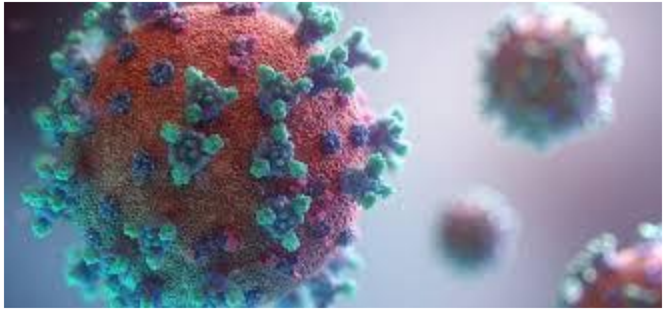

WATER
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# Wastewater-Based COVID 19 Epidemiology Surveillance for Non-Sewered Communities:

## A Case Study for Surveillance of Pathogen Indicators in the Environment

*G. Pocock. L. Coetzee, J. Mans & B. Genthe*

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

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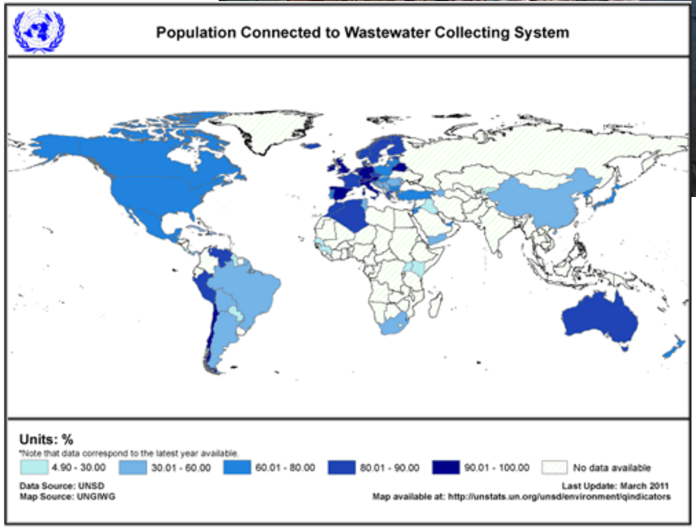
# Introduction

- Globally, only 45% of households are connected to sewerage systems
- In South Africa, more than 40% of the population does not have access to a municipal sewage system and these communities are usually the most vulnerable - lacking lack health care and financial resources
- In South Africa, about 81,9% of households in metropolitan areas live in formal dwellings, while 16,8% live in informal dwellings



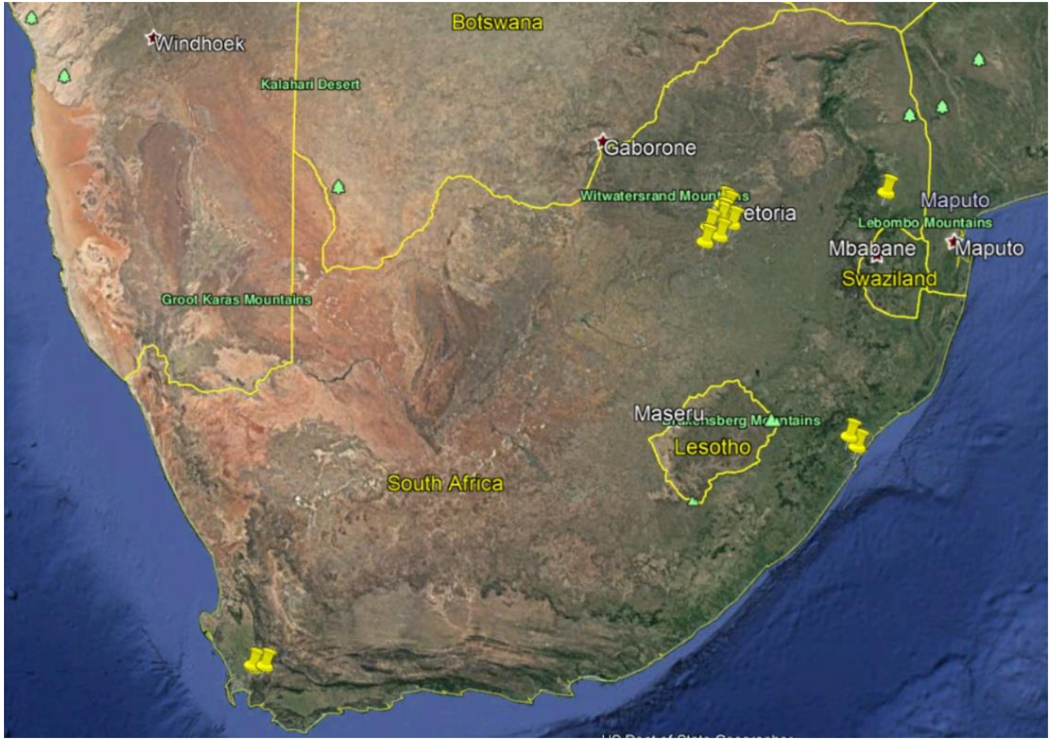
# Objectives

1. Develop a sampling framework for COVID-19 surveillance in non-sewered communities by determining
    - Ideal sampling points
    - Sampling methods
    - Sample types (Rivers, greywater run-off, on-site sanitation)
2. Develop and optimise the methodology for SARS-CoV-2 detection, quantification and monitoring in different types of samples from non-sewered environments
3. Provide the data and recommendations for the development of a surveillance reporting platform and undertake mapping and trend analysis
4. Support capability building for water quality-based SARS-CoV-2 epidemiology

# Sample Sites

Four Provinces including:-

- Gauteng
  - City of Tshwane (2)
  - City of Johannesburg (5)
  - East Rand (2)
  - Sedibeng (2)
- Western Cape – Cape Winelands
  - Stellenbosch (2)
  - Franschhoek (2)
  - City of Cape Town (1)
- KwaZulu Natal
  - eThekwini (6)
- Mpumalanga
  - Mbombela - Kanyamanzane (2)



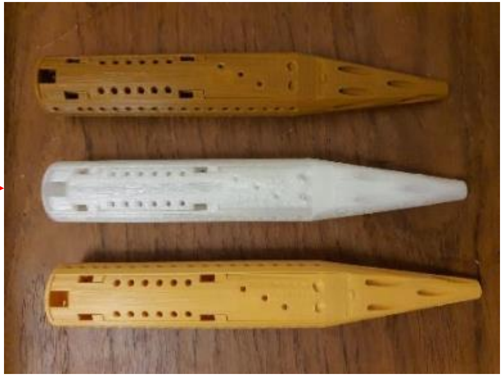
# Sampling Methodology: Grab samples

- **Surface water**
  - Informal settlements lacking access to sewered sanitation located in close proximity to a river were identified
  - Up and downstream river sample sites were chosen to determine the impact of the community on the background contamination of the stream
- **Grey water runoff from within non-sewered communities**
  - Standing pools around stand-pipes, ablutions and in drainage channels
- Bi-weekly samples of surface run-off from within communities and river up-and downstream taken for period of 5 months

- Basic water quality parameters analysed per sample
  - pH, SS, COD, EC, Ammonia and Faecal coliforms / *E. coli*

# Sampling Methodology: Passive Samples

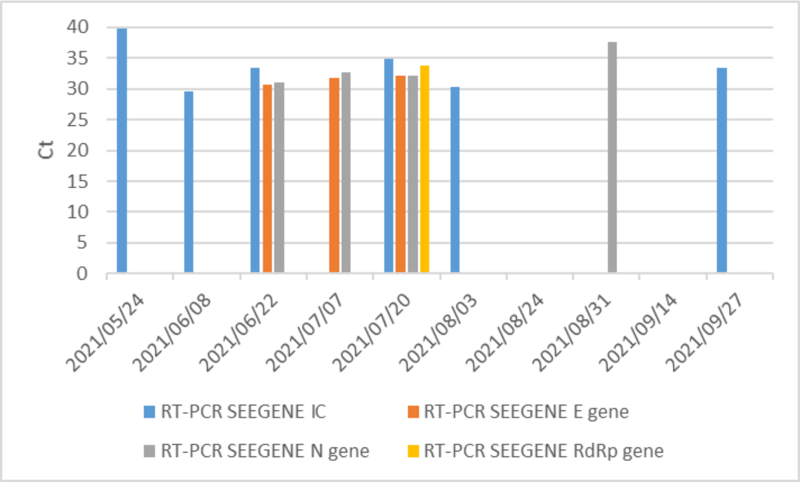
- Methodology developed by Shang et al. (December 2020): “Passive sampling of viruses for wastewater-based epidemiology: a case-study of 2 SARS-CoV-2”, using torpedo device design
    - Device covered in shadecloth and anchored at sample point for a specific period, then virus nucleic acid eluted in the lab
- Currently passive sampling
    - Rivers
    - run-off channels in informal settlements
    - Emptying tankers collecting waste from portable toilets

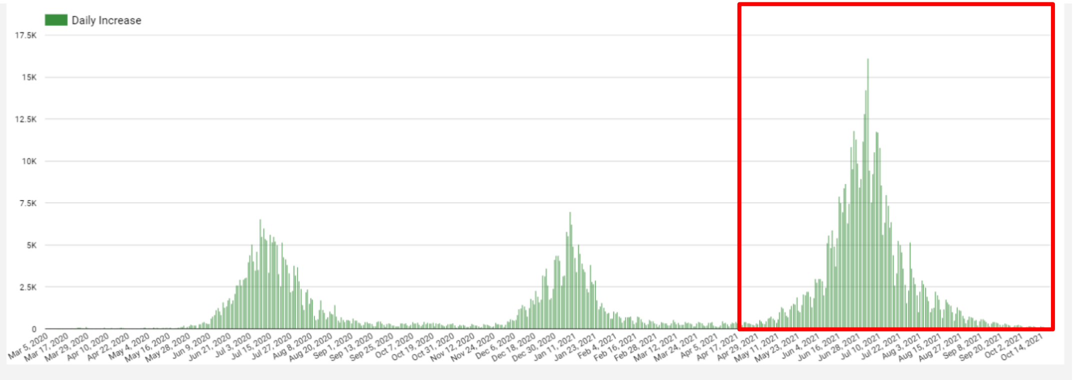


# Methodology: Virus Recovery, Extraction and Assay

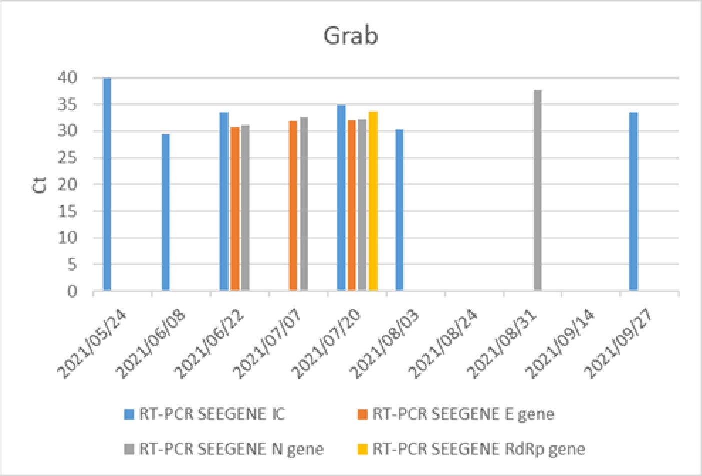
- Recovery method based on the simplest and most cost-effective method from proof-of-concept work
  - Skim milk flocculation
  - Virus recovery efficiency determined with mengovirus
  - 1-2 L sewage samples received and stored at 4°C until processing
  - 200 mL aliquot used for each recovery
- Nucleic acid extraction with QIAamp Ultrasens Virus Kit (Qiagen)
- Passive samples eluted in 10ml PBS with 0.05% Tween 80
- RT-PCR screening for SARS-CoV-2 with real time multiplex Seegene Allplex™ 2019-nCoV Assay RT-PCR
  - E gene
  - N gene
  - RdRp gene
  - Internal control
- Multiple gene targets were chosen for detection due to the environmental variability of the waters
- Ct values below 40 considered positive
- Dilutions of 1:10 are also included routinely due to inhibition of internal controls when screening surface samples

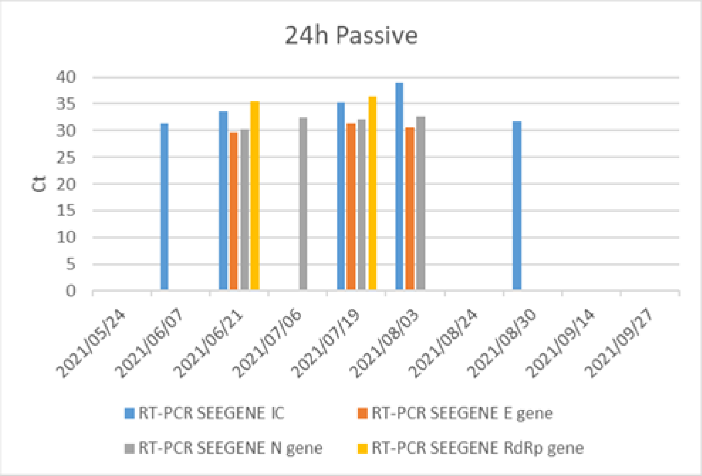
# Gauteng: Grab samples from Jukskei River Source in JHB CBD



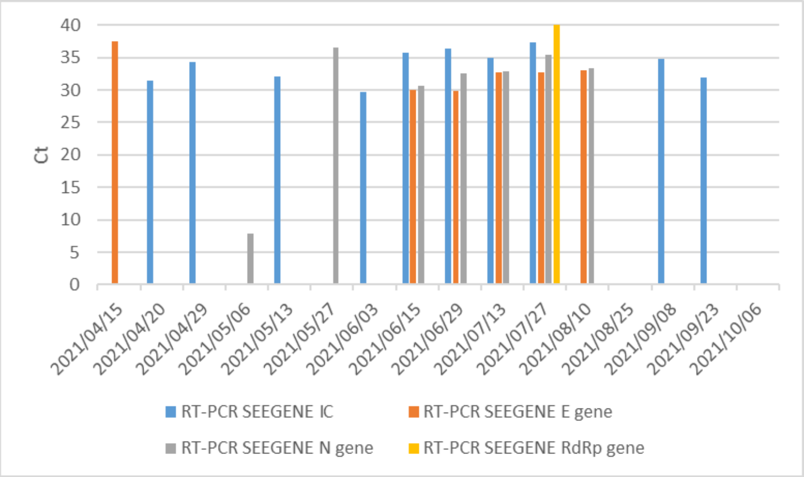


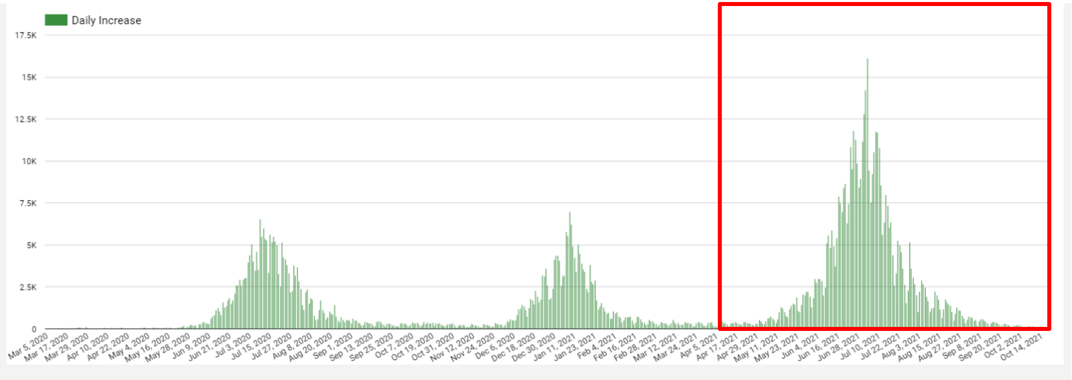
# Gauteng: Passive samples from Jukskei River Source



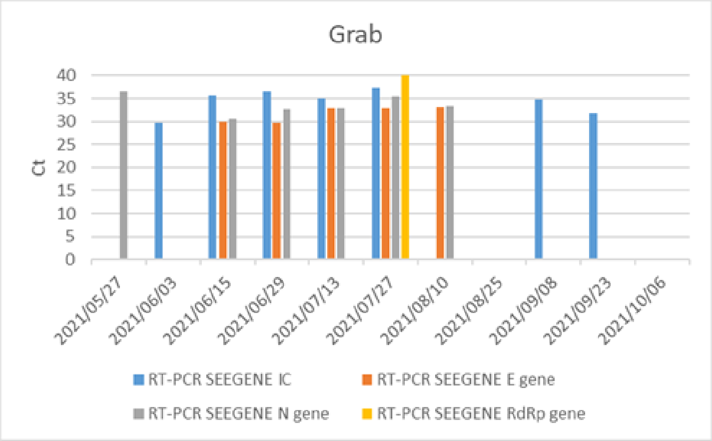


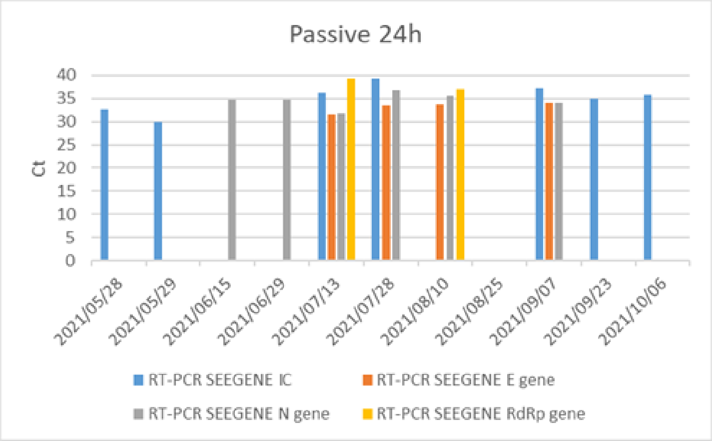
# Gauteng: Jukskei River grab samples downstream Alexandra Informal Settlements



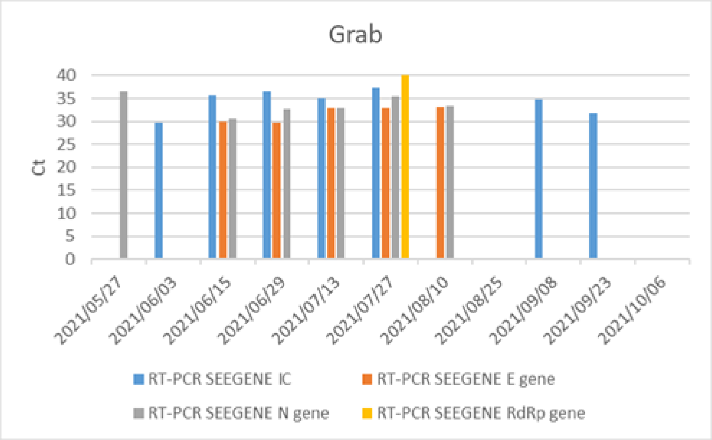


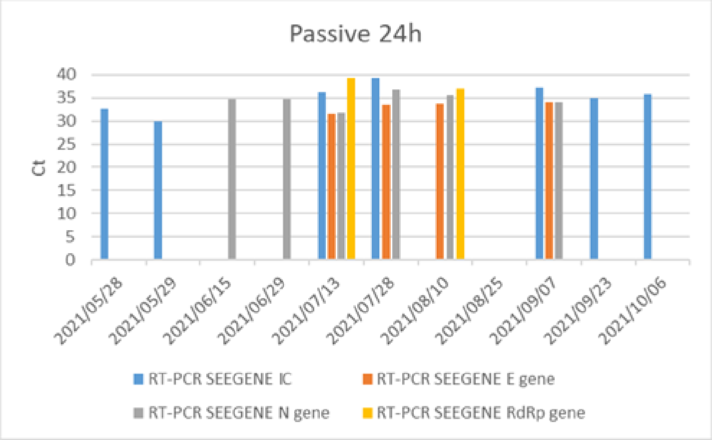
# Gauteng: Jukskei River passive samples (24h)



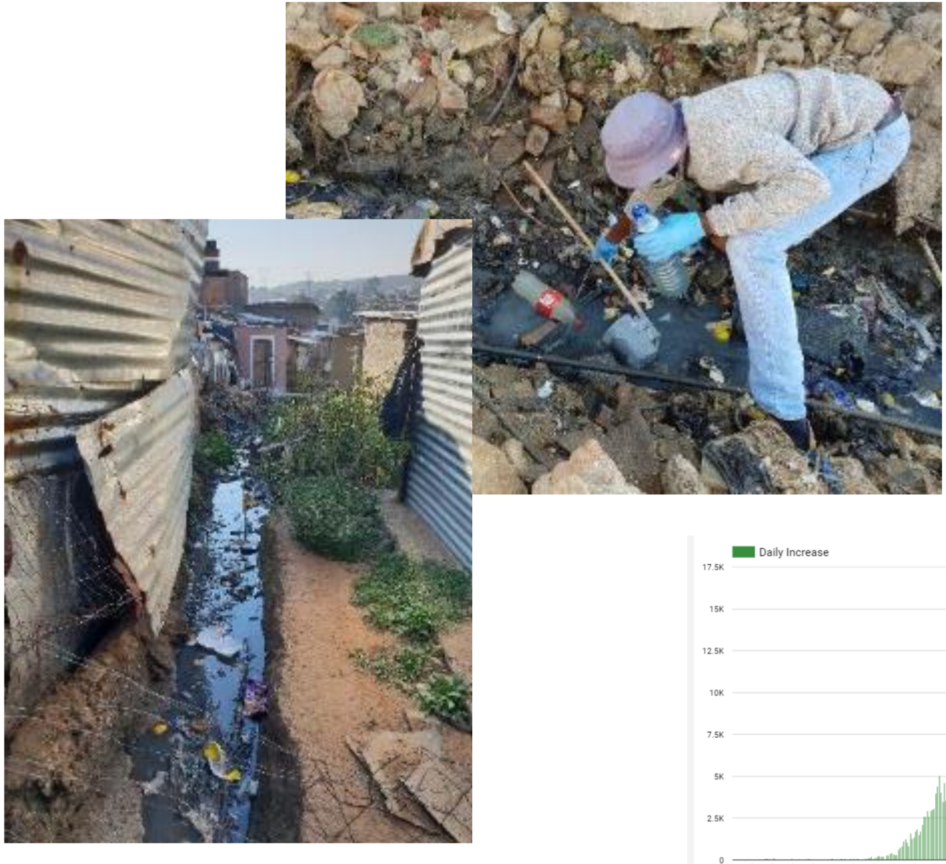


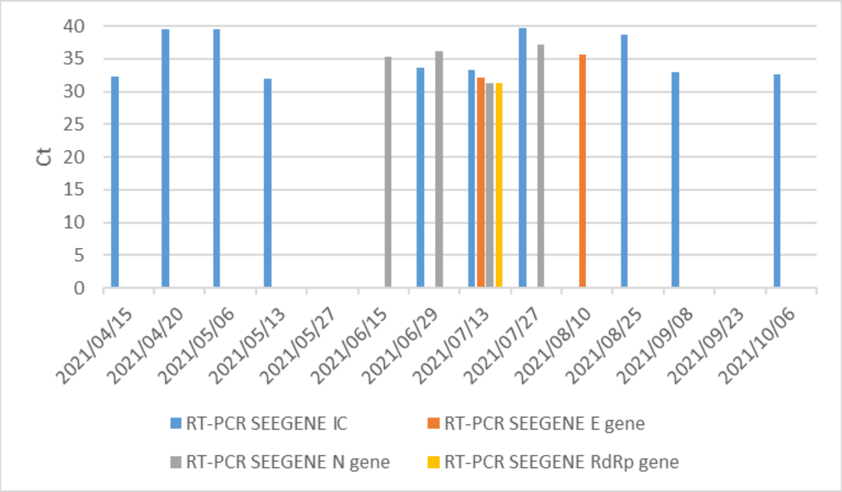
# Gauteng: Jukskei River passive samples (24h)

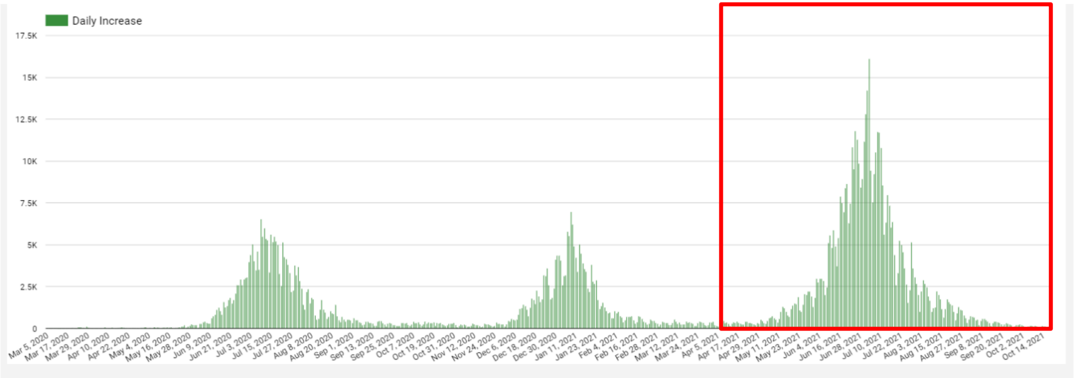




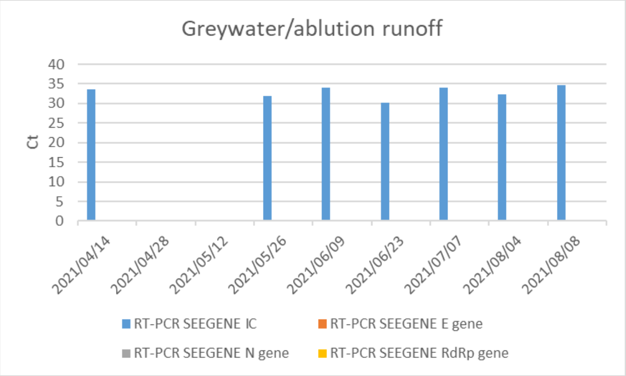
# Gauteng: Informal settlement greywater runoff grab samples

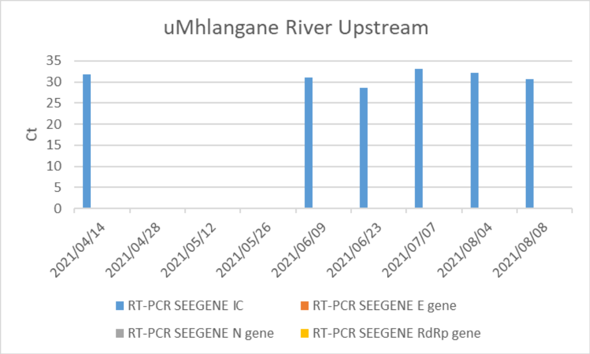


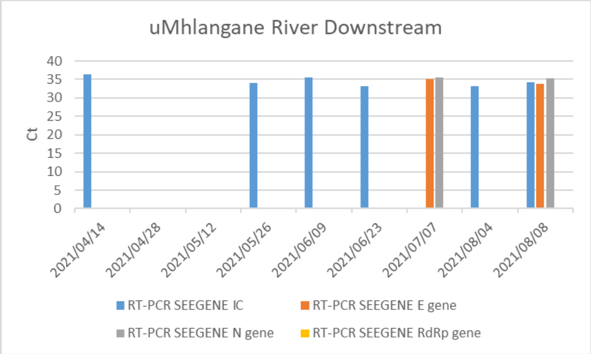


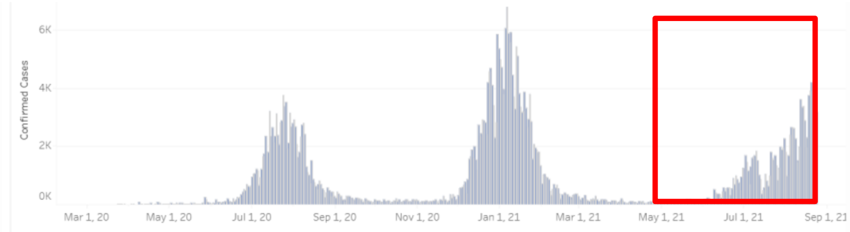


# KwaZulu Natal: eThekwini Grab sampling from Quarry Road Informal Settlement and Palmiet River

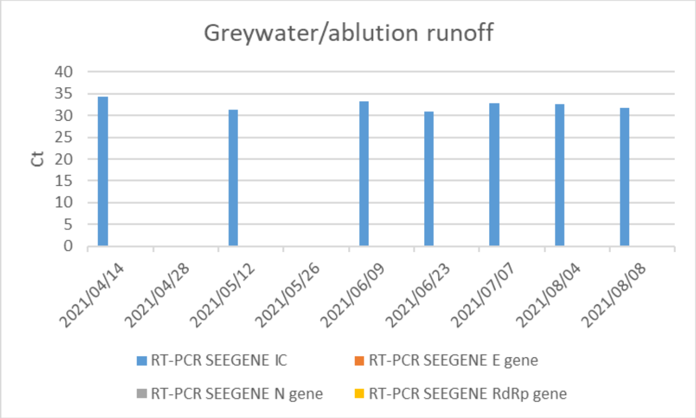

Greywater/ablution runoff

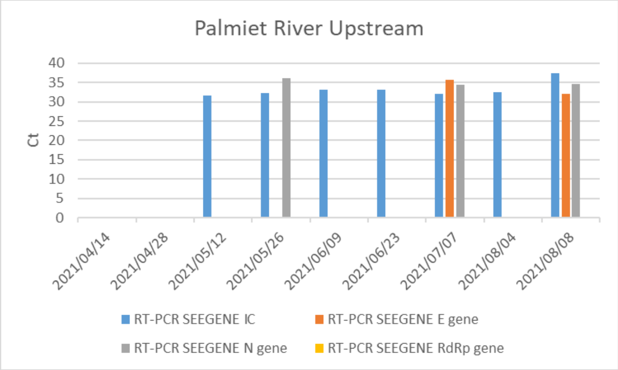

uMhlangane River Upstream

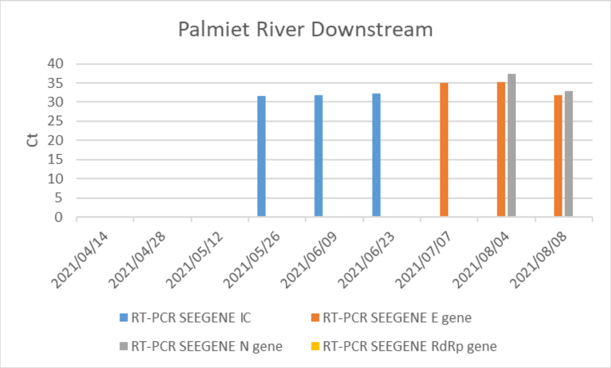

uMhlangane River Downstream

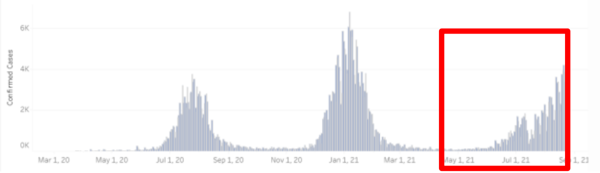


# KwaZulu Natal: eThekwini Grab sampling from Johanna Road Informal Settlement and uMhlangane River

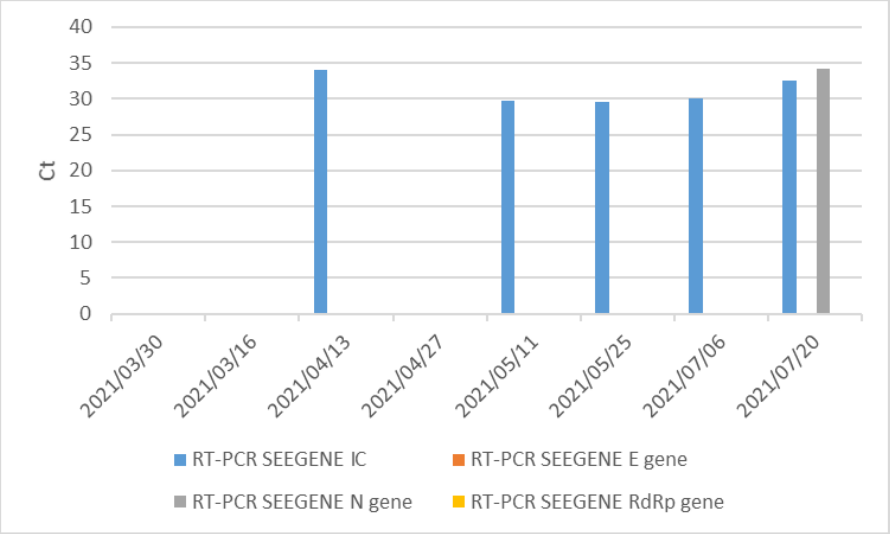


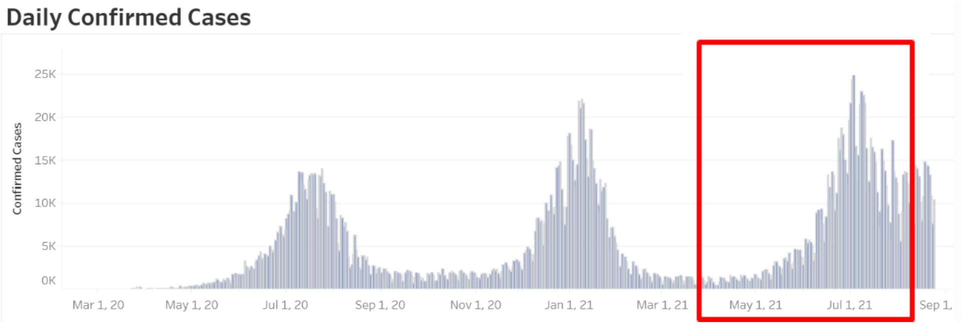




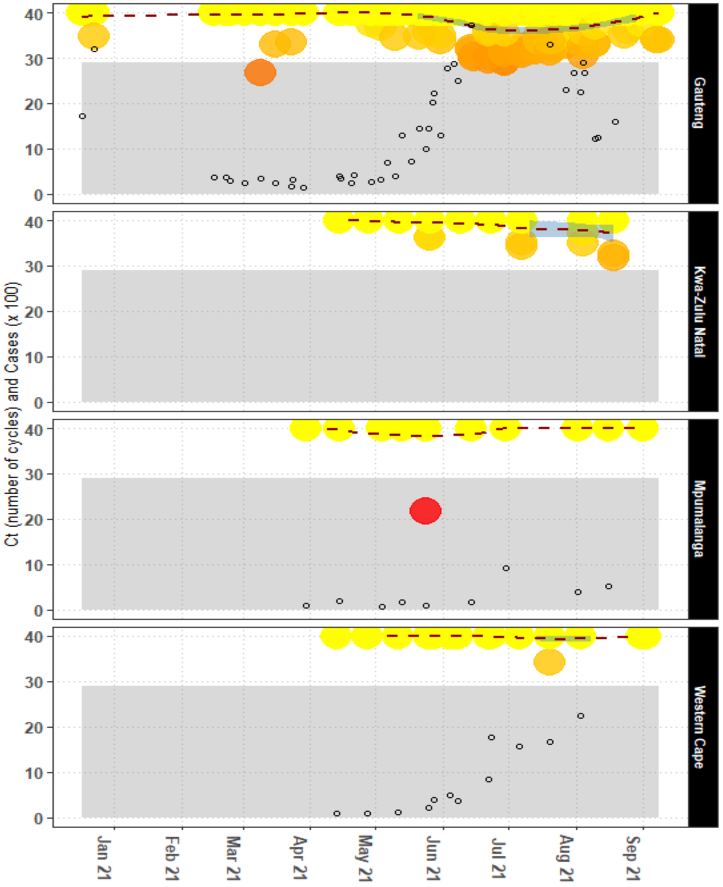


# Western Cape: Plankenbrug River Downstream Informal Settlements in Kayamandi





# National overview: COVID-19 infection in non-sewered communities



- The project team has been able to identify COVID-19 in non sewered community runoff, surface water and in the rivers which lie downstream of these communities
- The data also shows that the incidence of COVID-19 in these communities is reflected in the Ct values obtained in the rivers and surface run-off samples
- In Gauteng province where the informal settlements are dense and the rivers are highly polluted by faecal matter from these communities, the trends are even more evident
- It is difficult to correlate viral loads to clinical cases. However, inclusion of trend monitoring of SARS-CoV-2 prevalence in unsewered communities together with established WBE data collection from WWTW sampling can greatly expand knowledge base and serve to highlight the needs of vulnerable communities

# Challenges and Opportunities

- Sampling logistics are difficult and time consuming, requiring extensive support from municipalities, river action groups and community leaders
- Transport of large volumes of water is costly, and cold chain must be maintained out of rural areas
- Dilution during rainy season may hinder detection at practical processing volumes
- Passive sampling may overcome issues of low yield during high dilution periods, allow for easier and cheaper transport of samples, and improve consistency. Sample processing is much quicker.
- Passive samplers can potentially be used for wastewater-based epidemiology for a broader scope of pathogens than only SARS-CoV-2
  - project team investigating Norovirus and Hepatitus E in wastewater samples
- Sharing of resources to develop environmental specimen banks of extracted nucleic acid material could reduce the burden on individual research groups
- Opportunity for training and capacity building, and development of community “champions” to assist with sampling
- Storing of historical samples will allow for tracking of disease emergence and tracking of variants

# Future Work

- Passive sampling will continue to be conducted at several sample sites in parallel to the grab samples so as to compare more extensive data sets
- Positive samples with low Ct values will now be sequenced with the MiSeq next generation sequencing instrument to identify the specific variants present in those samples and allow for tracking of variants over time
- It is also proposed that indicators of human faecal contamination be monitored in the historical environmental samples
  - crAssphage assays have recently been shown to be effective for normalisation of viral load data
  - This will give information on the extent of human faecal contamination and stormwater dilution

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# Thank you

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