VIR*Seek* SARS-CoV-2 Mplex I Wastewater

TEST KIT FOR SARS-COV-2 DETECTION
(N1/N2-GENE TARGETS) USING
QUALITATIVE REAL-TIME RT-PCR IN WASTEWATER

Cat. No. 5728401801

For 48 RT-PCR reactions

VIR*Seek*

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1 INTRODUCTION

Wastewater surveillance for SARS-CoV-2 virus has been postulated as an efficient tool to help identify the risk of coronavirus disease (COVID-19) outbreaks. This tool can contribute to public health surveillance not only on a community level (wastewater treatment plants) but also on institutional level using collection points such as at schools or nursing homes.

As a consequence the European Commission is driving a harmonised approach for monitoring SARS-CoV-2 and its variants in wastewater, as a cost-effective, rapid and reliable source of information[1].

In detail, setting a sampling frequency and geographical coverage adapted to the epidemiological situation is recommended. Samples should be taken at the inlets to wastewater treatment plants or where relevant upstream at the wastewater collecting networks. Specific quality standards are given for real-time polymerase chain reaction including the inclusion of adequate controls to assess efficiency of the concentration/extraction steps as well as the absence of inhibition.

The VIR*Seek* SARS-CoV-2 Mplex I Wastewater kit detects genes to specifically identify SARS-CoV-2 together with primer/probes sets to evaluate process parameters such as the nucleic acid extraction efficiency and a water quality indicator.

The primer/probe combination of this real-time RT-PCR system is highly specific for SARS-CoV-2 and detects two targets on the N-gene (nucleocapsid) sequence, N1 and N2 in two distinct channels (N1 in HEX™; N2 in FAM™).

Furthermore, the VIR*Seek* SARS-CoV-2 Mplex I Wastewater kit provides an MS2 phage as an optional extraction control that allows monitoring the extraction procedure as well as potential PCR inhibition. The dedicated primer/probe combination of this real-time RT-PCR system is detected in the Cy5® channel. A water specific quality indicator can also be assessed by the VIR*Seek* SARS-CoV-2 Mplex I Wastewater kit, as PMMoV can be monitored in the ROX™ channel.

The VIR*Seek* SARS-CoV-2 Mplex I Wastewater kit is validated for use with the Agilent AriaMx™, Bio-Rad CFX96 Touch™ and CFX96 Touch™ Deep Well.

The kit is intended to be used by analytical laboratories for SARS-CoV-2 analysis in wastewater samples as part of quality control/quality assurance testing.

The kit is not intended for clinical diagnostics and should therefore be regarded as “For Research Use Only”.

1.1 Test Principle

In order to be able to sensitively detect SARS-CoV-2 virus in wastewater samples, a respective sample pre-treatment procedure needs to be implemented prior to isolation of the RNA and subsequent real-time RT-PCR analysis. This concentration step may contain a PEG-precipitation step as descriped in the 'Wastewater Treatment Quick Reference Guide' from Eurofins Technologies.

After the concentration of viral particles from wastewater samples, a subsequent isolation of the RNA from the viral particles is required, using an automated setup based on magnetic beads such as the VIR*Seek* RNA*Extractor* AE1 (see section 1.4) is recommended. The purified RNA is subsequently analysed using the VIR*Seek* SARS-CoV-2 Mplex I Wastewater.

The first step of a real-time RT-PCR is the reverse transcription (RT) of viral RNA to cDNA, which can then be amplified by real-time PCR. By means of specific primer nucleotide sequences of the SARS-CoV-2 N1 and N2 target regions are amplified during PCR from isolated and reverse-transcribed total RNA. Primers do not cross-react with transcribed RNA (cDNA) from other common foodborne virus species, such as norovirus genogroup I & II, hepatitis A & E virus, rotavirus, adenovirus or astrovirus.

The extraction control (EC) contains MS2 phage particles, which can be added to the sample lysis step during nucleic acid extraction. Detection of the respective target sequence in the subsequent RT-PCR confirms the successful extraction of viral particles from the sample, as well as the absence of inhibition in the PCR. Finally, the detection of PMMoV helps to evaluate the success of the viral concentration from wastewater as a sample matrix and serves as a water specific quality indicator. As a normalisation control PMMoV also allows to assess the SARS-CoV-2 incidence in the population.

1.2 Components of the Kit

Please pay attention to the storage condition and the maximum number of freeze/thaw cycles.

- **1x** **OligoMix VIRSeek SARS-CoV-2 Mplex I Wastewater**, vials with yellow - white caps, contains primers / probes for SARS-CoV-2 N-Genes / EC (MS2 phage) / PMMoV, 530 µL,store at -20 °C ± 2 °C, do not freeze / thaw more than 3 times.
- **1x** **BasicMix VIRSeek SARS-CoV-2 Mplex I Wastewater**, vials with white caps, 265 µL, store at -20 °C ± 2 °C, do not freeze / thaw more than 3 times.
- **1x** **Positive Control SARS-CoV-2 Mplex I Wastewater**, vial with red cap, 100 µL, store at -20 °C ± 2°C, do not freeze / thaw more than 3 times.
- **1x** **Negative Control**, vial with transparent cap, 500 µL, store at -20 °C ± 2 °C.
- **1x** **Extraction Control**, vial with yellow cap, contains MS2 phage for lysis/extraction and amplification control, 1000 µL, store at -20 °C ± 2 °C, do not freeze / thaw more than 3 times.

1.3 Additional Equipment, Consumables and Reagents Required

Equipment:

- 1x **Stepper pipette (1 mL),** (e.g. HandyStep® S (Brand®))
- 1x **Single channel pipette (1 mL, 100 µL),** (e.g. Transferpette® S 100 - 1000 µL (Brand®))
- 1x **Single channel pipette (100 µL, 10 µL),** (e.g. Transferpette® S 10 - 100 µL (Brand®))
- 1x **Single channel pipette (up to 10 µL),** (e.g. Transferpette® S, 0.5 - 10 µL (Brand®))
- 1x **Cooling block** for 1.5 mL tubes, (e.g. Biozym).
- 1x **96 well cooling block,** (e.g. Blue cooling block 96 well)
- 1x **Vortex mixer,** (e.g. VWR Collection)

Centrifuge for microtiter-plates / or -strips - depending on throughput:

- Capacity of 2x 8-well strips: (e.g. Carl Roth GmbH, Rotilabo® centrifuge with butterfly rotor)
- Capacity of 4x 8-well strips: (e.g. Mini Centrifuge IKA Mini G or VWR, MiniStar silverline).
- Capacity of two times 12x 8-well strips: (e.g. Benchmark Scientific, PlateFuge™ microplate microcentrifuge)

Real-time PCR Thermocycler:

- Agilent AriaMx™ with FAM™, HEX™, ROX™ and Cy5® filter set (AriaMx Software, up to version 1.5).
- Bio-Rad CFX96 Touch™ (CFX Manager™ Software / CFX Maestro™ Software).
- Bio-Rad CFX96 Touch™ Deep Well (CFX Manager™ Software / CFX Maestro™ Software).

Consumables:

- **RNase-free water** (molecular biology grade).
- **Nucleic acid / Nuclease-free pipette tips** with filters, need to be compatible with pipettes used.
- **RNase-free reaction tubes,** 1.5 mL (e.g. DNA LoBind Tubes, Eppendorf).
- **PCR plates or strips**, compatible with thermocycler used.
- **Optical 8-caps strip or equivalent seals** (compatible with thermocycler used).
- **RNase decontaminating reagent** (e.g. RNase AWAY® Carl Roth GmbH).
- **DNA degrading agent** (e.g. Roti® Nucleic Acid-free, Carl Roth GmbH).
- **Gloves,** powder free.

1.4 Complete Portfolio of the VIR*Seek* Solution

- **VIR*Type* SARS-COV-2 Mplex (N501Y, A570D)**, cat. no. 5728401301
  - RT-qPCR kit with 48 reactions for the detection of the N501Y and A570D single point mutations SARS-CoV-2 in food and environmental surface samples as well as in wastewater.
- **VIR*Type* SARS-COV-2 (A570D)**, cat. no. 5728401401
  - RT-qPCR kit with 48 reactions for the detection of the A570D single point mutations SARS-CoV-2 in food and environmental surface samples as well as in wastewater.
- **VIR*Type* SARS-COV-2 (L452R)**, cat. no. 5728401601
  - RT-qPCR kit with 48 reactions for the detection of the L452R single point mutations SARS-CoV-2 in food and environmental surface samples as well as in wastewater.
- **VIR*Type* SARS-COV-2 (E484K)**, cat. no. 5728401701
  - RT-qPCR kit with 48 reactions for the detection of the E484K single point mutations SARS-CoV-2 in food and environmental surface samples as well as in wastewater.

- **VIR*Seek* Murine Norovirus (MNV) Process Control**, cat. no. 5728200401
  - Real-time RT-PCR kit with 48 reactions for rapid detection of Murine Norovirus (MNV)/ Process Control Virus.
- **VIR*Seek* RNA*Extractor***, cat. no. 5524400101
  - Kit for extraction of viral RNA via silica-coated magnetic beads.
- **VIR*Seek* RNA*Extractor* AE1**, cat. no. 5524400801
  - Kit for 96 automated viral RNA isolations from environmental samples including wastewater. Validated for Thermo Fisher Scientific™ KingFisher™ Flex and GSD Auto-Pure96.

2 HOW TO USE THIS PRODUCTImportant Notes

- Store all reagents as indicated in section 1.2.
- During PCR set-up:

Keep all reagents on ice ice/cooling block.

Perform all pipetting steps on ice/cooling block.

- Do not use the reagents beyond the expiration dates printed on the labels.
- Never store kit components in the vicinity of samples or post-PCR products.
- Ideally perform PCR in a UV PCR cabinet.
- Do not mix kit components of different lot numbers.

2.2 General and Safety Precautions

- All samples should be handled with caution, ideally in a bio safety cabinet class II, as they are potentially infectious.
- Viruses should not be handled by pregnant women, children, elderly and immuno-compromised individuals due to the high infection risk and potentially fatal health consequences for this group, in particular for the unborn child in case of pregnant women.
- The VIR*Seek* kit contains glycerol and propane-1,2-diol which may cause mild skin irritation. For more information, please refer to the VIR*Seek* kit safety information.

2.3 Working Guidelines

- Comply with Good Laboratory Practice (refer to EN ISO 7218 standard).
- Refer to EN ISO 22174:2005 for the general requirements for the *in-vitro* amplification of nucleic acid sequences.
- Perform cleaning protocol (outlined in section 2.5).
- Use DNA-/RNA-, nuclease-free and sterile lab ware.
- Wear gloves and change frequently.

2.4 RNA Handling – Specific Working Guidelines

It is important to create and maintain an RNase-free environment when working with RNA. RNases are very thermostable enzymes degrading RNA – even in small quantities. Laboratory personnel are the main source for RNase contamination as RNases are expressed in human keratinocytes and are present on skin and hairs.

- Separate the different procedures spatially.
  Ideally use separate rooms for sample preparation and PCR setup laid-out to maintain a strict "one-way-system", thus avoiding cross-contamination in the work stream.
  At least dedicate different areas, equipment and consumables for each procedure.
- Establish a working area, designated as "RNase-free", in which only RNA work is performed. If the RNase-free working area is inside a lab with non-RNase-free working areas, clearly indicate RNase-free parts, e.g. using colour tape.
- Use dedicated RNase-free lab equipment (e.g. pipettes) for RNA-related work. Glassware has to be cleaned and decontaminated before use. For decontamination we recommend baking glassware at >200 °C for ≥4 hours.
- Only use RNase-free filter tips and consumables which are certified to be RNase-free.
- Control high risk areas for DNA / amplicon contamination on a regular basis (swabs / PCR analysis).
- Clean the real-time RT-PCR working area as described in the cleaning protocol (see section 2.5).
- Wear disposable gloves (latex or vinyl gloves) to prevent contamination with RNases which are present on human skin. Change gloves frequently during the procedure and / or after touching skin, hair, common surfaces etc.
- Wear a lab coat to prevent contamination from clothes.
- Always thaw RNA on a cooling block/ on ice and store RNA at -20 °C or below.
- Handle real-time RT-PCR enzyme mix as briefly as possible at 0 °C or above. Do not mix reagents from different kits and do not mix reagents from different batches. Return all reagents to -20 °C after usage.
- Store VIR*Seek* kit components for real-time RT-PCR in dedicated areas, and separate from sample storage.
- Only open one tube at a time and always change pipette tips between liquid transfers to avoid cross-contamination.

2.5 Cleaning Protocol

Before commencing work and after completing the work, ensure that the real-time RT-PCR working area is cleaned as follows:

Cleaning steps	Cleaning protocol
1.	Decontaminate surfaces with Roti® Nucleic Acid-free* or 1 % HCl (hypochlorite acid) to remove DNA / RNA contamination.
2.	Clean the work surfaces and non-disposable laboratory equipment (pipettes, shaker, thermo shaker etc.) with an RNase decontaminating solution* (e.g. RNase AWAY®, Carl Roth, cat. no. A998) to remove RNase contaminations.

** Follow the manufacturer's instructions.*

2.6 Waste Disposal

Dispose of any waste which is potentially contaminated with a pathogenic virus according to your internal and local regulations.

For disposal of reagents and chemicals please refer to safety information.

2.7 Before you Begin

Store the cooling block for real-Time RT-PCR at -20 °C overnight.

For RNA extraction use suitable RNA extraction kits, for optimal performance we recommend to use Eurofins GeneScan Technologies VIR*Seek* RNA*Extractor* AE1 kit (see section 1.4).

The extraction control (EC) is used for monitoring RNA extraction procedure and/or any potential PCR inhibition. Therefore, 20 µL of the EC have to be added prior to the nucleic acid extraction procedure into the lysis buffer (E^+). A negative extraction control of MS2 in pure lysis buffer is highly recommended (E^-)..

2.8 Real-time RT-PCR

2.8.1 Special Precautions during Real-time RT-PCR Analysis

RT-PCR includes the reverse transcription (RT) of RNA into cDNA. RNA is a molecule which is particularly at risk of degradation due to abundant free RNases in the environment. Prior to RT, special emphasis has to be put on RNase-free environments (see section 2.4).

PCR is an exponential reaction. Therefore, after RT and amplification, the detection of single DNA targets is possible. The extreme sensitivity requires special precautions for handling and equipment. After a successful amplification, several billion amplicons are present in the reaction tube. Each of them might lead to a false positive result when contaminating sample material, i.e. by spreading as aerosols.

2.8.2 PCR Setup

Keep all components (OligoMix and BasicMix) permanently on ice/cooling block during PCR setup.
Prepare final reaction mix fresh each time and immediately before starting the real-time RT-PCR run.

Calculate required number of reactions and pipette all components (OligoMix and BasicMix) together and mix for the final reaction mix. The final real-time RT-PCR reaction mix is prepared with an additional 10 % volume.

Frequent freezing and thawing might cause inactivation of the reagents. Do not freeze / thaw kit components more than three times.

Components of final reaction mix	**Amount per reaction**	**e.g. for 10 RT-PCR reactions (+ 10 %)**
BasicMix	5 µL	55 µL
OligoMix	10 µL	110 µL
Total volume	15 µL	165 µL

Before starting the hands-on working steps make sure you have switched on the computer, the PCR instrument and ensure the sample layout for the PCR plate is suitably documented and programmed (see below “Plate Setup”).

1. Place PCR plate or strips into the 96-well cooling block which has been cooled at -20 °C.
2. Add 15 µL of final reaction mix to each test well.
3. Add 5 µL Positive Control SARS-CoV-2 Mplex I wastewater (C+), Negative Control (C-), negative extraction control sample (E-) and extraction control sample (E+) to the corresponding wells.
4. Add 5 µL of each sample to the corresponding reaction well of the PCR plate.
5. Use optical caps or foil to seal the PCR plate / strips.
6. **Option 1**: Brief vortexing (10-30 sec) and spin-down of PCR strips/ plate

   Option 2: Manual shaking (5x) and spin-down of PCR strips/plate
7. Transfer the PCR plate / strips in cooled condition to the real-time RT-PCR instrument and start the run according to the thermocycler’s instructions.
8. Store samples at -20 °C or below in case PCR needs to be repeated.

Samples and Controls for VIR*Seek* SARS-CoV-2 Mplex I Wastewater Assay

Designation	Volume of reaction mix	Addition of
Test samples	15 µL	5 µL of sample
Positive control (C^+)	15 µL	5 µL of Positive Control SARS-CoV-2 Mplex I wastewater
Negative control (C^-)	15 µL	5 µL of Negative Control
Negative extraction control (E^-)	15 µL	5 µL of negative extraction control sample
Extraction control (E^+)	15 µL	5 µL of extraction control sample

Plate Setup

The following PCR plate setup is recommended, if samples are analysed for SARS-CoV-2

	1	2	3	4	5	6	7	8	9	10	11	12
A	C^+	S1-1										
B	C^-	S1-2										
C	E^-	Sn-1										
D	E^+	Sn-2										
E												
F												
G												
H												

- C^+: positive control (of the target of interest)
- C^-: negative control
- E^-: negative extraction control
- E^+: extraction control
- S1-1 – Sn-2: test samples in duplicates

Thermal Profile for AriaMx™ and Bio-Rad CFX96 Touch™

1 HOLD	**1 HOLD**	**40 CYCLES**	
Reverse transcription	Enzyme activation & Reverse transcriptase inactivation	Denaturation	Annealing & Extension
10 min at 50 °C	3 min at 95 °C	10 sec at 95 °C	30 sec at 60 °C
No data collection	No data collection	No data collection	Data collection

For Bio-Rad CFX96 Touch™ Standard and Deep Well use default ramp rate.

Probe / Detection System

VIR*Seek* SARS-CoV-2 Mplex I wastewater	**Fluorophore (Dye)**
SARS-CoV-2 N1	HEX™
SARS-CoV-2 N2	FAM™
Extraction Control (EC)	Cy5®
Normalisation control (PMMoV)	ROX™

3 DATA INTERPRETATION

Data is analysed by using the appropriate software provided by the cycler manufacturer or the FastFinder automated PCR analysis software.
For evaluation with the cycler software, we recommend the following settings:

Real-time RT-PCR Thermocycler	Threshold	Baseline
Agilent AriaMx™	Auto[1)]	Adaptive
Bio-Rad CFX96 Touch™	Auto (Check visually)	Baseline Subtracted Curve Fit[2)]
Bio-Rad CFX96 Touch™ Deep Well		Baseline Subtracted Curve Fit[2)]

[1)] *If appropriate, auto calculated threshold with default background based threshold settings can be used: Cycle range: 5 thru 9; Sigma multiplier: 10.*

[2)] *Always apply fluorescence drift correction*

- If the threshold is set incorrectly in automatic mode, adjust it manually.
- For orientation the amplification curve of the positive control should be used.
- The threshold should be set at the beginning of the exponential phase of this curve.

3.1 Export of Raw Data

For raw data export please follow the instruction in the corresponding cycler analysis software.

3.2 Evaluation of Results

3.2.1 Threshold setting

The interpretation of real-time PCR runs depends on automated algorithms that analyse and interpret the measured fluorescence data.

These algorithms are thoroughly validated by the manufacturers of the PCR cyclers and the respective evaluation software and provide reliable results for the vast majority of analyses.

In rare cases, when the automated threshold calculation of the cycler software fails, manual adjustments are reasonable and justified in order to get a correct interpretation of the measured data (e.g. automatic threshold settings might calculate a threshold which is not set in the exponential phase of the amplification curves).

3.2.2 Examples of threshold settings for Agilent AriaMx™ and Bio-Rad CFX96 Touch™:

In both software platforms the threshold can be adjusted via drag & drop if the automatic threshold calculation fails.

In case of failure of the automatic threshold settings, it is possible to review the curves and set the threshold within the exponential phase (phase 2; Figure 1 and Figure 2) of the amplification curve (signal intensity doubling in each cycle) before it gets into a phase with steady linear increase of the signal intensity (phase 3; Figure 1 and Figure 2).

Note: The adjustment is described for the linear view. For AriaMx™ make sure Graph Type “Linear” is selected. For Bio-Rad CFX96 Touch™ ensure that the box “Log Scale” is unchecked.

Agilent AriaMx™ software:

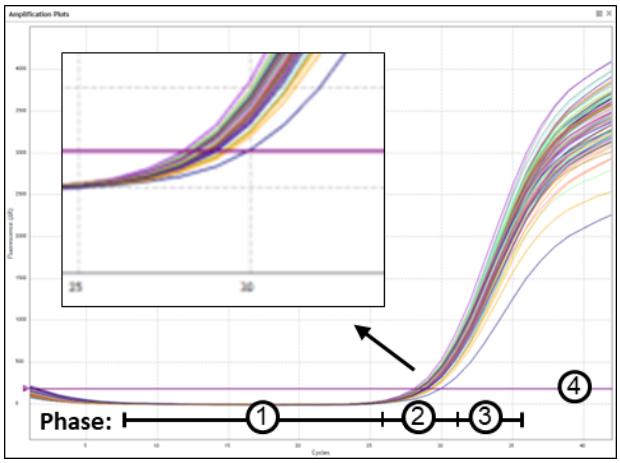

Figure 1. Exemplary amplification plot of a real-time PCR in linear scale (Graph Type “Linear”). 1, background; 2, exponential amplification phase; 3, linear amplification phase; 4, threshold line.

Bio-Rad CFX™ software:

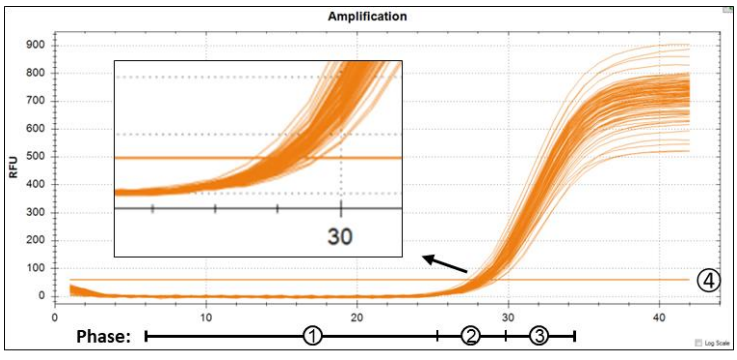

Figure 2. Exemplary amplification plot of a real-time PCR in linear scale (Box “Log Scale” is unchecked). 1, background; 2, exponential amplification phase; 3, linear amplification phase; 4, threshold line.

The following tables provide an overview of the criteria to evaluate the run files:

Control evaluation

Control type	Cq FAM™ value	Cq HEX™ value	Cq ROX™ value	Cq Cy5® value	Overall results
Positive control (C+) VIRSeek SARS-CoV-2 Mplex I Wastewater	25 ≤ Cq ≤ 35	25 ≤ Cq ≤ 35	25 ≤ Cq ≤ 35	No Cq	Valid
	Cq < 25	Not relevant	Not relevant	Not relevant	Invalid*
	Cq > 35	Not relevant	Not relevant	Not relevant	Invalid*
	Not relevant	Cq < 25	Not relevant	Not relevant	Invalid*
	Not relevant	Cq > 35	Not relevant	Not relevant	Invalid*
	Not relevant	Not relevant	Cq < 25	Not relevant	Invalid*
	Not relevant	Not relevant	Cq > 35	Not relevant	Invalid*
	Not relevant	Not relevant	Not relevant	Cq ≤ 40	Invalid*
Negative control (C-) Negative extraction control (E-)	No Cq	No Cq	No Cq	No Cq	Valid
	Cq ≤ 40	Not relevant	Not relevant	Not relevant	Invalid*
	Not relevant	Cq ≤ 40	Not relevant	Not relevant	Invalid*
	Not relevant	Not relevant	Cq ≤ 40	Not relevant	Invalid*
	Not relevant	Not relevant	Not relevant	Cq ≤ 40	Invalid*
Extraction control (E+)**	No Cq	No Cq	No Cq	Cq ≤ 33	Valid
	Not relevant	Not relevant	Not relevant	Cq > 33	Invalid*
	Not relevant	Not relevant	Not relevant	No Cq	Invalid*
	Cq ≤ 40	Not relevant	Not relevant	Not relevant	Invalid*
	Not relevant	Cq ≤ 40	Not relevant	Not relevant	Invalid*
	Not relevant	Not relevant	Cq ≤ 40	Not relevant	Invalid*

**Check amplification curve for sigmoid amplification signals, software background calculation and threshold settings*

***RNA extraction of 20 µL MS2 (approximately 2 x 10^3 MS2 phage particles per µL)*

Scoring of samples, extraction control and normalisation control

Target name	Fluorophore (Dye)	Cq result	Target specific results
SARS-CoV-2 N1	HEX™	Cq ≤ 40	Positive
		No Cq	Negative
SARS-CoV-2 N2	FAM™	Cq ≤ 40	Positive
		No Cq	Negative
Extraction Control (MS2 phage)	Cy5®	Cq Sample < Cq (E+) + 4	Valid
		Cq Sample > Cq (E+) + 4 or No Cq	Invalid. Sample processing failed or sample inhibited.
Normalisation control (PMMoV)	ROX™	Cq Sample ≤ 40	Valid
		No Cq	Sample processing failed or sample inhibited.

Scoring of samples Agilent AriaMx™ and Bio-Rad CFX96 Touch™ Standard

Preliminary Target Result SARS-CoV-2 N1	**Preliminary Target Result SARS-CoV-2 N2**	**Extraction control (MS2)**	**Normalisation Control (PMMoV)**	**Final results**	**Result interpretation**	**Next Steps**
Positive for SARS-CoV-2 N1	Not relevant	Not relevant	Not relevant	Positive for SARS-CoV-2	SARS-CoV-2 specific N1 target RNA detected in wastewater	n/a
Not relevant	Positive for SARS-CoV-2 N2	Not relevant	Not relevant	Positive for SARS-CoV-2	SARS-CoV-2 specific N2 target RNA detected in wastewater	n/a
Negative for SARS-CoV-2 N1	Negative for SARS-CoV-2 N2	Valid	Valid	Negative for SARS-CoV-2 (N1/N2)	No detection of SARS-CoV-2 specific N1/N2 target RNA. Sample does not contain detectable amounts of specific N1/N2 target RNA.	n/a
Negative for SARS-CoV-2 N1	Negative for SARS-CoV-2 N2	Invalid	Not relevant	Inhibited real-time RT-PCR or sample processing failed	No evaluation possible	Test 1:5 dilution of undiluted RNA extract. As option: test also 1:10 dilution of the undi-luted RNA extract. If 1:5 (and 1:10) dilution is still inhibited, repeat the wastewater sample preparation.

Negative for SARS-CoV-2 N1	Negative for SARS-CoV-2 N2	Not relevant	Invalid	Inhibited real-time RT-PCR or sample processing failed	No evaluation possible	Test 1:5 dilution of undiluted RNA extract. As option: test also 1:10 dilution of the undilut-ed RNA extract. If 1:5 (and 1:10) dilution is still inhibited, repeat the wastewater sample preparation.

Result interpretation for sample duplicates

Replicate 1	**Replicate 2**	**Final Result**
Positive for SARS-CoV-2	Positive for SARS-CoV-2	Positive for SARS-CoV-2
Positive for SARS-CoV-2	Negative for SARS-CoV-2	Positive for SARS-CoV-2
Negative for SARS-CoV-2	Positive for SARS-CoV-2	Positive for SARS-CoV-2
Negative for SARS-CoV-2	Negative for SARS-CoV-2	Negative for SARS-CoV-2

4 REFERENCES

1. Commission Recommendation, 17.3.2021, On a common approach to establish a systematic surveillance of SARS-CoV-2 and its variants in wastewaters in EU

5 PRODUCT WARRANTIES, SATISFACTION GUARANTEE

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6 PRODUCT USE LIMITATIONS

This kit is developed, designed, and sold for research purposes only. It is not to be used for diagnostic purposes unless expressly cleared for that purpose by the competent regulatory authorities in the country of use. All due care and attention should be exercised in the handling of the materials described in this text.

7 IMPORTANT NOTES

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TECHNICAL SUPPORT SERVICE

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