SCIENCE BRIEF: 10

The efficacy of passive samplers for water quality based COVID 19 epidemiology surveillance

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- Wastewater-based surveillance of communities for SARS-CoV-2 viral prevalence by sampling from wastewater treatment works is a powerful complementary epidemiological tool. However, in South Africa almost 40% of the population is not connected to a waterborne sewerage system.
- Sampling and surveillance of grey water and faecal waste within our non-sewered areas may give an early warning of the presence of COVID-19 infections in these communities, where there is both the risk of rapid spread and low likelihood of conventional testing.
- Passive sampling of environmental sites has shown promise, and may overcome issues of low yield when viral load is low and during high dilution periods, particularly in rivers downstream of unsewered settlements.
- Passive samplers have the advantage of allowing for easier and cheaper transport of samples compared to grab samples that require the costly transport of large volumes of water maintenance of the cold chain out of rural areas. Sample processing is also much quicker compared to concentration of water samples.
- Inclusion of trend monitoring of SARS-CoV-2 prevalence in unsewered communities together with established *Wastewater-based epidemiology* (WBE) data collection from WWTW sampling can greatly expand the knowledge base and serve to highlight the needs of vulnerable communities in South Africa. These passive samplers can now also potentially be used for wastewater-based epidemiology for a broader scope of pathogens than only SARS-CoV-2.
- The success of passive samplers from this initiative has demonstrated the future potential and opportunity to enable more widespread environmental water quality monitoring due to its low cost and ease of application in the collection of samples.

INTRODUCTION

The Coronavirus pandemic caused by the *Severe Acute Respiratory Syndrome Coronavirus* 2 (SARS-CoV-2) virus was declared a Public Health Emergency of International Concern on the 30 January 2020 by the *World Health Organisation* (WHO) and was named *Coronavirus Disease 2019* (COVID-19). Since then, COVID-19 has swept across the world infecting 233,356,026 people and causing 4,776,055 deaths globally as of 30 September 2021 (https://coronavirus.jhu.edu/map.html). It has also had a severe impact on the world economy and international trade. As part of the efforts to stop the spread of this virus, the detection of SARS-CoV-2 in municipal sewage has successfully been proven both internationally (Medema et al., 2020) and in South Africa (Pocock et al., 2020). This has assisted in developing maps of hot spots of SARS-CoV-2 within the boundaries of sewered communities.

According to Statistics South Africa's General Household Survey, 2018, only 61% of people living in South Africa have access to a flush toilet connected to a public sewerage system. At the time of publication of the

report in 2018, flush toilets connected to public sewerage systems were most common in the most urbanised provinces, namely Western Cape (89.1%) and Gauteng (88.6%). Only 26.5% of households in Limpopo had access to any type of flush toilet, the lowest of any province, with 70.2% of households in Limpopo using pit latrines. In the Eastern Cape, 40.3% of households used pit toilets.

Therefore, whilst wastewater-based surveillance of communities for SARS-CoV-2 viral prevalence by sampling from wastewater treatment works is a powerful complementary epidemiological tool, in South Africa almost 40% of the population will not be covered. These are also usually the most vulnerable communities who do not have access to sufficient health care or financial resources.

It is vital to develop a framework and methods for sampling and surveillance of grey water and faecal waste within our non-sewered areas to ensure a timeous response to an upsurge in SARS-CoV-2 within these vulnerable communities. Since grey water and wastewater is discharged eventually to the nearest river, the rivers near non-sewered communities are used in this study as the most sustainable and reliable sampling point for the exposure of these communities to COVID-19. Samples are drawn at defined points, particularly where known non-point sources of sewage contamination are occurring from non-sewered informal housing communities. Greywater runoff polluted by sewage in non-sewered communities is also sampled as a potential epidemiological indicator when available. While it is not necessarily possible to relate viral loads in surface water to a defined population or possible case numbers, sampling of rivers may provide a means to monitor the spread of SARS-CoV-2 within informal settlements by monitoring river quality over time, as well as monitoring trends in viral loads to identify possible infection spikes in communities upstream of the sample point (Rimoldi et al., 2020, Guerrero-Latorre et al., 2020) This may give an early warning of the presence of COVID-19 infections in these communities, where there is both the risk of rapid spread and low likelihood of conventional testing. This will enable deployment of rapid response teams into these areas to conduct more intensive testing and quarantining of infected individuals to curb the spread of the virus.

During the project, the application of passive samplers was validated based on a study from Australia (Shang et al., 2020). The use of passive sampler units made from readily available consumables have been compared with grab samples from selected sites.

ABOUT PASSIVE SAMPLERS

Passive samplers are perforated plastic housings for gauze or similar material, which are placed in situ into the path of flowing water. The gauze allows the water to drain while retaining sediments and contaminants which adsorb to the gauze,. In our case this includes Covid-19 RNA shed by infected individuals. The gauze is then harvested and sent to the lab for analysis. These samplers were easily produced through 3D printing according to the torpedo shaped pattern developed by Shang et al. (2020). This design performed best due to its streamlined design, which proved to be optimal for minimising fouling.

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The plastic housing was filled with six standard 75mm x 75 mm medical gauze swabs and wrapped in 50% shade cloth, also to help to prevent excessive surface fouling (Figure 1~~Figure 1~~). Passive samplers were using a 3mm nylon rope, fishing line, or binding wire, as per availability and suitability at site. Shang et al. (2020) indicated 8h exposure of the passive sampler in a sewer manhole, but the optimum exposure time of exposure in the aquatic environment is being explored, with exposure times of 24-48h depending on the sampling environment. More dilute streams or rivers impacted by rainfall will likely need longer exposure times *in situ*. Parallel grab samples were taken at the end of each passive sampling period to compare recoveries.

Torpedo style passive sampling devices

Torpedo filled with gauze and wrapped in shade cloth ready for deployment.

Figure 1: Passive sampling devices

Elution of potential viral nucleic acids is carried out using a modified methodology described by Shang et al (2020). An aliquot of 10ml of PBS with 0.05% Tween 20 is added to the gauze samples, which are then massaged for 3 minutes. The anti-foaming agent was found to be unnecessary and was excluded (Archer et al., 2020). This eluted material is then directly extracted with the QIAamp Ultrasens Virus Kit (Qiagen).

Reverse Transcription–Polymerase Chain Reaction (RT-PCR) screening for SARS-CoV-2 with real time multiplex Seegene Allplex™ 2019-nCoV Assay. The assay targets the envelope (E), nucleocapsid (N) and RNA dependent RNA polymerase (RdRp) genes of SARS-CoV-2 and contains an internal control to monitor inhibition. The multiplex assay (Seegene) is used for this environmental work to enable detection of multiple gene targets due to the amount of variability observed in this sample matrix. The real time RT-PCR is performed on a QuantStudio™ 5 Real Time PCR System (Applied Biosystems, Foster City, CA). Ct values below 40 considered positive. Dilutions of 1:10 are also included routinely due to inhibition of internal controls when screening surface samples.

Initial deployment of the passive samplers has taken place at the source of the Jukskei River in Ellis Park, Johannesburg, an informal settlement in Alexandra, the Jukskei River downstream of Alexandra, and in the Western Cape in the Plankenbrug River in Stellenbosch, Langrug near Franschhoek and downstream of the informal settlements at Kayamandi. Also under investigation is the utilisation of a passive sampler in a honeysucker tanker which is used to collect sewage from toilets or septic tanks in rural areas which may not be connected to the sewage network.

PRELIMINARY RESULTS

The Jukskei river is already significantly contaminated at the point of daylight due to sewage contamination of stormwater and groundwater from hi-jacked and un-serviced buildings in the Johannesburg Central Business District (CBD). Deployment of the passive sampler is presented in Figure 2~~Figure 2~~. The initial deployment 48h period, with significant fouling of the device and gauze observed. A second deployment of 24h was undertaken with less fouling observed.

Passive sampler deployed in the source of the Jukskei River

After 48 hours, sample swab is significantly fouled

Figure 2: Passive sampling device installed in the source of the Jukskei river in Braamfontein, Johannesburg

Deployment of the samplers in the Alexandra informal settlement and the Jukskei River downstream of Alexandra is presented in Figure 3~~Figure 3~~. These samplers were recovered 24h after installation with low fouling of the device and gauze observed.

Finally, 24h deployment was also undertaken in the Plankenbrug River in Stellenbosch downstream of the Kayamandi Informal Settlement (Figure 4~~Figure 4~~). The conventional Moore swab method was also applied to observe the degree of fouling and determine whether it is prohibitive in an environmental sampling context.

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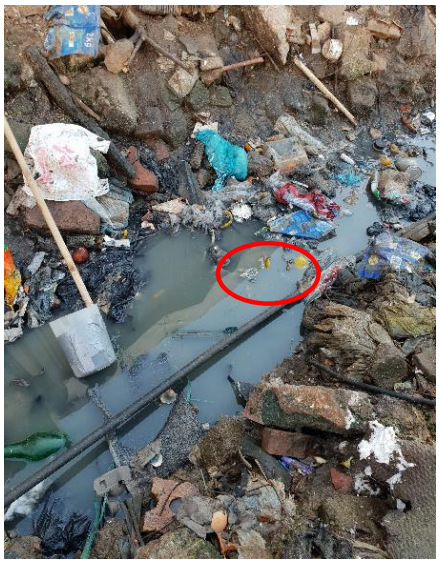
Second deployment in the Jukskei River downstream of Alexandra



In a greywater stream in an informal settlement in Alexandra

“Torpedos” retrieved 24 after installation

Gauze ready for processing

Figure 3: Passive sampling in the Silvertown Informal Settlement in Alexandra, Gauteng, and in the Jukskei River downstream of the Silvertown Settlement

Deployment of sampler in the Plankenbrug River downstream of Kayamandi informal settlements

Recovered gauze swabs

Figure 4: Passive sampling in the Plankenbrug River in Stellenbosch downstream of the Kayamandi Informal Settlement

Daily COVID-19 caseloads for Gauteng are illustrated in Figure 5~~Figure 5~~. The period of passive sampling of the Gauteng sample sites from May to September is indicated with the red block, illustrating the third wave of infections in the province.

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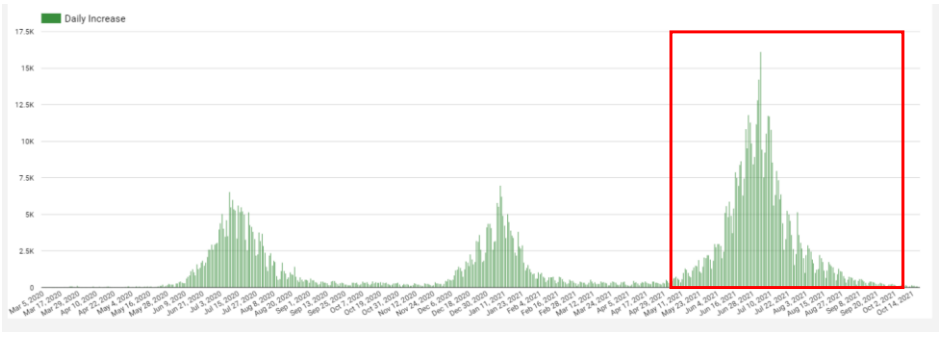

Figure 5: Gauteng daily COVID-19 caseloads, period of passive sampling through the third wave indicated in red (https://www.covid19sa.org/provincial-breakdown)

A comparison of the assay results from the grab and 24h passive samples from the Jukskei Source and Jukskei River downstream of Alexandra are presented in Figure 6~~Figure 6~~ and Figure 7~~Figure 7~~. Blue bars on the positive control. For most samples, similar results were seen in both the grab and passive, in terms of indicating a positive result, with the impact of the third wave clearly visible. In the 24h passive samplers in the Jukskei River downstream of Alexandra, a positive result was found for the sample taken from the 6-7 September 2021, which was not seen in the grab sample. By this stage the third wave had waned and the viral load was expected to be low, so the passive sampler had the advantage of 24h exposure to the water.

Figure 8~~Figure 8~~ illustrates a comparison between 24h and 48h passive sampling in the Jukskei River Alexandra for four bi-weekly sampling events starting at the end of August 2021. Positive results were seen for three of the 48h samples, but only one of the 24h samples. By this stage the third wave was waning, so it appears that the longer contact time was preferrable for additional viral particle adsorption under these conditions. Longer contact time may also be required during high dilution periods with high rainfall.

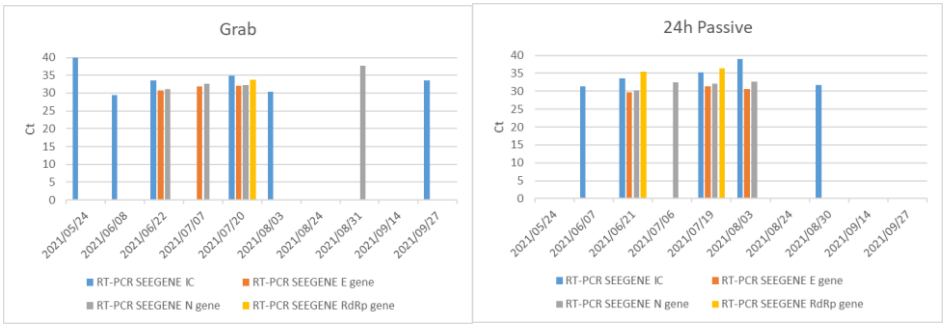

Figure 6: A Comparison between Seegene assay Ct values for grab and 24h passive sample results from the Jukskei Source, sampled bi-weekly

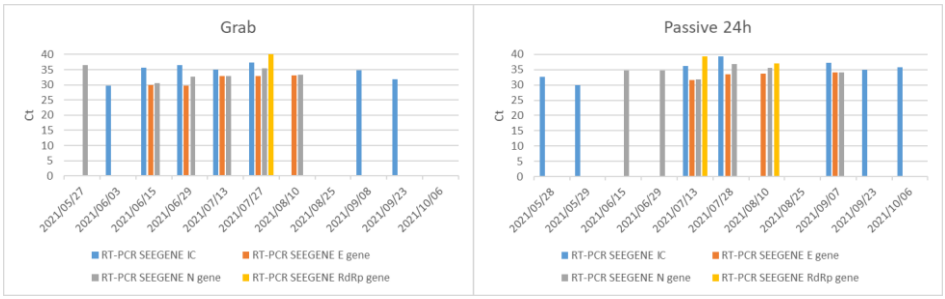

Figure 7: A Comparison between Seegene assay Ct values for grab and 24h passive sample results from the Jukskei River downstream of Alexandra, sampled bi-weekly

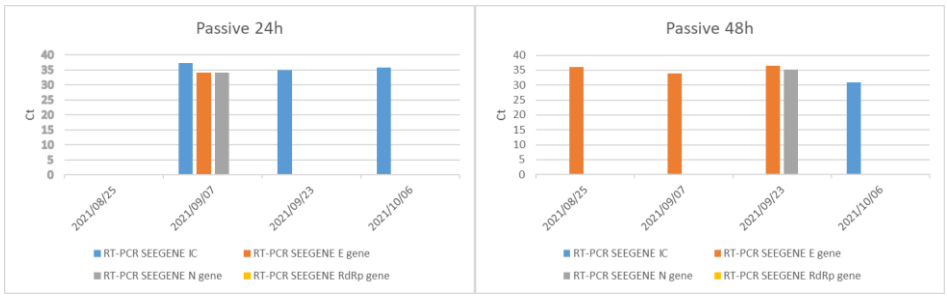

Figure 8: A Comparison between Seegene assay Ct values for 24h and 48h passive sample results from the Jukskei River downstream of Alexandra, sampled bi-weekly

A comparison between the grab and 24h passive samples taken from the contaminated runoff in the informal settlement in Alexandra is presented in Figure 9~~Figure 9~~. While positive samples were found in the grab from June to August during the third wave, no positive samples were found in the 24h passive samples. It is expected that there is a high level of inhibition in these samples due to the presence of soaps, oils and ash from household activities. The design of the passive sampler also excludes solids from exposure to the gauze. It is thought that the raw nature of the faecal material in this matrix may have also prevented exposure of the gauze to the viral particles. Passive sampling of greywater in unsewered communities may therefore not be a recommended methodology

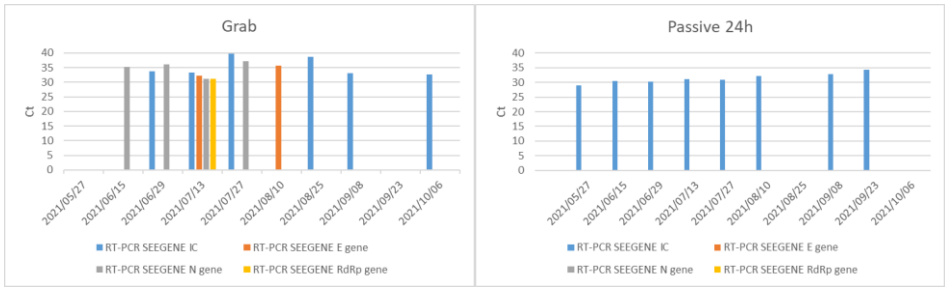

Figure 9: A Comparison between Seegene assay Ct values for grab and 24h passive sample results from run-off from an informal settlement in Alexandra, sampled bi-weekly

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 study samples with a Ct value below 40 were considered positive.

CONCLUDING REMARKS

Passive sampling of environmental sites has also shown promise, and may overcome issues of low yield when viral load is low and during high dilution periods, particularly in rivers downstream of unsewered settlements. Passive samplers have the advantage of allowing for easier and cheaper transport of samples compared to grab samples that require the costly transport of large volumes of water maintenance of the cold chain out of rural areas. Sample processing is also much quicker compared to concentration of water samples. It is therefore proposed that passive sampling continue to be conducted at several sample sites in parallel to the grab samples to compare more extensive data sets.

Access to these environmental samples, particularly sample points within informal settlements is difficult and time consuming and requires significant planning and community engagement for the programme to be a success. One of the disadvantages of passive sampling is that it is more labour intensive than grab sampling, and requires the sampler to be installed for 24-48h (or more depending on dilution) before removal for processing. Collaborative relationships have been built with various river action groups, community leaders and Universities and research facilitators to enable the collection of samples from identified sites. There is an opportunity for training and capacity building through this programme, and development of youth and community "champions". It is proposed that sites be selected for more in-depth training of these community surveillance champions in terms of sample collection methodologies (particularly how to correctly and safely install and remove a passive sampler), an understanding of the results and how they will be used for public health benefits, and how to communicate with their fellow community members.

Inclusion of trend monitoring of SARS-CoV-2 prevalence in unsewered communities together with established *Wastewater-based epidemiology* (WBE) data collection from WWTW sampling can greatly expand the knowledge base and serve to highlight the needs of vulnerable communities in South Africa. These passive samplers can now also potentially be used for wastewater-based epidemiology for a broader scope of pathogens than only SARS-CoV-2.

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