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The WRC operates in terms of the Water Research Act (Act 34 of 1971) and its mandate is to support water research and development as well as the building of a sustainable water research capacity in South Africa.



## COVID progress

### ***WRC Proof of Concept Study: Wastewater-based epidemiology for surveillance of the spread of Covid-19 in South African communities***

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*After a four-week period of wastewater sampling for the COVID-19 virus from ten wastewater treatment works (WWTW) serving hotspot areas in Metropolitan areas in five provinces, the WRC study draws to the following conclusions:*

- *SARS-CoV-2 RNA was detected in 98% of wastewater samples collected. This confirms and demonstrates the power of wastewater surveillance of the SARS-CoV-2 virus responsible for COVID-19, and has demonstrated the proof of concept.*
- *This study illustrates that wastewater-based epidemiology can be applied as a complementary surveillance tool for management of the COVID-19 pandemic.*
- *Continued sampling of these sites already involved will allow for the expansion of trend monitoring, and it is recommended that more WWTW be added to the sampling protocol so as to move to a pilot phase study.*
- *Wastewater surveillance offers a cost-effective and less invasive means of continuous screening. Where increasing trends in viral load are noted then additional clinical test methods could be rolled out based on an early warning system. This investment is better than going into an economic lockdown.*
- *Positive results were found when sampling defined populations such as prisons and hospitals upstream of wastewater treatment works (WWTW), and assays successfully detected SARS-CoV-2 at small WWTW serving industries and mines.*
- *Results indicated an increase in the viral load in the samples being tested with time which corresponded to the increase in case numbers in the hotspots as the peak infection phase of the pandemic was entered.*
- *This initiative has built a robust collaborative platform of scientists and laboratories which has thrust South Africa forward to be one of the world leaders in this area.*

## INTRODUCTION

Since the beginning of the 21<sup>st</sup> century, three coronaviruses have crossed the species barrier to cause deadly pneumonia in humans, including Severe Acute Respiratory Syndrome (SAR-CoV-1), Middle-East Respiratory Syndrome (MERS), and now Severe Acute Respiratory Syndrome 2 (SARS-CoV-2). This CoV is the newest of the family of coronaviruses associated with human infections that are grouped into the beta-CoV genus, with 79% genetic similarity to SARS-CoV-1. Coronaviruses are enveloped, with a lipid membrane envelope around the surface of the virus. The lipid envelope makes coronaviruses more fragile than other viruses and is relevant to understanding their sensitivity to disinfection and their persistence in the environment and transmission.

The outbreak was declared a Public Health Emergency of International Concern on 30 January 2020 and on 11 February 2020 the World Health Organisation (WHO) announced a name for the new coronavirus disease: COVID-19. On March 11, WHO upgraded the status of the COVID-19 outbreak from epidemic to pandemic.

The concept of screening municipal wastewater and environmental water quality as an epidemiological tool for viruses is not a new concept, and has been used to help inform broader infectious disease epidemiological surveillance and mitigation efforts such as the Global Polio Eradication Initiative<sup>1,2</sup>. Environmental surveillance has also been used and recommended for other infections, such as typhoid<sup>3</sup>, early warning of hepatitis A and norovirus outbreaks<sup>4</sup>, as well as for antimicrobial resistance<sup>5</sup>, with modelling techniques used to assist both the design and interpretation of those efforts<sup>6,7</sup>. Wastewater-based epidemiology (WBE) is also commonly used in the surveillance of licit and illicit drugs as well as various chemical contaminants which may impact human health<sup>8</sup>.

Many COVID-19 infections are asymptomatic and, unless tested, remain undetected. Recent studies have shown that environmental surveillance of SARS-CoV-2 (signals or biomarkers (typically RNA by means of Reverse transcriptase Polymerase Chain Reaction (RT-PCR)<sup>9</sup>) can be a low-cost solution for tracking Covid-19 outbreaks in communities. This is because SARS-CoV-2 is shed at relatively high titres in the stool of some individuals. Viral gastrointestinal infection, or at least shedding, can remain for some time after clearance of the virus in the respiratory tract<sup>10,11</sup>. The presence / absence of SARS-CoV-2 in wastewater treatment plant influent can determine the presence of infected individuals in a community and can be used as an epidemiological indicator, especially where community testing is not possible.

The detection of SARS-CoV-2 RNA in untreated domestic wastewater has been reported internationally, including Australia<sup>12</sup>, the Netherlands<sup>13</sup>, USA<sup>14,15,16</sup>, France<sup>17</sup>, China<sup>18</sup>, Israel<sup>19</sup>, Turkey<sup>20</sup>, Spain<sup>21,22</sup>, Italy<sup>23</sup> and Japan<sup>24</sup>. This study's overall objective was a Proof of Concept to detect the RNA signal of the SARS-CoV-2 virus in wastewater samples

in South Africa as a means of assessing the presence of infective individuals in a community to consider as a complimentary epidemiological surveillance tool.

## METHODOLOGY

Wastewater was collected as 24-hour composite samples from 10 wastewater treatment works (WWTW) in 5 provinces, over a period of 4 weeks. Additional duplicate grab samples were taken from selected sites during the morning peak flow period. Grab samples were taken from a sewer manhole downstream of a hospital with known COVID-19 as well as a prison. Finally, 24-hour manual composite samples were taken from small WWTW serving industries and mines.

Three virus recovery methods were tested based on their ease of use and cost effectiveness, namely, PEG 8000/ NaCl precipitation, skim milk flocculation and aluminium hydroxide adsorption-flocculation. Virus recovery efficiency was determined making use of the mengovirus. 1-2 L sewage samples were received and stored at 4°C until processing. Samples were mixed thoroughly and a 200 mL aliquot was used for each of the three recovery methods.

Viral nucleic acid extraction took place making use of the QIAamp Ultrasens Virus Kit (Qiagen). Screening for SARS-CoV-2 was done using real time Allplex™ 2019 nCoV assay (Seegene Inc. Seoul, South Korea) and repeated with the QuantiFast® Pathogen RT-PCR + IC kit (Qiagen). The Seegene kit used 3 targets (E gene, N gene, RdRp gene and an internal control), whereas the Quantifast kit included 2 target genes (N1 and N3) as well as an internal control. The RT-PCR detection took place on QuantStudio5 real time PCR platform. Ct values below 40 were considered positive.

A total of 49 WWTW samples were collected, which included 19 duplicate samples to include a total of 68 wastewater samples, of which 29 samples were recovered with PEG/ NaCl, 19 samples were recovered with milk and 20 samples were recovered with Al(OH)<sub>3</sub>. An additional 6 wastewater samples from industry package WWTW, a prison and hospital were included in the testing. Five gene targets for each recovered sample were tested in three assays, totalling 204 RT-PCR reactions.

## SUMMARY OF RESULTS

In terms of virus recovery, grab samples performed as well or better than 24-hour composite samples. This is an advantage when sampling from plants where no composite sampler is available.

All three virus recovery methods tested were effective in the recovery of the SARS-CoV-2 virus (Figure 1). For wastewater, of the three recovery methods applied in this study, the skim milk flocculation and aluminium hydroxide adsorption-precipitation methods are preferred, as they are both faster and cheaper than the PEG method, and only require low

speed centrifugation. The three methods could be used interchangeably between laboratories, although due to the slight variation in results it is recommended that one method be used consistently when monitoring a site to enable the visualisation of trends.

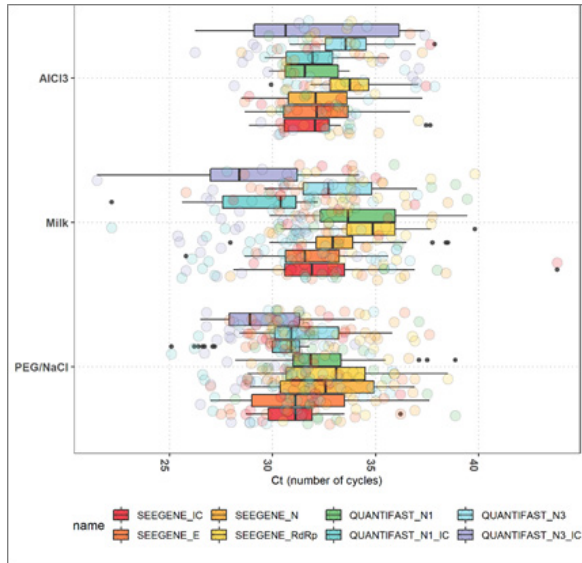


Figure 1: Comparison of Ct values by recovery method and assay

Of the total of 68 wastewater samples, 50 were positive for all 5 targets (70.4%), 16 were positive for 4 targets (20.4%), 9 were positive for 3 targets (13%), 2 were positive for 1 target (2.94%) and only 1 (1.47%) was negative.

Of the 10 defined community wastewater samples tested (industries, mines, hospital, prison), 8 were positive for all 5 targets (80%), 1 was positive for 4 targets (10%), and 1 was negative.

Virus quantification was successfully carried out with genome copies/mL ranging between  $1,2 - 2,7 \times 10^4$  for N1 and  $4,2 - 5,5 \times 10^4$  for N3 target genes.

Both the Seegene and Quantifast kits detected the virus RNA (Figure 2). The commercial Seegene multiplex assay was found to be more sensitive than the Quantifast N1 assay, had the advantage of amplifying 3 targets in one reaction, and is more likely to be more consistent across laboratories. This assay is recommended for further work. The disadvantage is that the Ct value must then be used to approximate viral load, as the copy number cannot be determined by comparison with a standard curve.

Plotting of weekly sample results expressed as Ct values appeared to be sufficient to indicate trends (Figure 3), as such weekly sampling of identified sites for national surveillance is recommended. It is recommended that viral load be quantified making use of the Ct number with a proposed categorical data analysis recommended based on the Global Polio Surveillance scheme.

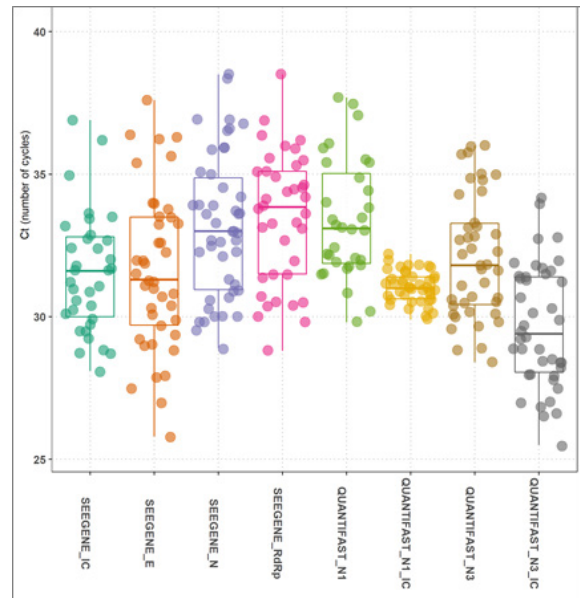


Figure 2: Comparison of Ct values for all samples per assay method

The Ct value is a relative measure of the concentration of target in the PCR reaction. The Ct value increases with a decreasing amount of template. Lower Ct values (typically below 29 cycles) indicate high amounts of target sequence. Higher Ct values (above 38 cycles) mean lower amounts of your target nucleic acid. For the purposes of the proof of concept samples with a Ct value below 40 were considered positive.

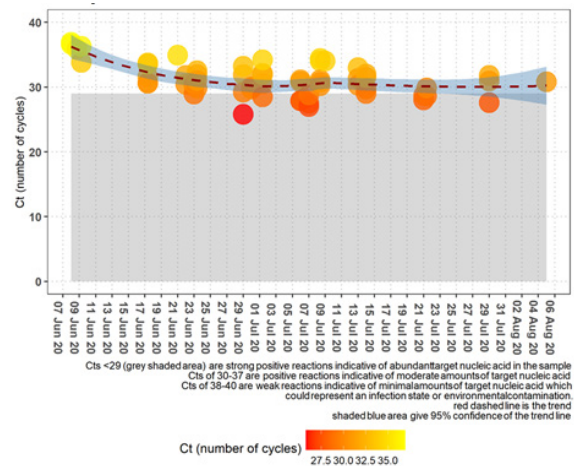


Figure 3: Plotting of Ct values over time for all sites  
Concluding remarks

The detection of SARS-COV-2 RNA in 98% of wastewater samples collected, has demonstrated the proof of concept. This study illustrates that wastewater-based epidemiology can be applied as a complementary surveillance tool for management of the COVID-19 pandemic. Continued sampling of those sites already involved will allow for the expansion of trend monitoring, and it is recommended that more WWTW be added to the sampling protocol so as to move to a pilot phase study. South Africa is through its first

peak of the pandemic, but the experience of other countries teaches us that second and even third waves of infection are likely, if not inevitable, as the economy and intra- and international borders re-open.

Sampling of combined sewage for a defined population such as a prison, hospital or hostel can be useful for surveillance of increased viral load to give early warning of a possible surge in infections. It is important however that regular samples be taken over time to establish trends and baselines, due to the inherent variability of sampling from smaller populations compared to a regional WWTW. This could provide a cost effective and less invasive means of continuous screening. Where increasing trends in viral load are noted then additional clinical test methods could be rolled out based on an early warning system.

Translating the viral titres from wastewater into the actual number of cases within a community is highly challenging, if not impossible. This type of calculation relies on many assumptions, which still remain poorly quantified, for example the amount and dynamics of viral shedding in faeces, viral persistence in the sewer network and variation in wastewater flow and temperature due to climatic conditions. Although wastewater surveillance of SARS-CoV-2 provides a powerful tool to evaluate disease incidence at the community level, it is clear that they also need to be integrated into other public health initiatives, for example campaign-based and randomised testing of individuals (presence of pathogen or antibodies), clinical case reporting, and mobile-based contact-tracking and self-reporting systems. One of the benefits of wastewater, is that it has limited sociological bias with few if any ethical issues.

On the way forward, Phase two of this initiative – the pilot scale monitoring – will begin from the 1 October 2020. Partnerships for pilot-scale monitoring have been established through the phase one between laboratories, municipalities, DWS, private sector and research organisations. This phase will see the scaling and commissioning of a collaborative monitoring initiative in provincial hotspots) using the sampling and testing protocols developed in phase one.

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