Water SARS-CoV-2 RT-PCR Test

English Version

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

IDEXX

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English Version

Water SARS-CoV-2 RT-PCR Test

Intended Use

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

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

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

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 U.S. 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 on 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.

If the IDEXX Water Internal Control is added to the extraction lysis solution during nucleic acid purification, the Water SARS-CoV-2 RT-PCR test also provides an internal positive control. The Internal Control is used to verify successful purification of nucleic acids from each individual sample and to evaluate whether PCR amplification may have been inhibited by substances in that sample. Detection of both SARS-CoV-2 and the Internal Control occurs simultaneously in a single RT-PCR reaction, thereby providing a quality control result for each sample tested. The Internal Control is detected on the HEX (VIC) fluorescent channel. The Internal Control has been validated for use with the IDEXX Water SARS-CoV-2 RT-PCR Test and is not compatible with other RT-PCR tests for SARS-CoV-2.

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

<table>
<tr><th rowspan="2">Identification/
general information</th><th rowspan="2">Cap color</th><th>Quantity</th><th colspan="2">Storage</th><th rowspan="2">Freeze/thaw cycles</th></tr>
<tr><th>100 tests</th><th>At receipt</th><th>After reconstitution</th></tr>
<tr><td>SARS-CoV-2 Mix, dried</td><td>Red</td><td>1 x 1.0 mL</td><td>–25 to 8°C</td><td>–25 to –15°C</td><td>≤6</td></tr>
<tr><td colspan="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.</td></tr>
<tr><td>RNA Master Mix (RNA MMx)</td><td>Black</td><td>1 x 1.0 mL</td><td>–25 to –15°C
(Long-term)</td><td>N/A</td><td>≤6</td></tr>
<tr><td colspan="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.</td></tr>
</table>

Identification/ general information	Cap color	Quantity	Storage		Freeze/thaw cycles
		100 tests	At receipt	After reconstitution	
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	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

- DNase/RNase free molecular grade water
- Vortex mixer
- DNase/RNase free aerosol-resistant pipette tips
- DNase/RNase free microcentrifuge tubes
- Microcentrifuge capable of reaching 1500 – 3000 x RCF
- Pipettes (5–1000 μL); dedicated pipettes for preparation of PCR Mix
- Personal protective equipment (gloves, safety glasses, lab coat)
- 96- or 384-well format PCR plates and optical adhesive film/plate or suitable alternative.
- Real-time PCR instrument (Applied Biosystems® 7500, Applied Biosystems® 7500 Fast Dx, 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. Contact IDEXX Technical Support for guidance on use of this instrument.
- Centrifuge with rotor and adapters for multi-well plates (optional).
- Quantitative SARS-CoV-2 RNA containing the N1 and N2 target regions (for example, ATCC No. VR-3276SD, or suitable alternative).
- TE pH 8.0. A low EDTA formulation containing 0.1 mM EDTA and 10 mM Tris is recommended.

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 samples 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.
- Keep reagents and 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.

Reconstitution and Use of Components

Reconstitute the SARS-CoV-2 Mix and Positive Control by pipetting PCR Grade Water to the volume indicated on the component label. Incubate 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

Controls 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 batch is valid. The positive control should be included on each PCR run and should test positive for the SARS CoV-2 target on the FAM channel. The PCR Positive Control will also give a positive signal for the Internal Control on the HEX channel. 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 on the FAM channel and the Internal Control on the HEX channel.

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 (Material Number: 0505-0168), 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.
- 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.

Controls that are provided with the Water SARS-CoV-2 RT-PCR Test and are recommended but not required are listed below:

- Internal Control (IC): If the IDEXX Water Internal Control was included during nucleic acid purification, then a multiplexed internal positive control is available for each sample to demonstrate successful purification of nucleic acids and indicate the potential for PCR inhibition. During RT-PCR, the Internal Control is detected on a different fluorescence channel from SARS-CoV-2. When the Internal Control is used, the Extraction Negative Control will continue to provide a negative result for SARS-CoV-2 on the FAM channel (see below). The Extraction Negative Control will also provide a positive result for the Internal Control on the HEX channel. The HEX channel Ct from the Extraction Negative Control may also be used as a reference value (termed "IC^{Ref}") to assist with interpretation of Internal Control results (see sections below: "Batch Validity Criteria" and "Quantification of SARS-CoV-2"). For best results, the Extraction Negative Control should be included in the same extraction run as the unknown samples to be analyzed with the Internal Control.

Note: Alternate materials may be used in place of the recommendations above. For more information, please refer to Available and Compatible Controls for Quantifying SARS-CoV-2 in Wastewater with IDEXX Test Kits or contact IDEXX Technical Support.

The interpretation of control results described in the "Data Analysis" section below.

PCR Test Procedure

Prepare the PCR Mix

1. Mix the thawed RNA MMx by inversion or gentle vortex. **Note:** The RNA MMx is a viscous solution; always pipette it slowly.
2. 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. **Note:** 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 pipette tip containing MMx.
3. Gently vortex the solution to ensure the components are mixed well.
4. Load the PCR plate within 20 minutes or store at 2 to 8°C for up to 4 hours. **Note:** it is recommended to use a cold block if the plate loading time may exceed 20 minutes.
   The PCR Mix can be stored at –25 to –15°C for up to 2 weeks. Protect from light.

Combine PCR Mix and Sample

5. Slowly pipette 20 μL of the PCR Mix into the required wells of the multi-well plate.
6. For each wastewater sample, add 5 μL of purified RNA to the appropriate well. The final reaction volume is 25 μL.
7. 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.
8. Cover the plate. Briefly spin, if necessary, to settle contents and remove air bubbles.

PCR Instrument set up

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

Settings for Reporter and Quencher

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

***Note:** Some RT-PCR systems do not require BHQ to be specified in instrument settings.

****Note:** The use of the HEX (VIC) channel is only required when the Water Internal Control is used. If the Internal Control is not used, the HEX (VIC) channel does not need to be supported by the RT-PCR system.

*****Note:** The fluorescence characteristics of HEX and VIC are very similar and may be used interchangeably in instrument settings.

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.

Data Analysis

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 and accurate quantitative results, analysis for SARS CoV-2 target and the optional Internal Control 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 data analysis.

Batch Validity Criteria

The following control results must be obtained for each batch of samples processed together during extraction and PCR for the results to be deemed valid. If the batch controls are not valid, the sample results cannot be interpreted, are not valid, and must be repeated.

Control	SARS-CoV-2		Internal Control*	
	FAM Ct Value	FAM Result	HEX Ct Value	HEX Result
PCR Positive Control	<40	Positive	<36	Positive
PCR Negative Control	No Signal	Negative	No Signal**	Negative
Extraction Positive Control*	<40	Positive	<36	Positive
Extraction Negative Control*	No Signal	Negative	<36***	Positive

*If used, expected results are indicated. If the Internal Control is not used, only the FAM results for each control are used to determine batch validity.

**The PCR Negative Control is expected to give negative results for both the SARS-CoV-2 and Internal Control targets. Trace environmental human nucleic acid contamination can result in late HEX Ct values (>36). This is because the internal control target is a human nucleic acid sequence and trace amounts can be present in the laboratory environment. Trace levels of contamination observed in the PCR Negative Control (Ct>36) will not affect interpretation of the Internal Control results and does not invalidate the PCR run.

***The HEX Ct value obtained from the Extraction Negative Control may be used as a reference value (IC^{Ref}) to assist with interpretation of Internal Control results for unknown samples (see "Quantification of SARS-CoV-2 section" below).

Interpretation of Results With Optional Internal Control

Sample Validity: Assessment of wastewater sample results should be performed only after the positive and negative batch controls for both PCR and extraction have been examined and deemed valid. Positive samples should display a characteristic amplification curve on both the FAM and HEX channels. The validity of each sample is judged according to its Internal Control result as shown in the table below.

Results Interpretation **with** optional Internal Control

Wastewater Sample Result	SARS-CoV-2 FAM Ct Value	Internal Control HEX Ct Value	Interpretation
SARS-CoV-2 Negative	>40 or No Ct value	≤ 36	SARS-CoV-2 RNA is absent or below the limit of detection. Detection of the Internal Control indicates a valid test.
SARS-CoV-2 Positive	≤ 40	≤ 36	SARS-CoV-2 RNA is present and above the limit of detection. Detection of the Internal Control indicates a valid test. Result may be used for quantification.
Invalid Sample*	Any Ct value	> 36 or No Ct value	Absence of Internal Control signal indicates a problem and the sample should be retested.

*An invalid sample can indicate failed sample addition, failed extraction and/or PCR, or contamination. To test for PCR inhibition, it is recommended to dilute the purified nucleic acid, for example using a five-fold dilution into PCR-grade water, and test the dilution alongside the original undiluted sample. A proportionally higher result from the diluted sample, after accounting for the dilution factor, indicates the presence of inhibitors. If the retest is still not valid a new extraction is recommended.

Interpretation of Results Without Optional Internal Control

Sample Validity: Assessment of wastewater sample results should be performed only after the positive and negative batch controls for both PCR and extraction have been examined and deemed valid. Each sample is considered valid when analyzed on a plate demonstrating the expected control results on the FAM channel as described in the Batch Validity Criteria. Positive samples should display a characteristic amplification curve on the FAM channel. Results from the HEX channel are not used.

Results Interpretation **without** optional Internal Control

Wastewater Sample Result	SARS-CoV-2 FAM Ct Value	Interpretation
SARS-CoV-2 Negative	>40 or No Ct value	SARS-CoV-2 RNA is absent or below the limit of detection.
SARS-CoV-2 Positive	≤ 40	SARS-CoV-2 RNA is present and above the limit of detection. Result may be used for quantification.

Quantification of SARS-CoV-2

General Considerations

Absolute quantification of SARS-CoV-2 in wastewater can be performed using a standard curve produced with an appropriate nucleic-acid reference material. The standard curve is created by diluting the reference material in a series of known concentrations and then measuring a SARS-CoV-2 Ct value by RT-qPCR (FAM channel) for each concentration. The calculated relationship can then be used to convert a Ct value measured for an unknown sample into a concentration of RNA expressed as genome copy number per volume.

Sample Considerations

For the most accurate quantification results, the optional Internal Control may be used to reveal negative factors, for example a problem during extraction or the presence of PCR inhibitors, that may affect quantification accuracy. To perform the comparison, the reference Internal Control HEX Ct value (IC^{Ref}; see "Batch Validity Criteria" above, in the "Data Analysis" section) obtained from the Extraction Negative Control is subtracted from the Internal Control HEX Ct value obtained for each sample. Typically, a difference of 2 cycles or less indicates no significant issues and the SARS-CoV-2 result may be used for quantification. An Internal Control difference greater than 2 cycles indicates that sample result accuracy may be significantly affected. In this case it is recommended to dilute and retest the sample as described above under "Interpretation of Results **with** Optional Internal Control".

Preparing a Standard Curve

A variety of reference materials and dilution approaches may be used to produce a standard curve.

For best results, the following is recommended:

- A standard curve should be determined within each RT-PCR run to avoid inaccuracies resulting from changes in procedures, reagents and equipment that may vary from run-to-run.
- Use a reference material quantified by an appropriate analytical method.
- Use consistent methods for preparation of standard curve dilutions and analysis of results.
- Verify results meet recommended quality acceptance criteria (see below).
- Use RNA reference material to control for both the reverse transcription and polymerase amplification steps of the reaction.
  - For example, Quantitative Synthetic SARS-CoV-2 RNA from ATCC (part number VR-3276SD), is verified to be compatible with the IDEXX Water SARS-CoV-2 RT-PCR test. This product provides a single-stranded RNA containing SARS-CoV-2 N1 and N2 sequences in a ready-to-use solution, and a Certificate of Analysis is available with a measured target concentration.
  - Note that DNA reference materials may also be used, however they may not provide the same level of accuracy. If DNA is used, it is recommended to use a linear molecule.
- Prepare dilution levels that encompass the full range of Ct values that will be obtained from wastewater samples and the assay limit of detection (approximately 1 RNA copy per reaction).
- Serial ten-fold dilution levels are recommended. For higher accuracy, each dilution level may be tested in duplicate or triplicate.

Note: Nucleic-acid solutions, and especially RNA, are susceptible to degradation. The following approaches are recommended to increase the stability of reference materials:

- Prepare single-use aliquots of nucleic-acid stock solutions. It is recommended to store RNA solutions frozen, at or below -70°C.
- Freeze and thaw nucleic-acid solutions as few times as possible.
- Keep nucleic-acid solutions and associated materials cold during use.
- Prepare dilutions using TE pH 8.0 (10 mM Tris, 0.1mM EDTA). A low 0.1 mM concentration of EDTA is recommended.

Expected Results

For the series of standard curve dilutions, a plot of the observed Ct value (y-axis) against the log of the nucleic-acid copy number (x-axis) should show a linear relationship. The equation for this relationship is determined through linear regression. Refer to specific instrument's user manual for more information on calculating the formula for a standard curve from Ct results. The quality of the resulting standard curve should be judged according to the following metrics:

- The difference in Ct value between pairs of dilutions of equal magnitude should be consistent. For example, dilutions separated by a 10-fold difference in nucleic-acid copy number should give Ct values separated by approximately 3.32 cycles.
- The regression line should intersect the data points for all dilutions, and the calculated R^2 statistic should be >0.990. If replicates were tested for each dilution, there should be close agreement among the replicate Ct values.
- The slope of the regression line should be between -3.6 and -3.1.

Calculations

To calculate the number of copies of SARS-CoV-2 virus detected in the PCR reaction, the following formula is used:

$$\text{genome copy number} = 10^{\frac{Ct-b}{m}}$$

where:

- Ct = threshold cycle value measured on FAM channel for the unknown sample
- b = y-intercept of the standard curve
- m = slope of the standard curve

To determine the concentration of SARS-CoV-2 in an unknown sample, the genome copy number calculated above is expressed relative to the proportion of original wastewater sample volume that was tested in the PCR reaction. This can be determined using the formula below and the respective volumes used at each step in the sample concentration and purification process. An example calculation is given for when the recommended PEG concentration procedure is used, and nucleic acids are purified from the wastewater concentrate using the IDEXX DNA/RNA Magnetic Bead kit:

virus genome copies per L

$$= \text{genome copy number} * \left(\frac{RNA^{total}}{RNA^{PCR}}\right) * \left(\frac{concentrate^{total}}{concentrate^{extracted}}\right) * \left(\frac{1000\text{ mL}}{wastewater}\right)$$

$$= \text{genome copy number} * \left(\frac{0.1\text{ mL}}{0.005\text{ mL}}\right) * \left(\frac{0.4\text{ mL}}{0.2\text{ mL}}\right) * \left(\frac{1000\text{ mL}}{105\text{ mL}}\right)$$

$$= \text{genome copy number}*381$$

where:

- RNA^{total} = total volume of RNA eluted from magnetic-bead extraction (0.1 mL)
- RNA^{PCR} = volume of purified RNA tested in PCR (0.005 mL)
- $concentrate^{total}$ = total volume of wastewater concentrate (0.4 mL)
- $concentrate^{extracted}$ = volume of wastewater concentrate from which RNA was extracted (0.2 mL)
- wastewater = volume of original wastewater sample processed (105 mL)

Limitations

- Performance of the Water SARS-CoV-2 RT-PCR Test has only been established in wastewater using primary effluent and raw influent 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 IDEXX procedures. 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

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

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