



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

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Overview of IDEXX-Validated SARS-CoV-2 Test Process

IDEXX has validated an end-to-end protocol for detecting SARS-CoV-2 in wastewater and offers a combination of materials and procedures for each required step in the process. The complete recommended procedure, summarized in Table 1, incorporates important laboratory controls for wastewater processing, including: (1) Pepper Mild Mottle Virus (PMMoV) as a Matrix Recovery Control¹, (2) an Internal Control to monitor reaction validity and the potential for RT-qPCR inhibition, and (3) PMMoV as a Human Fecal Control. A summary of all available and compatible controls, including additional controls for RT-qPCR and nucleic acid extraction process steps, is provided in Table 2. For more information on recommended laboratory controls, please refer to guidance from the EU Commission².

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 described below, changes can be made to meet requirements in different laboratories. 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 procedure indicated below. Similarly, the IDEXX Water SARS-CoV-2 RT-PCR Test can be used with nucleic acids prepared by other extraction methods. Because each component of the test can be used independently, procedures for each component are detailed in separate documents, as described in the table below.

¹ An alternate procedure is also available for use of exogenous Bovine Respiratory Syncytial Virus (BRSV) as the Matrix Recovery Control. For more information, please refer to the [IDEXX Water Matrix and Fecal Control Kit Product Insert](#).

² https://ec.europa.eu/environment/pdf/water/recommendation_covid19_monitoring_wastewaters.pdf

Table 1. IDEXX Validated Procedures for Quantification of SARS-CoV-2 in Wastewater

Note: Process Steps below match the protocol steps in the Matrix and Fecal Control Kit product insert

Process Step	Procedure	Description	Protocol Document
2.A. Sample processing	1. Reserve unconcentrated portion of each wastewater sample	A portion of each wastewater sample is processed without concentration to measure the initial concentration of PMMoV in each sample	IDEXX Water Matrix and Fecal Control Kit Product Insert
	2. Sample concentration	Concentration of viral particles and nucleic acids present in wastewater to improve limit of detection of the test.	Example Concentration Protocol for Wastewater Surveillance for SARS-CoV-2 by PEG Precipitation
	3. Internal Control	A synthetic nucleic acid is added to verify successful performance of nucleic acid purification and RT-qPCR steps.	IDEXX Water Internal Control Product Insert
	4. Nucleic-acid purification	Total nucleic acids are extracted and purified from both the concentrated and unconcentrated wastewater samples.	IDEXX Water DNA/RNA Magnetic Bead Kit Product Insert
2.B. RT-qPCR and Result Interpretation	1. Measurement of PMMoV	Used to quantify the amount of PMMoV RNA in the extracted sample via amplification of specific genetic sequences	IDEXX Water Matrix and Fecal Control Kit Product Insert
	2. Multiplex measurement of SARS-CoV-2 and Internal Control	Used to quantify the amount of SARS-CoV-2 RNA in the extracted sample via amplification of specific genetic sequences. Detection of Internal Control in each sample determines result validity and possibility of PCR inhibition.	IDEXX Water SARS-CoV-2 RT-PCR Test Product Insert
2.C. Calculations	1. Calculation of PMMoV Matrix Recovery Efficiency	The proportion of virus recovered during sample processing, including concentration, is determined for process monitoring.	IDEXX Water Matrix and Fecal Control Kit Product Insert
	2. Quantification of SARS-CoV-2	The concentration of SARS-CoV-2 RNA is calculated using a standard curve produced from an appropriate quantitative reference material.	IDEXX Water SARS-CoV-2 RT-PCR Test Product Insert
	3. Quantification of PMMoV	The concentration of PMMoV RNA is calculated using a standard curve produced from an appropriate quantitative reference material.	IDEXX Water Matrix and Fecal Control Kit Product Insert*

* A supplemental protocol is also available that provides information on preparing custom synthetic nucleic acid for use as PMMoV reference material.

Table 2. Available and Compatible Controls for Quantifying SARS-CoV-2 in Wastewater with IDEXX Test Kits

Control	Purpose	Validated and Provided Materials	Validated Materials	Instructions for use
PCR positive	Demonstrate valid PCR reagent performance	Positive Control (PC) (included in 99-0015314 and 98-0016622-00)		IDEXX Water SARS-CoV-2 RT-PCR Test product insert
PCR negative	Detect nucleic acid template contamination in reagents or materials	PCR Grade Water (included in 99-0015314 and 98-0016622-00)		IDEXX Water Matrix and Fecal Control Kit Product Insert
Extraction positive control	Demonstrate valid extraction reagent performance, i.e. recovery of RNA from an enveloped viral particle		One of the following options can be used: 1. Endogenous PMMoV in wastewater 2. BRSV Matrix Recovery Control 3. Seracare AccuPlex™ SARS-CoV-2 Verification Panel (Material Number: 0505-0168)	IDEXX Water DNA/RNA Magnetic Bead Kit Product Insert IDEXX Water SARS-CoV-2 RT-PCR Test product insert IDEXX Water Matrix and Fecal Control Kit Product Insert
Extraction negative control	Detect nucleic acid template contamination in extraction reagents or materials (discriminate from PCR reagents and materials)		PCR Grade Water	
Internal Control	<u>For each sample tested:</u> 1. Demonstrate valid extraction and PCR reactions. 2. Indicate potential for PCR inhibition.	Internal Control (IC) (included in 99-0015314)		IDEXX Water Internal Control Product Insert
SARS-CoV-2 Quantitative Reference Material	Provide standard curve for quantification of SARS-CoV-2 RNA		ATCC Quantitative Synthetic SARS-CoV-2 RNA (Material Number VR-3276SD)	IDEXX Water SARS-CoV-2 RT-PCR Test product insert
PMMoV Quantitative Reference Material	Provide standard curve for quantification of PMMoV RNA		Custom synthetic nucleic acid *	IDEXX Water Matrix and Fecal Control Kit Product Insert
Matrix Recovery Control	Evaluate viral recovery during sample processing/concentration	Matrix and Fecal Control Kit (98-0016622-00)	BRSV vaccine product	IDEXX Water Matrix and Fecal Control Kit Product Insert
Human Fecal Control	Measure the relative amount of human feces in a sample	Matrix and Fecal Control Kit (98-0016622-00)		IDEXX Water Matrix and Fecal Control Kit Product Insert

* A supplemental protocol is also available that provides information on preparing custom synthetic nucleic acid for use as PMMoV reference material. Contact IDEXX Technical Support for more information.

Additional materials not listed in the table may be compatible with the IDEXX Water SARS-CoV-2 Test and Water DNA/RNA Magnetic Bead Kit. Contact IDEXX Technical Support for details.

Supplemental Information

The following documents are available that provide additional information to support the central test procedures described above. Please contact IDEXX Technical Support for more information.

Table 3. Supplemental Information

Document	Description
Preparation and dilution of synthetic PMMoV Reference Material for quantification	Provides information on preparing a custom synthetic nucleic acid for use as PMMoV reference material, as well as an example dilution series for a PMMoV standard curve.
Dilution of SARS-CoV-2 Reference Material for Quantification	Provides an example dilution series that can be used for SARS-CoV-2 specifically.

Positive Control Version Summary and Test Compatibility

With the release of the new IDEXX Water Matrix and Fecal Control Kit, a new Positive Control (PC) is available that is compatible with both the IDEXX Water Matrix and Fecal Control Kit and the IDEXX Water SARS-CoV-2 RT-PCR Test kit. The new PC version 2.0 can be used as the PC sample for RT-PCR reactions performed with both IDEXX kits. The following table summarizes the synthetic targets provided with each version of the PC. Starting with Version 2.0, the PC tube label indicates both the version and part number.

Table 4. Positive Control Compatibility

Positive Control Version	Part Number	Provided Targets	Description
1.0	44-56623-00	SARS-CoV-2 N1 Water Internal Control	Original release for use with the IDEXX Water SARS-CoV-2 RT-PCR Test.
2.0	44-56624-00	Version 1.0 plus: BRSV PMMoV	Update for compatibility with the Water Matrix and Fecal Control Kit.

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