Sample Concentration Protocol for Wastewater Surveillance for SARS-CoV-2

Used to prepare and concentrate wastewater samples prior to nucleic acid purification and quantification of SARS-CoV-2

IDEXX

English Version

Sample Concentration Protocol for Wastewater Surveillance for SARS-CoV-2

Overview of IDEXX Materials and Procedures Validated for Quantification of SARS-CoV-2 in Wastewater

IDEXX has validated an end-to-end protocol for detecting SARS-CoV-2 in wastewater and offers materials and/or procedures for each required step in the process, as detailed below. IDEXX has validated that these protocols and materials reliably quantify SARS-CoV-2 in untreated wastewater. Validation data is available and demonstrates strong repeatability and sensitivity of the end-to-end protocol and materials. Please contact IDEXX Technical Support (contact information below) to request the validation report.

While IDEXX has validated the entire procedure, individual components of the procedure can be used independently. For example, the IDEXX Water DNA/RNA Magnetic Bead Kit and SARS-CoV-2 RT-PCR Test can be used with a different concentration method than the example PEG concentration. Similarly, the IDEXX Water SARS-CoV-2 RT-PCR Test can be used with different extraction methods, and so on.

Because each component of the test can be used independently, procedures for each component of the test are detailed in separate documents. To use the entire, end-to-end and validated procedure, it is recommended to reference the following documents in the order listed in the table below.

Test component	Description	Document
Concentration	Used to concentrate the viral particles and nucleic acids present in a wastewater sample to improve limit of detection	This document
Nucleic Acid Extraction	Used to extract and purify nucleic acids from the concentrated sample	IDEXX Water DNA/RNA Magnetic Bead Kit Product Insert
Reverse Transcription Quantitative Polymerase Chain Reaction (RT-qPCR)	Used to quantify the amount of SARS-CoV-2 RNA in the extracted sample via amplification of specific genetic sequences	IDEXX Water SARS-CoV-2 RT-PCR Test Product Insert

In addition to the documentation detailed in the above table, a description of available and compatible controls is also available: Available and Compatible Controls for Quantifying SARS-CoV-2 in Wastewater with IDEXX test kits

Protocol for Wastewater Sample Concentration

The following protocol has been validated to provide repeatable quantification of SARS-CoV-2 in untreated wastewater when used in conjunction with the IDEXX Water DNA/RNA Magnetic Bead Kit and SARS-CoV-2 RT-PCR Test. It is provided as an example that may be used in conjunction with those tests. Other concentration methods may also be used with the IDEXX Water DNA/RNA Magnetic Bead Kit and SARS-CoV-2 RT-PCR Test.

Materials Not Provided

- Sterile 50 mL centrifuge tubes rated for a relative centrifugal force (RCF) of at least 12,000 x RCF (maximum)
- Refrigerated centrifuge, with rotors and needed accessories for centrifugation at 12,000 x RCF (maximum RCF)
- Polyethylene glycol (PEG), average molecular weight 8000, molecular biology grade or equivalent
- NaCl, molecular biology grade or equivalent
- Nuclease free water, molecular grade
- Nuclease-free, aerosol-resistant pipette tips
- Microcentrifuge tubes (DNase/RNase free)
- Pipettes, including micropipettes and serological pipettes, as needed.
- Personal protective equipment consistent with current guidelines for handling infectious samples
- Water bath for pasteurization (optional)

Warnings and Precautions

- Follow all local regulatory and safety guidelines for the handling of wastewater samples. In addition, follow local health authorities' recommended procedures for handling and processing of wastewater samples associated with SARS-CoV-2. One source of information is the U.S. Centers for Disease Control & Prevention (CDC) Interim Laboratory Biosafety Guidelines for Handling and Processing Specimens Associated with Coronavirus Disease 2019 (COVID-19). (https://www.cdc.gov/coronavirus/2019-ncov/lab/lab-biosafety-guidelines.html).
- Use personal protective equipment (PPE) consistent with current guidelines for the handling of potentially infectious samples.
- Do not eat, drink, smoke, apply cosmetics or handle contact lenses in areas where reagents and human specimens are handled.
- Dispose of waste in compliance with the local, state, and federal regulations.

Wastewater Sample Collection

- Wastewater samples should be collected in an appropriate container to provide enough sample volume for testing.
- Refer to the Water Research Foundation and the US Centers for Disease Control and Prevention for more information on collecting untreated wastewater for quantification of SARS-CoV-2:
  - Water Research Foundation: Best Practices for Collection and Storage of Wastewater Samples to Support Wastewater Surveillance of the COVID-19 Signal in Sewersheds
  - US Centers for Disease Control and Prevention: Developing a Wastewater Surveillance Sampling Strategy
- Keep samples cold but unfrozen (<8°C) during transportation of the sample to the laboratory for processing. Wastewater samples can be stored at 2–8°C for up to 72 hours after collection.

Wastewater Concentration Protocol

Pasteurization (Optional)

1. Decontaminate exterior surface of container. Surface disinfection may be performed, for example, with 70% isopropanol and/or exposure to short-wave (UV-C) light using appropriate contact or exposure times, respectively.
2. Incubate sample in a 60±1°C water bath for 1.5 hours, mixing once during incubation. The water level must cover the container sufficiently for the container and sample volume used to ensure that the target temperature is reached.
3. Cool sample to 2–8°C before proceeding with concentration. Pasteurized sample may be stored overnight at 2–8°C.

Concentration

1. Mix sample well, then add 35±1 mL to each of three (3) empty 50 mL centrifuge tubes.
2. Centrifuge all three tubes at 4700 RCF for 30 minutes at 4±1°C. A swinging bucket rotor is recommended to provide a stable bacterial pellet. Use little or no braking force to prevent the bacterial pellet from being disturbed.
3. Add 3.5±0.1 g PEG 8000 and 0.788±0.01 g NaCl into three (3) empty 50 mL centrifuge tubes. **Note:** complete this step in advance or while step 2 is in process.
4. Promptly and gently, remove centrifuge buckets from centrifuge. Carefully decant supernatant, smoothly and in one consistent motion, from each tube into a 50 mL tube containing PEG and NaCl to prevent the pellet from being disturbed.
5. Mix the tubes containing PEG and NaCl at ambient temperature until completely dissolved.
6. Open tubes carefully (see recommended technique in the Concentration Procedural Notes below) and decant liquid into a new 50 mL centrifuge tube. This step prevents leaks during centrifugation due to powder interference with the cap seal.
7. Mark a location near the bottom of the tube and orient to the outside of the rotor during centrifugation. This will aid the resuspension of viral pellets that are not clearly visible
8. Centrifuge at 12,000 RCF for 120 minutes at 4±1°C. Use little or no braking force to prevent the viral pellet from being disturbed.
9. Promptly and gently, remove rotor from centrifuge. Carefully decant and discard most of the supernatant from each tube. Decant smoothly in one consistent motion to prevent the pellet from being disturbed. It is not necessary to remove all liquid as the remainder will be removed in the next step.
10. Centrifuge at 12,000 RCF for 5 minutes at 4±1°C. Use little or no braking force to prevent the viral pellet from being disturbed.
11. Promptly and gently, remove rotor from centrifuge. Use a pipette to carefully remove and discard the remaining supernatant from each tube. Do not contact or disturb pellet with pipette tip.
12. Use a pipette to transfer 0.4 mL nuclease-free water to one of the tubes containing a viral pellet.
13. Resuspend the viral pellet by repeatedly pipetting to rinse the inside surface of the tube around the expected location of the pellet. Rinse a wide area surrounding the expected pellet location to ensure all precipitated virus is recovered. Some of the resuspension liquid will adhere to the sides of the tube. This liquid will be recovered in the next step.
14. Flash spin the tube at 2,000 RCF (+/-1000) to collect all the liquid at the bottom of the tube.
15. Pipette up and down several times to homogenize the concentrate, then transfer the entire volume to the second tube containing a viral pellet. Repeat steps 13 and 14 to resuspend the pellet and collect all the liquid.
16. Pipette up and down several times to homogenize the concentrate, then transfer the entire volume to the third tube containing a viral pellet. Repeat steps 13 and 14 to resuspend the pellet and collect all the liquid.
17. Transfer the recovered concentrate to a RNase free microtube. The volume should be approximately 0.4 mL
18. Proceed with extraction immediately, or store concentrate overnight at –25 to –15°C.

Procedural Notes

- Larger or smaller volumes of wastewater can be analyzed by modifying the basic procedure with appropriate centrifugation equipment, including centrifuge, rotor, tubes or bottles, and any other required accessories. Appropriate fill volumes must be used to ensure the sample remains contained during centrifugation.
- Maintain samples at cold temperatures near 4°C throughput the procedure. It is recommended to use refrigerated centrifuge equipment and keep rotors, buckets, and other processing equipment cold where practical. It is recommended to minimize handling time and avoid delays while working with tubes outside of the centrifuge to minimize warming of the sample and centrifuge rotor.
- PEG and NaCl may be electrostatically attracted to plastic centrifuge tubes. Adherence of the powders to the tube rim must be avoided to prevent interference with the cap seal.
- After PEG and NaCl dissolution, liquid adhering to the inner cap surface may unexpectedly be transferred to outside of the tube when the cap is removed. To prevent this, the following technique is recommended when opening the tubes: let tube sit undisturbed for 1 minute to allow excess fluid to drain from the cap; loosen cap 1/2 turn; pause for one second; continue to loosen cap until threads are disengaged; then carefully lift cap straight up off tube.
- Flash spins may be performed by bringing the tubes momentarily up to approximately 2,000 RCF (±1000) then quickly stopping the rotor with strong braking force.

Concentration Method Verification

IDEXX recommends a verification procedure be performed to ensure the full method, including concentration, extraction, and PCR are working correctly. This procedure can be performed when first adopting the method and afterwards, when needed to meet laboratory quality standards. Contact IDEXX Technical Support for more information.

Next Steps

Extraction or nucleic acid purification can be performed with the Water DNA/RNA Magnetic Bead Kit (98-0014719-00, WCOV2MAG). Reference the Water DNA/RNA Magnetic Bead Kit product insert for more information and detailed instructions for use.

Other extraction or lysis methods may also be used once validated by the laboratory.

For Technical Support, please call:
North / South America: 1 207 556 4496 / 1 800 321 0207
Europe: 00800 4339 9111
UK: +44 (0) 1638 676800
China: +86 21 61279528
Japan: 03 5301 6800
Australia: 1300 443 399

06-0015425-00