

Detection of SARS-CoV-2 in wastewater.

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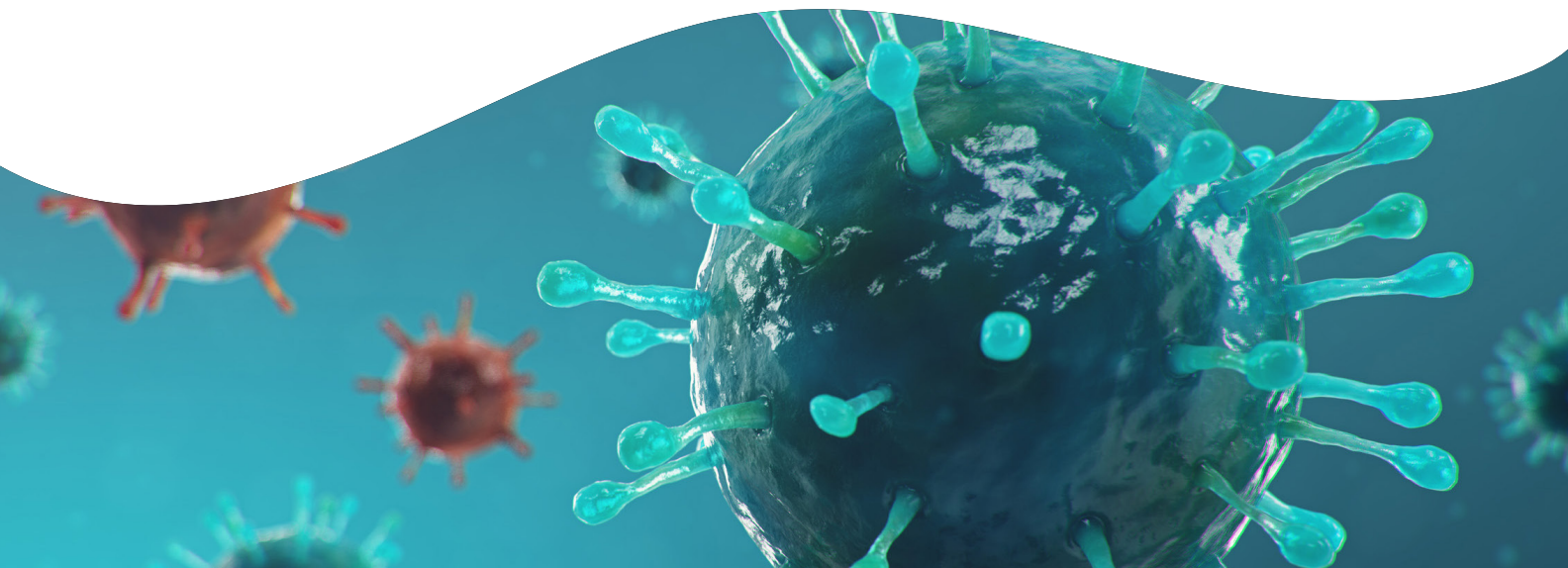
Revvity, Inc.

Introduction

Since the COVID-19 pandemic has had a serious impact on world health, society, and the economy, understanding the extent of the COVID-19 pandemic is an ongoing key challenge for public health officials. Monitoring sewage for traces of pathogens can enable effective surveillance and help limit the spread of the disease^{1,2}.

The novel coronavirus, SARS-CoV-2, seems to behave similarly to the other viruses and it has been detected in wastewater. This was expected given the ability of the virus to infect ACE2-expressing cells in the small intestine³. Concentrations of viral RNA in feces vary from patient to patient and over the course of the illness, but the signal can be detected for up to a couple weeks⁴. Studies have established that wastewater can be used as a lead indicator of SARS-CoV-2 infection in a population, as the detection of SARS-CoV-2 by PCR methods allow to achieve signals of presence of the RNA of the virus even if it is present at low concentration⁵.

The detection of SARS-CoV-2 in a city's sewage water can be used as an indicator of the extent of the local pandemic in terms of street value 'lines of SARS-CoV-2', as it has already been demonstrated to be for cocaine by a KWR Institute⁶. Nucleic acid detection is of major importance when conducting surveillance analysis based on molecular technologies. Molecular analysis can reveal the presence of the SARS-CoV-2 genetic fingerprint. As SARS-CoV-2 can be found 3-7 days in advance of symptoms through wastewater testing, this methodology can provide an early indication of an outbreak at a community level⁴. The molecular analysis of wastewater sampling is non-invasive and cost-effective.



To address this challenge Dante Labs has developed a comprehensive solution for the molecular monitoring of wastewater that offers a reliable, highly sensitive identification of the virus from a 250 mL of wastewater. The Dante Labs wastewater solution is standardized, validated, and based on Revvity's SARS-CoV-2 RT-PCR solutions.

Methods

Sample Preparation

1. Collection and concentration of untreated wastewater

A proper sampling and collection of the wastewater sample is critical for the downstream success of the analysis since factors causing degradation, presence of impurities, and issues with storage can impact the quality of the data. The time period and the number of samples collected depend on the size of the site of interest and the occupancy of the building for which the collection is required. As a general principle, one sample should be collected for every hour in which the building is occupied. The collection of untreated wastewater samples, the composite sample, should be carried out from the waste stream or collection point with the use of autosamplers. The automatic collection results into a 1 liter of raw wastewater collected in the autosampler tank. From this, 250 mL is required for downstream molecular analysis. Transport the collected samples using a 4°C cold chain.

2. Sample concentration in the laboratory

With the need to start from a highly concentrated sample, in the laboratory, the wastewater sample is scaled down to 20 mL using a two-part concentration protocol including polyethylene glycol precipitation (modified WHO poliovirus protocol⁷ with a final matrix obtained after a centrifugation and ultracentrifugation step. Elute and filter the final matrix using filters with a pore size ranging between 0.1 µm and 0.05 µm, until a final volume of 2 mL is obtained.

Method performance

The Dante Labs SARS-CoV-2 test for wastewater is based on real-time reverse transcription polymerase chain reaction (RT-PCR), a laboratory technique that detects the presence of a specific nucleic acid by using specific probes, the primers.

As it is based on the New Coronavirus Nucleic Acid Detection kit, the assay identifies the presence of SARSCoV-2 by detecting the presence of the (N) gene and ORF1ab gene, which are the two highly conserved genes of the SARS-CoV-2 genome.

High quality viral RNA isolation extraction is also important when conducting molecular analysis. The Dante Labs SARS-CoV-2 test for wastewater uses the Revvity chemagic™ Viral DNA/RNA kit run on the chemagic™ 360 instrument for extraction of viral RNA.

The extraction is followed by reverse transcribing the RNA into cDNA followed by RT-PCR performed using the New Coronavirus Nucleic Acid Detection kit. This kit enables the detection and presence of the viral genes with the use of specific reporter dyes, FAM and HEX, that generate a fluorescent signal with a specific colour when the presence of the virus is detected.

Positive and negative controls are included in the assay. This assay uses three controls:

- A no-template control, (NTC), designed to eliminate the possibility of sample contamination and serves as a general control for extraneous nucleic acid contamination.
- A positive template control (PTC), designed to verify that the assay run is performing as intended. The positive template control consists of quantified synthetic DNA oligonucleotides containing the N and ORF1ab viral genes.
- An internal control targeting RPP30 gene, to demonstrate that nucleic acid is present in every sample.

At the end of the RT-PCR thermal cycling run, the digital results are interpreted and validated. The interpretation follows specific instructions based on the Revvity assay. The control requirements are described in the table below.

Table 1: The table describes the guidelines for the interpretation of the results

Control type	Control name	Used to monitor	Expected results & Ct values
Negative	NTC	Assay or extraction reagent contamination	Negative Ct ND
Positive	SARS-CoV-2- Positive Template Control (PTC)	Improper assay setup, reagent failure including primer and probe degradation	Positive Ct < 32 for N gene & Ct < 35 ORF1ab gene
Extraction control	Negative Human Clinical Sample (HCS)	Cross-contamination during extraction, inefficient lysis of specimen, poor specimen collection, improper assay setup, extraction failure	Positive Ct ≤ 35.0

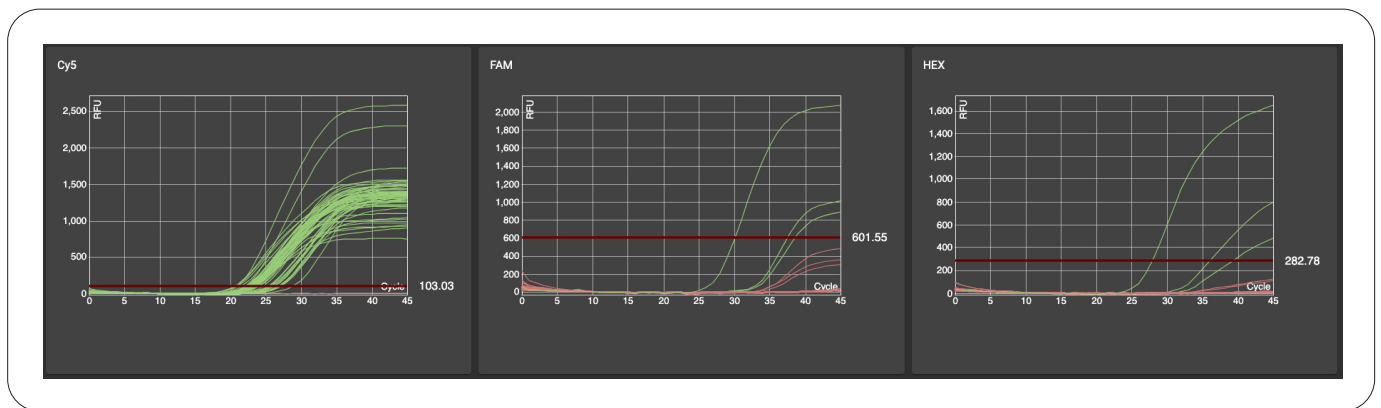


Figure 1: High quality RT-PCR analysis of viral SARS-CoV-2 RNA extracted from wastewater

RNA was extracted from wastewater using the chemagic 360/MSM I instrument and the chemagic™ Viral DNA/RNA 300 Kit H96. RT-PCR was carried out by using the FDA EUA authorized kit for PCR, Revvity New Coronavirus Nucleic Acid Detection Kit with a Bio-Rad® CFX RT-PCR Instrument. HEX, Cy5 and FAM represent the fluorophore probes. FAM is associated with the presence of the N gene and HEX is associated with the presence of the ORF1ab gene. Cy5 is associated with the presence of the internal control, a human housekeeping gene. On the y-axis the relative

fluorescence unit is represented, on the x-axis represents the cycle number at which the emission wavelength of the fluorophore. The red horizontal line is the baseline. The software is based on the proprietary interpretation and reporting platform of Dante Labs, the Immensa™ platform. Interpretation is based on the table above mentioned. According to the interpretation algorithm: C02 is positive (positive for both the FAM and HEX signal); C07, C05 and C012 are inconclusive (only positive for either FAM or HEX).

Conclusion

The importance of wastewater monitoring also known as wastewater-based epidemiology (WBE) for SARS-CoV-2 early detection and surveillance of COVID-19 is important. WBE provides a complimentary perspective to lab surveillance, hospital admissions and prescription data, biomonitoring and mortality rates. It yields insights on current infection situation, viral spread trends and hot spots.

The demand for highly sensitive, mass-scale, non-invasive solutions for the surveillance of the infection continues to be great. Dante Labs has demonstrated that the identification of the SARS-CoV-2 virus within wastewater samples is achievable through the use of the chemagic Viral DNA/RNA kit special H96 and the SARS-COV-2 RT-PCR kit from Revvity.

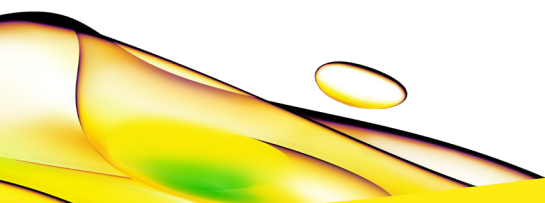
The use of this technique will allow the detection of the virus from wastewater according to Dante Labs Wastewater protocol method and Revvity PCR kits and automated workflow solutions.

About Dante Labs

Dante Labs is a global genetic testing and data analysis platform, specialized in advanced genomic solutions for health, food and environment. Specialized in NGS, Dante Labs has offices in Europe (Italy), United Kingdom (London and Wolverhampton), USA (New York and Austin). Dante Labs operates globally. Dante Labs mission is to make advanced genomic solutions accessible to everyone.

Reference

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