Water SARS-CoV-2 RT-PCR Test

English Version

Used for real-time PCR identification of SARS-CoV-2 RNA extracted from wastewater samples.

IDEXX

Table of Contents

Intended Use....1

Product Description....1

Materials and Storage....2

Materials Not Provided....2

Warnings and Precautions....3

Wastewater Sample Collection....4

Water Concentration Protocol....4

Extraction....5

PCR....5

Reconstitution of Dried Components....5

Quality Controls....6

Test Procedure....7

Limitations....8

English version

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Intended Use

The Water SARS-CoV-2 RT-PCR Test is a real-time fluorescent reverse transcription polymerase chain reaction test for the detection of nucleic acids from SARS-CoV-2 in wastewater. The Water SARS-CoV-2 RT-PCR Test is intended to be used by qualified and trained laboratory personnel specifically instructed and trained in the techniques of real-time nucleic acid amplification.

Product Description

The Water SARS-CoV-2 RT-PCR Test is a real-time reverse transcription polymerase chain reaction (RT-qPCR) test that uses the N1 and N2 primer and probe sequences which are described by the CDC design[1]. The SARS-CoV-2 Mix includes primers and probes for the detection of SARS-CoV-2 RNA when amplified with the included RNA Master Mix (RNA MMx). SARS-CoV-2 RNA targets (N1 and N2) are both detected in the FAM channel.

The RT-qPCR process reverse transcribes viral RNA into cDNA which is subsequently amplified in a real-time PCR cycling protocol. Fluorescence intensity is monitored at each PCR cycle by one of the real-time PCR instruments listed in Section “Materials Not Provided”.

In addition, the Water SARS-CoV-2 RT-PCR Test utilizes the Positive Control (PC) and PCR Grade Water (Negative Control). The Positive Control (PC) contains SARS-CoV-2 synthetic material and works as a positive control for the reaction. PCR Grade Water is used as the RT-PCR negative control, as well as to reconstitute the dried SARS-CoV-2 Mix and the PC.

1. “Coronavirus Disease 2019 (COVID-19) Real-Time rRT-PCR Panel Primers and Probes.” Centers for Disease Control and Prevention, 6 Mar. 2020, www.cdc.gov/coronavirus/2019-ncov/lab/rt-pcr-panel-primer-probes.html

Materials and Storage

Identification/ General Information	Cap color	Quantity	Storage		Freeze/Thaw cycles
		100 tests	At receipt	After reconstitution	
SARS-CoV-2 Mix, dried	Red	1 x 1.0 mL	–25 to 8°C	–25 to –15°C	≤6
REF 61-56616-00 Contains N1 and N2 primers and probes. Reconstitute to 1 mL in PCR Grade Water. Store the SARS-CoV-2 Mix in the dark. The expiration date on the vial is valid for either the dry or reconstituted form.					
RNA Master Mix (RNA MMx)	Black	1 x 1.0 mL	–25 to –15°C (Long-term)	N/A	≤6
REF 61-56618-00 Concentrated master mix that includes reverse transcriptase and hot-start polymerase. The RNA MMx is more viscous than most master mixes– see the Test Procedure section for handling recommendations. A reference dye (ROX) has been added for normalizing volume inaccuracies. Protect the RNA MMx from light.					
Positive Control, dried (PC)	Blue	1 x 200 µL	–25 to 8°C	–25 to –15°C	≤6
REF 44-56617-00 The PC contains the targets for SARS-CoV-2 (N1 target region). Reconstitute to 200 µL in PCR Grade Water. The expiration date on the vial is valid for either the dry or reconstituted form.					
PCR Grade Water	Clear	2 x 1.0 mL	–25 to 8°C		N/A
REF 61-56619-00 PCR Grade Water has been qualified for reverse transcription-PCR (RT-PCR) use. It is used for the reconstitution of the SARS-CoV-2 Mix and PC. It is also used as the PCR negative control for each test run. Do not transfer PCR Grade Water vials between PCR work areas. Separate vials of water are needed for each area to avoid contamination risk.					

Note: See table at the end of the insert for a description of symbols used on the insert and labels.

Materials Not Provided

- 50 mL centrifuge tubes rated for a relative centrifugal force (RCF) of at least 12,000 x g (maximum)
- Refrigerated centrifuge, with rotors and needed accessories for centrifugation at 12,000 x g (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
- Vortex mixer
- Nuclease-free, aerosol-resistant pipette tips
- Microcentrifuge tubes (DNase/ RNase free)
- Microcentrifuge capable of reaching 1500 – 3000 x g
- Pipettes (5–1000 µL); dedicated pipettes for preparation of PCR Mix
- Water DNA/RNA Magnetic Bead Kit (98-0014719-00, or alternative extraction or lysis materials)
- Seracare AccuPlex™ SARS-CoV-2 Verification Panel (Material Number: 0505-0129), or suitable alternative
- Personal protective equipment consistent with current guidelines for handling infectious samples
- Water bath for pasteurization (optional)

Additional Materials for PCR

- 96- or 384-well format PCR plates and optical adhesive film/plate or suitable alternative.
- Real-time PCR instrument (Applied Biosystems® 7500, Applied Biosystems® ViiA™ 7, Applied Biosystems QuantStudio 5, Agilent Mx3000P™, Agilent Mx3005P™, Agilent AriaMx, Bio-Rad CFX96 Touch™, Bio Molecular Systems Mic qPCR Cycler, QIAGEN Rotor-Gene (72-Well Rotor only), Roche LightCycler® 480 or equivalent).

  Note- the Roche LC480 instrument requires additional calibration and software settings. IDEXX Technical Service can provide guidance for use of this instrument with RealPCR reagents.
- Centrifuge with rotor and adapters for multi-well plates (optional).

Warnings and Precautions

General

- 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.

PCR

- Reagents must be stored and handled as specified in these instructions for use. Do not use reagents past expiration date.
- The entire procedure must be performed under nuclease-free conditions.
- Wear powder-free gloves when working with the reagents and nucleic acids.
- Always use pipette tips with aerosol barriers. Tips that are used must be sterile and free from DNases and RNases.
- Keep reagents and PCR Mix tubes capped or covered as much as possible.
- To avoid cross-contamination, use nuclease-free, aerosol-resistant pipette tips for all pipetting, and physically separate the workplaces for nucleic acid extraction/handling, PCR setup and PCR.
- Work surfaces, pipettes, and centrifuges should be cleaned and decontaminated with cleaning products such as 10% bleach, "DNAZap™" or "RNase AWAY®" to minimize risk of nucleic acid contamination. Residual bleach should be removed using 70% ethanol.

Wastewater Sample Collection

- Wastewater samples should be collected in an appropriate container to provide enough sample volume for testing. Refer to *Standard Methods for the Examination of Water and Wastewater* (Section 9060) for suggestions on wastewater sampling procedures.
- 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.

Water 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:

Alternative concentration methods may also be used to prepare wastewater samples for use with the Water SARS-CoV-2 RT-PCR Test.

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.
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 pipet 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 1,000 to 3,000 RCF 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 (Concentration):

- This procedure is recommended for concentration of SARS-CoV-2 from 105 mL of wastewater. Larger or smaller water volumes 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 (1-800-321-0207).

Extraction Protocol

The Water DNA/RNA Magnetic Bead Kit (98-0014719-00, WCOV2MAG) is used to purify nucleic acids, including SARS-CoV-2 RNA from wastewater concentrates. Other extraction or lysis methods may also be used once validated by the laboratory.

PCR Protocol

Reconstitution of Dried Components

Reconstitute the SARS-CoV-2 Mix and Positive Control by pipetting PCR Grade Water to the volume indicated on the component label. Allow to sit at 18 to 26°C for at least 10 minutes; mix and microcentrifuge briefly prior to use. Once the SARS-CoV-2 Mix and the Positive Control are reconstituted, aliquot as appropriate and store the solutions frozen. When handling frozen components, thaw at 18 to 26°C for approximately 15 to 30 minutes, mix gently and then microcentrifuge briefly to collect liquids at the bottom of the tube (2,000 ±1000 RCF).

Quality Controls

Control(s) that are provided with the Water SARS-CoV-2 RT-PCR Test are listed below:

- PCR Positive Control (PC): A positive template control is needed to confirm the PCR plate is valid. The positive control should be included on each PCR run and should test positive for the SARS CoV-2 target. The PCR Positive Control is not included during extraction.
- PCR Negative Control (PCR Grade Water): A "no template" (negative) control is needed to confirm the PCR plate is valid. PCR Grade water is used and should be included for each PCR run. The negative control should test negative for the SARS CoV-2 target.

Controls that are recommended, but not required to perform the test nor provided with the Water SARS-CoV-2 RT-PCR Test are listed below:

- Extraction Positive Control (Accuplex SARS-CoV-2 Verification Panel): An extraction control containing SARS-CoV-2 RNA should be extracted and tested with each set of samples. The extraction positive control is used to demonstrate successful recovery of RNA during the extraction process and should test positive for the SARS CoV-2 target. IDEXX recommends using the Accuplex Verification Panel (see ordering information), which contains a recombinant virus engineered to harbor SARS-CoV-2 RNA sequences; this provides a full extraction control that requires lysis and recovery of single-stranded RNA from an encapsulated viral particle to produce a positive result. To perform the extraction positive control, use 200 μL of the "Member 1" dilution provided in the Verification Panel for the sample during extraction. Results are interpreted according to the table below.
- Alternatively, samples that have been confirmed positive for SARS-CoV-2 may be used for the extraction positive control. Such samples should be available in enough volume to be used across multiple runs, tested prior to use, and used at an appropriate dilution to ensure the expected positive results are obtained.
- Extraction Negative Control (PCR Grade Water): A "no template" (negative) control containing no nucleic acids should be extracted and tested with each set of wastewater samples to verify the absence of nucleic acid contamination in the extraction reagents and materials. The Extraction Negative Control should give a negative result for the SARS CoV-2 target. To perform the extraction negative control, use 200 μL of PCR Grade Water as the sample during extraction.

PCR Test Procedure

1 Preparation of the PCR Mix.

- Mix the thawed RNA MMx by inversion or gentle vortex.
- The RNA MMx is a viscous solution; always pipette it slowly.
- To prepare the PCR Mix add 10 µL SARS-CoV-2 Mix and 10 µL RNA MMx for each reaction. Include reagents for all control reactions.
- When preparing the PCR Mix, first pipette SARS-CoV-2 Mix into the tube and then add the RNA MMx. Pipette up and down a few times to rinse the MMx pipette tip.
- Gently vortex the solution to ensure the components are mixed well.

Load the PCR plate within 20 minutes or store at 2 to 8°C for up to 4 hours. The PCR Mix can be stored at –25 to –15°C for up to 2 weeks. Protect from light.

2 Slowly pipette 20 µL of the PCR Mix into the required wells of the multi-well plate.

3 For each wastewater sample, add 5 µL of purified RNA to the appropriate well. The final reaction volume is 25 µL.

4 For each control reaction, add 5 µL to the appropriate well. The final reaction volume is 25 µL. Include the PCR Positive Control (5 µL PC), PCR Negative Control (5 µL PCR Grade Water), Extraction Positive Control (5 µL), and Extraction Negative Control (5 µL) for each test run.

5 Cover the plate and briefly spin the plate, if necessary, to settle contents and remove air bubbles.

6 Set up real-time PCR instrument with Cycling Program below.

Settings for Reporter and Quencher

Target	Reporter	Quencher
SARS-CoV-2	FAM™	BHQ® (none)
Passive Reference	ROX™	N/A

Cycling Program (used for all instruments)

Step	Temperature	Time	Cycles
Reverse transcription (RT)	50°C	15 min.	1
Denaturation	95°C	1 min.	1
Amplification**	95°C 60°C	15 sec. 30 sec.	45

**Ensure the instrument is set to record fluorescence following the 60°C amplification step.

7 Analyze data

All test controls should be examined prior to interpretation of results. If the controls are not valid, the results cannot be interpreted.

To obtain appropriate Ct values, analysis for SARS CoV-2 target should be performed by manually setting the threshold. The threshold should be adjusted to the inflection point for the exponential phase of the curve and above background signal. This is best done while viewing all amplification curves for a given run, on a logarithmic scale. It is important to follow the same procedure run to run when setting the manual threshold.

Refer to specific instrument's user manual for guidance on how to analyze data.

Plate Validity Criteria

The following control results must be obtained for each PCR run in order for the run to be deemed valid. If the plate controls are not valid, the results cannot be interpreted, are not valid, and the plate must be repeated.

Control/Sample	SARS-CoV-2 FAM Ct Value	SARS-CoV-2 FAM Result
Positive Control	<40	Positive
PCR Negative Control	No Signal	Negative
Extraction Positive Control*	<40	Positive
Extraction Negative Control*	No Signal	Negative
Wastewater Sample	<40	Positive

Sample Validity: Each sample is considered valid when analyzed on a plate demonstrating the expected control results as described in the table above. Positive samples should display a characteristic amplification curve.

*If used, expected results are indicated.

Limitations

8 Limitations

- Performance of the Water SARS-CoV-2 RT-PCR Test has only been established in wastewater using primary effluent samples. Other sample types have not been evaluated.
- Samples must be collected, transported, and stored using appropriate procedures and conditions. Improper collection, transport, or storage of samples may affect the test performance.
- Validation data was obtained with concentration, extraction and amplification of nucleic acid from wastewater samples performed according to the specified methods listed in this procedure. Other concentration, extraction approaches, and processing systems have not been validated.
- A false negative result may occur if a sample is improperly collected, transported or handled. False negative results may also occur if amplification inhibitors are present in the sample.
- If the virus mutates in the test target region, SARS-CoV-2 RNA may not be detected or may be detected less predictably.
- False-positive results may arise from cross contamination during sample handling, preparation, nucleic acid extraction, PCR assay set-up or product handling.

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Symbol Descriptions

	Batch Code (Lot)
	Catalog Number
	Use by date
	Manufacturer
	Temperature limitation

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