tray(s) containing the prepared cartridges onto the Maxwell® Instrument platform.
e. Inserting the Maxwell® deck tray(s): Hold the deck tray by the sides to avoid dislodging cartridges from the deck tray. Ensure that the deck tray is placed in the Maxwell® Instrument with the elution tubes closest to the door. Angle the back of the deck tray downward and place into the instrument so that the back of the deck tray is against the back of the instrument platform. Press down on the front of the deck tray to firmly seat the deck tray on the instrument platform. If you have difficulty fitting the deck tray on the platform, check that the deck tray is in the correct orientation. Ensure the deck tray is level on the instrument platform and fully seated. Note: Check the identifier on the 24-position Maxwell® deck trays to determine whether they should be placed in the front or back of the instrument. Deck trays are keyed and will only fit in their intended positions.
f. Touch Start to begin the extraction run. The platform will retract, and the door will close.
g. Follow the on-screen instructions at the end of the method to open the door. Verify that the plungers are located in well #8 of the cartridge at the end of the run. Remove elution tubes containing nucleic acid, and cap the tubes. store at –30 to –10°C. Avoid multiple freeze-thaw cycles. TNA purified using this method can be directly used for RT-qPCR.

Quantitation of Viral matter in Wastewater

20

$$\text{Concentration of Viral genome in wastewater (copies / L)} = \frac{\text{Copies in RT-qPCR reaction (copies)}}{\text{Volume of nucleic acid extract used for RT-qPCR (ml) *} \times \text{Concentration factor}^{\$\$}} \times 1000$$

* If 5ul of the nucleic acid extract is used in RT-qPCR assay the value in ml is 0.005

$$^{\$\$}\text{Concentration factor} = \frac{\text{Wastewater sample volume used (ml)}}{\text{Volume of nucleic acid extracted (ml)}}$$

Citation: subhanjan.mondal , Subhanjan Mondal, Nathan Feirer (03/28/2021). Isolation of SARS-CoV-2 RNA from Wastewater: MaxwellÃÂ® RSC Environmental TNA Kit. https://dx.doi.org/10.17504/protocols.io.btqtnmwn