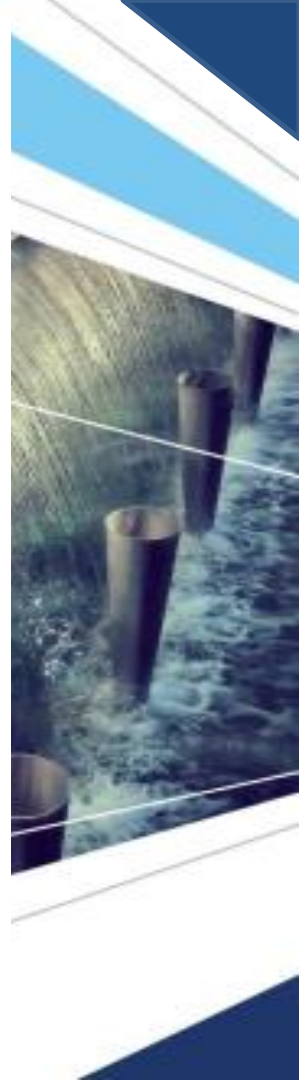


Development of a Framework for Wastewater-Based COVID 19 Epidemiology Surveillance for Non-Sewered Communities

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

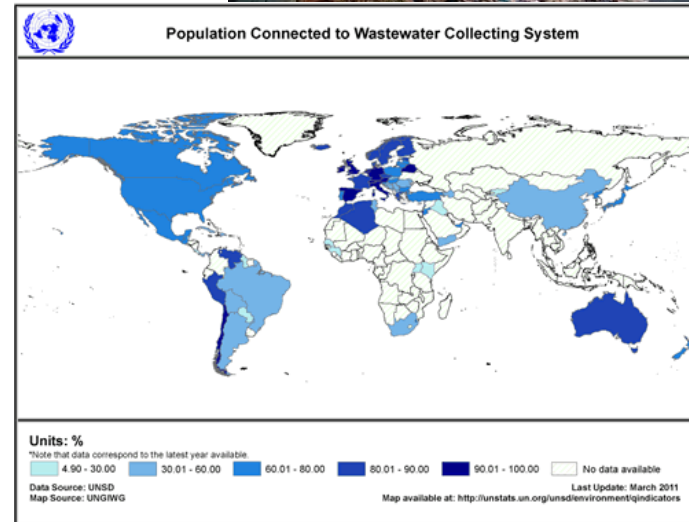


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Introduction

- 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



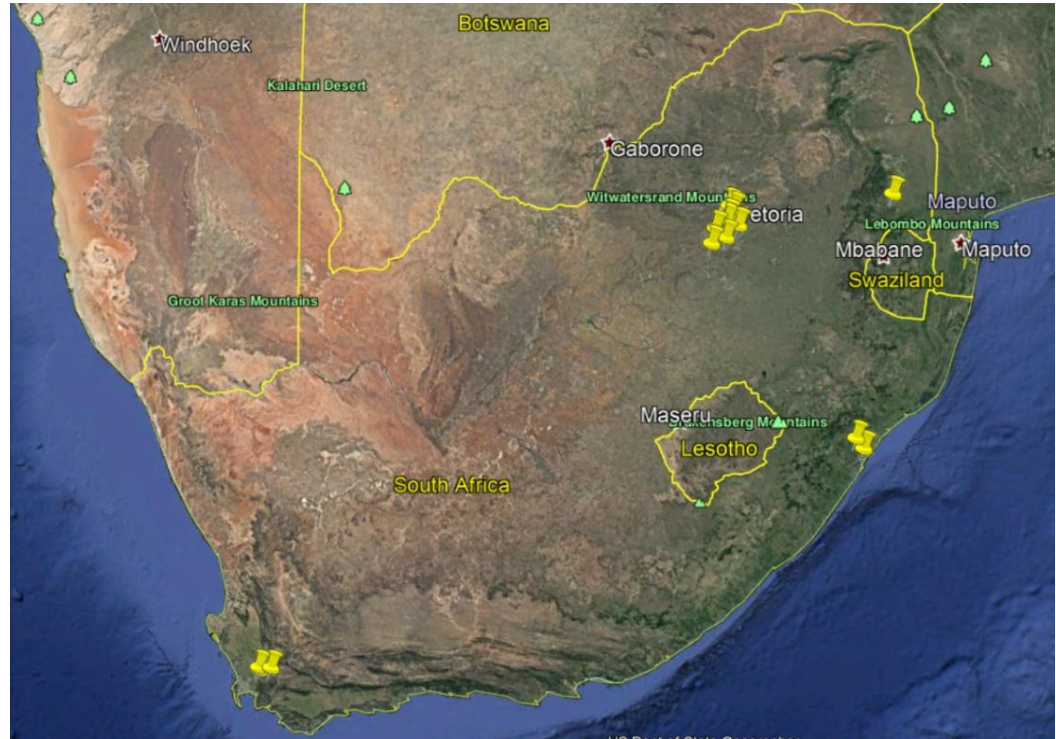
Objectives

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**
 - eThekweni (6)
 - Maphephetheni area North of eThekweni
- **Mpumalanga**
 - Mbombela - Kanyamanzane (2)



Sampling Methodology: Grab samples

- **Surface water**
 - Informal settlements lacking access to sewerage 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
- **On site sanitation**
 - Composite samples from urine diversion toilet systems combining material from 4-5 households
 - Chemical toilets and portable toilets
- These sources may serve to provide early warning of the spread of the virus within the community which has a high likelihood of rapid spread and low likelihood of conventional testing
- Basic water quality parameters analysed per sample
 - pH, SS, COD, EC, Ammonia and Faecal coliforms / *E. coli*

Sampling Methodology: Passive Samples

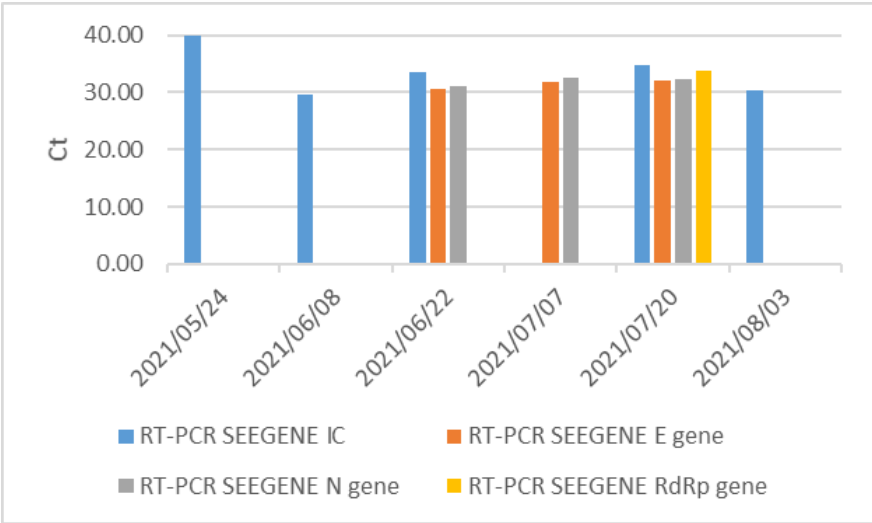
- Methodology developed by Shang et al. (December 2020): “Passive sampling of viruses for wastewater-based epidemiology: a case-study of 2 SARS-CoV-2”, using torpedo device design
 - Device covered in shade cloth and anchored at sample point for a specific period, then virus nucleic acid eluted in the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



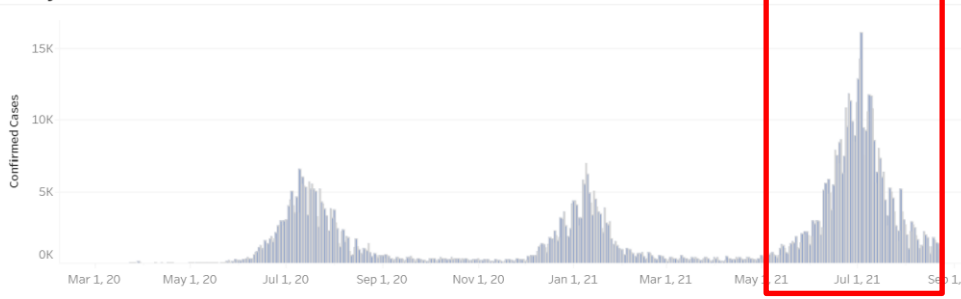
Methodology: Virus Recovery, Extraction and Assay

- 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
 - Disadvantage of multiplex assay: Cannot be used for quantification
- Ct values below 40 considered positive
- Dilutions of 1:10 are also included routinely due to inhibition of internal controls when screening surface samples
- Screening of positive samples with a singleplex RT-PCR assay (N1 /N3) for quantification using SARS CoV-2 N1 and N3 standard curve will be generated using the 2019_nCoV_N positive control plasmid

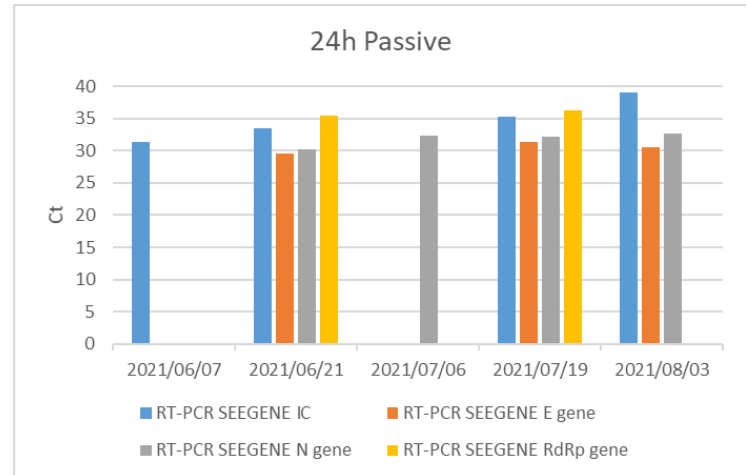
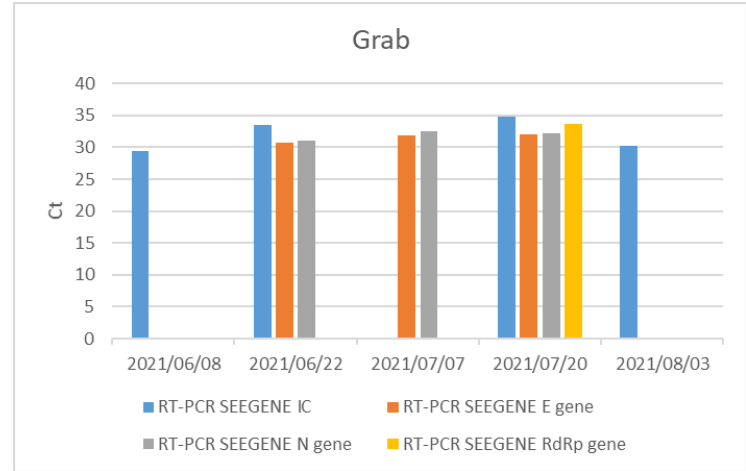
Gauteng: Grab samples from Jukskei River Source in JHB CBD



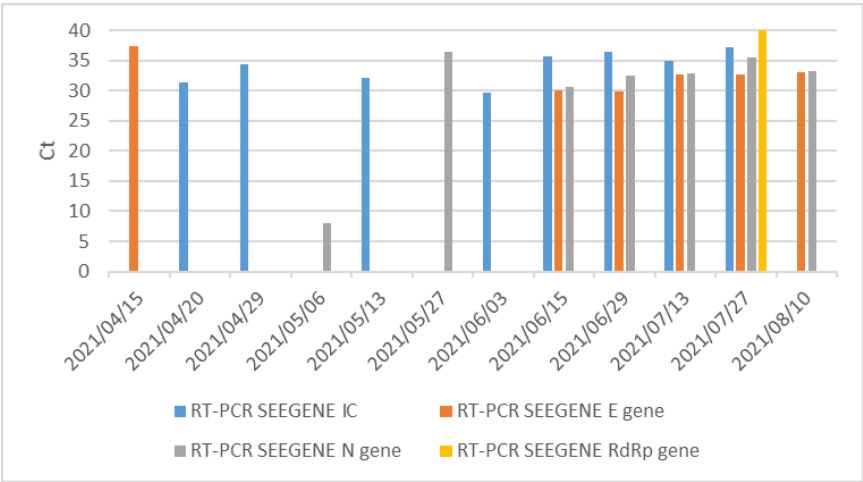
Daily Confirmed Cases



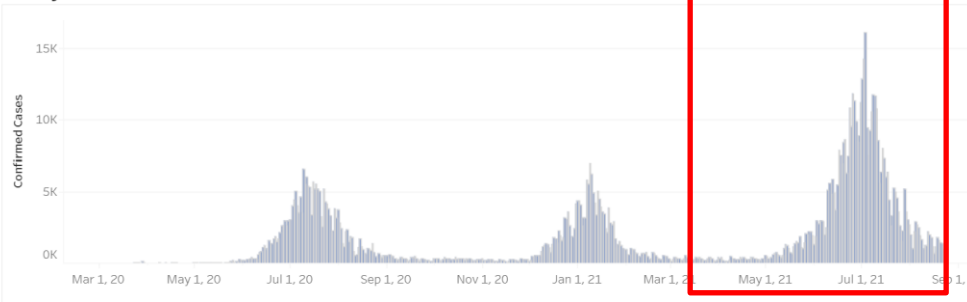
Gauteng: Passive samples from Jukskei River Source



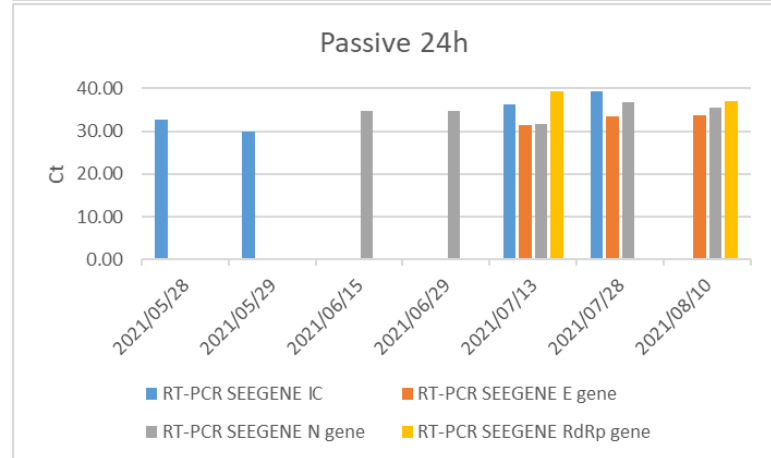
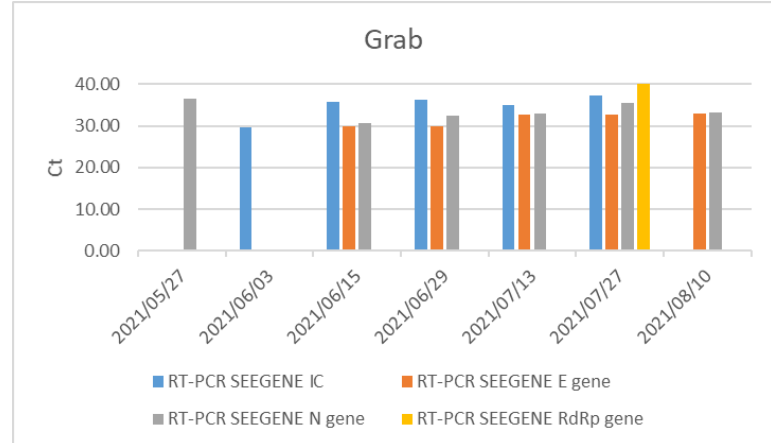
Gauteng: Jukskei River grab samples downstream Alexandra Informal Settlements



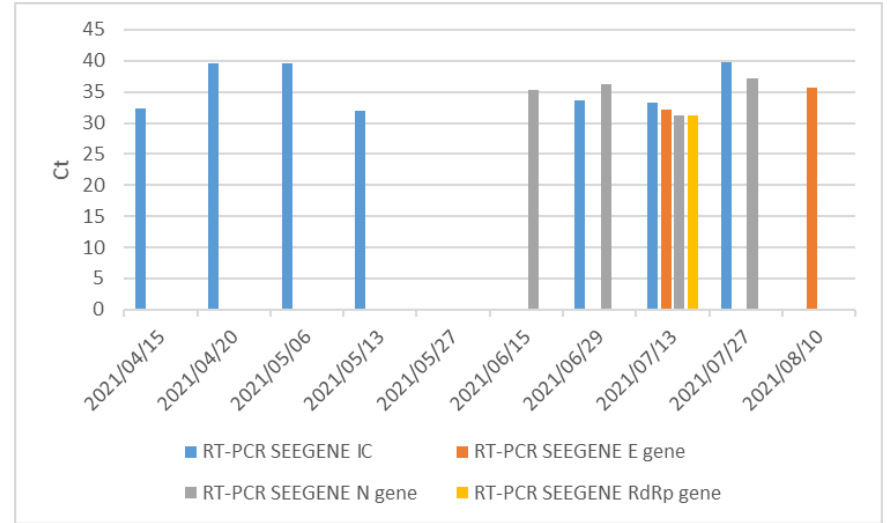
Daily Confirmed Cases



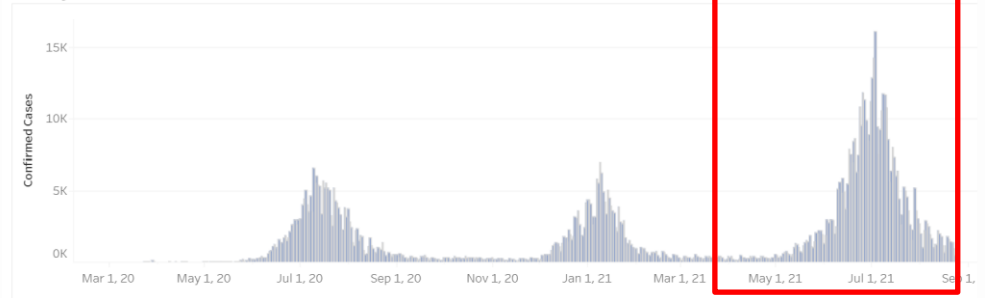
Gauteng: Jukskei River passive samples (24h)



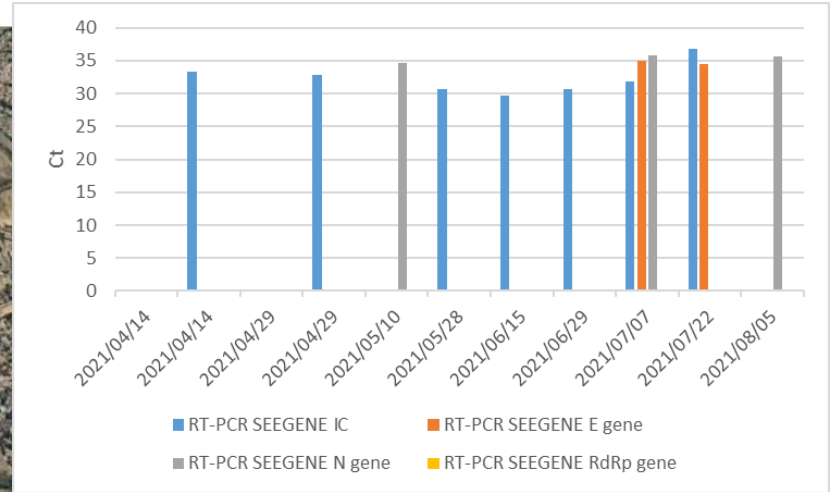
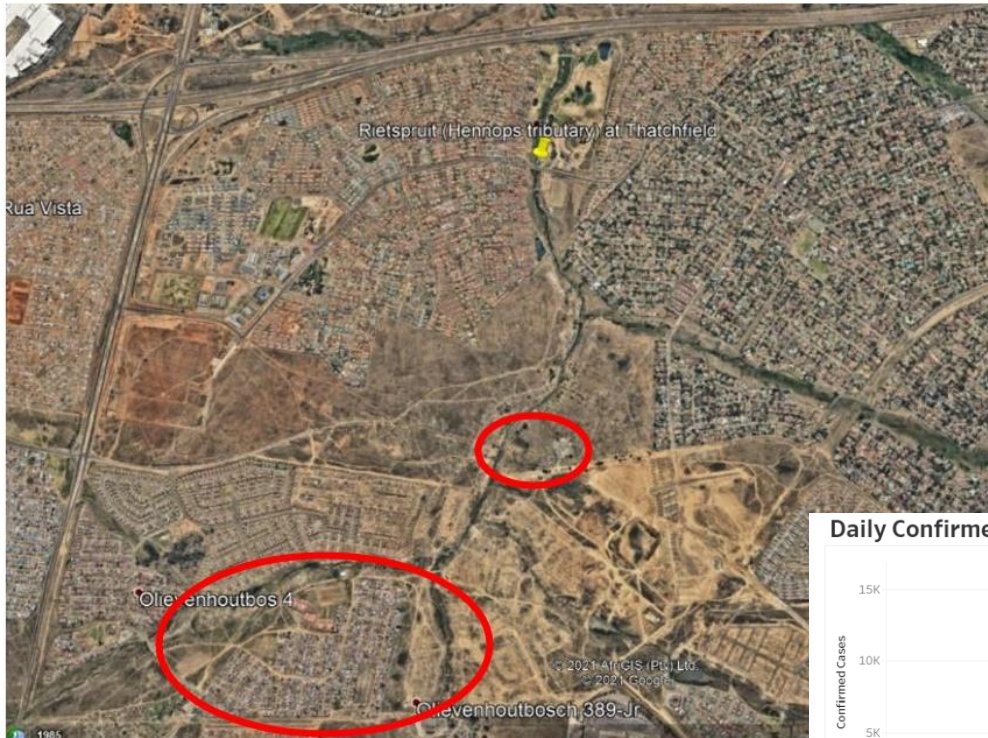
Gauteng: Informal settlement greywater runoff grab samples



Daily Confirmed Cases



Gauteng: Tshwane River Samples, Rietspruit at Thatchfield

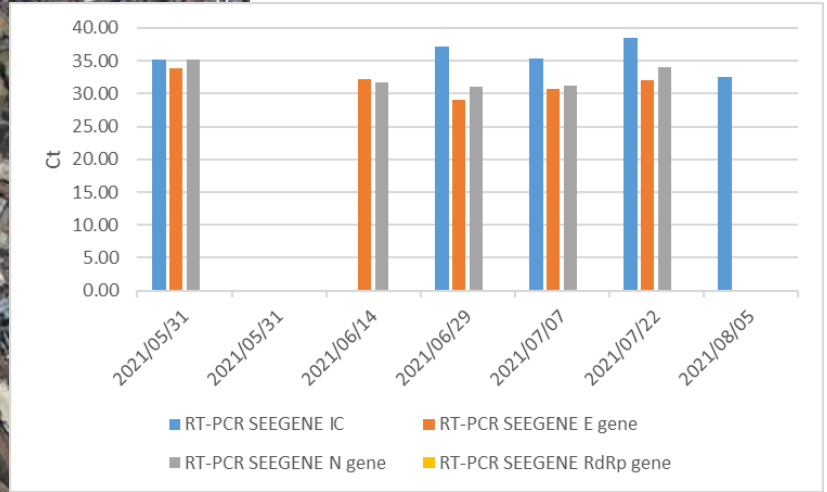


Daily Confirmed Cases

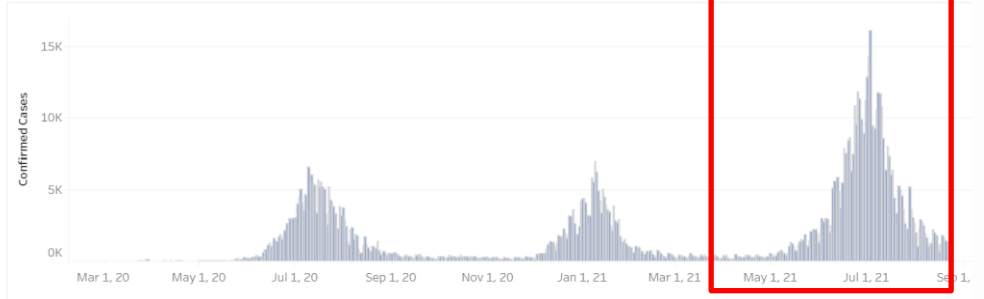


Rietspruit at Thatchfield downstream informal settlements at Olivenhoutbos

Gauteng: Tshwane River Samples, Kaalspruit

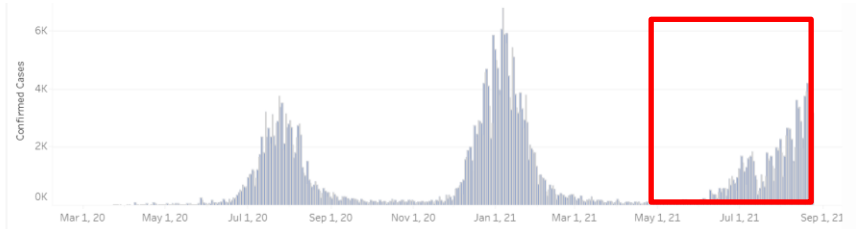
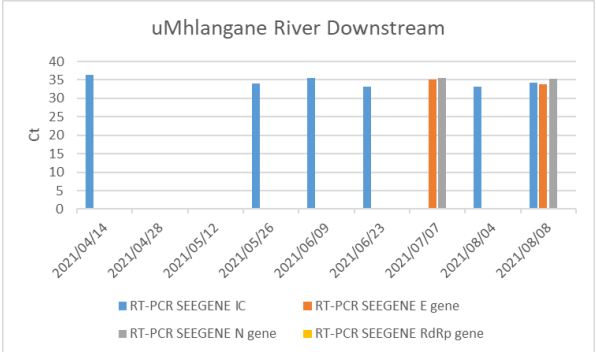
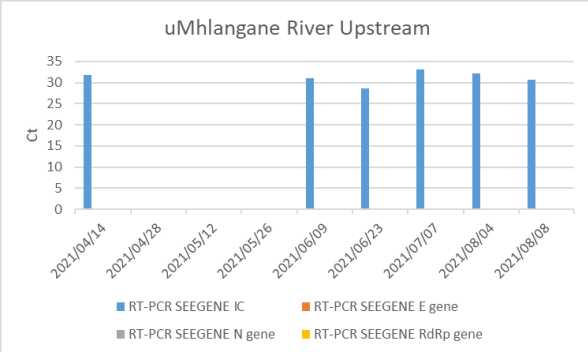
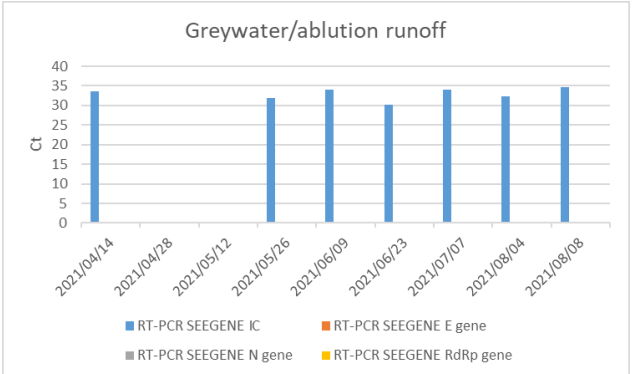


Daily Confirmed Cases

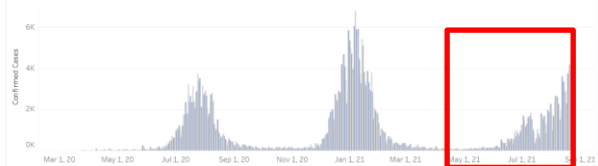
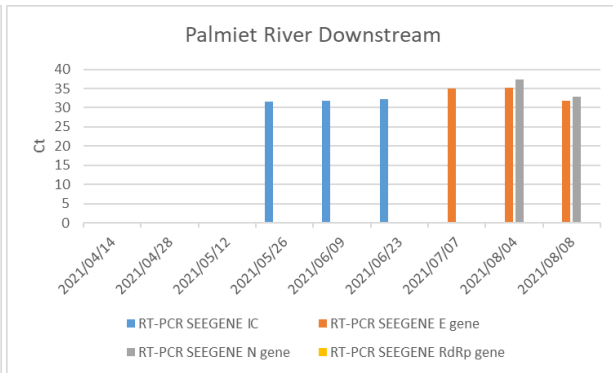
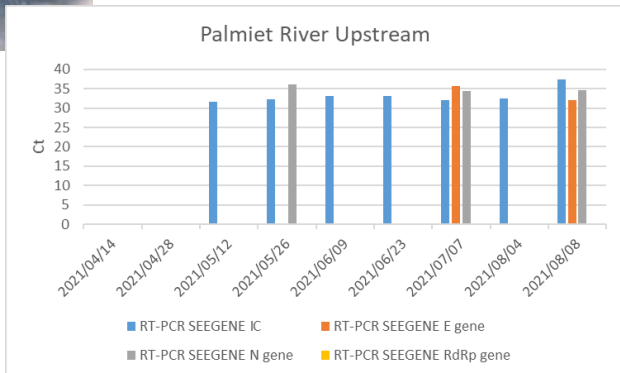
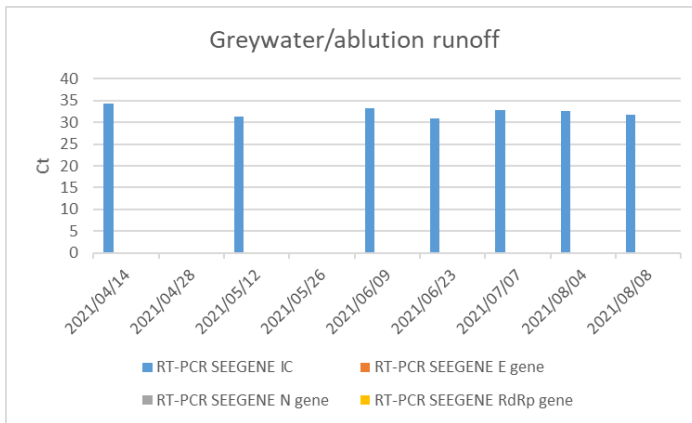


Kaalspruit (Hennops tributary) downstream Thembisa

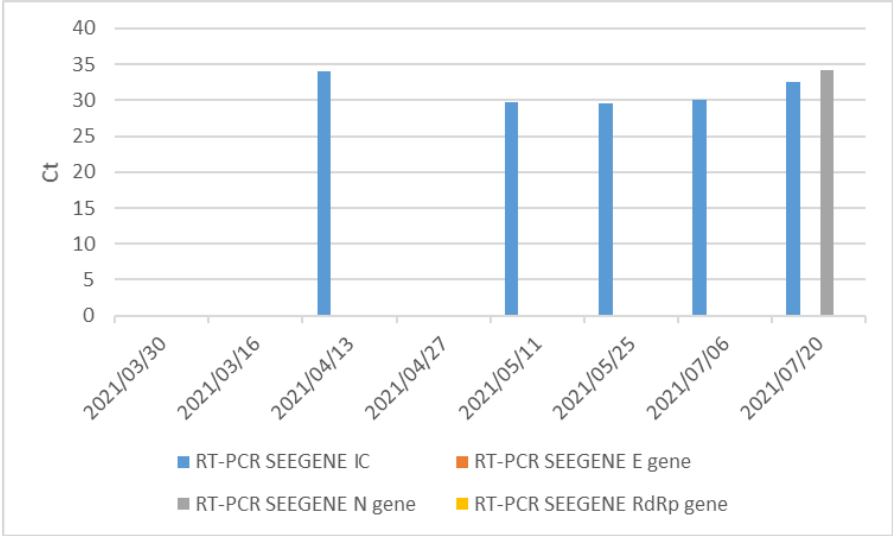
KwaZulu Natal: eThekweni Grab sampling from Quarry Road Informal Settlement and Palmiet River



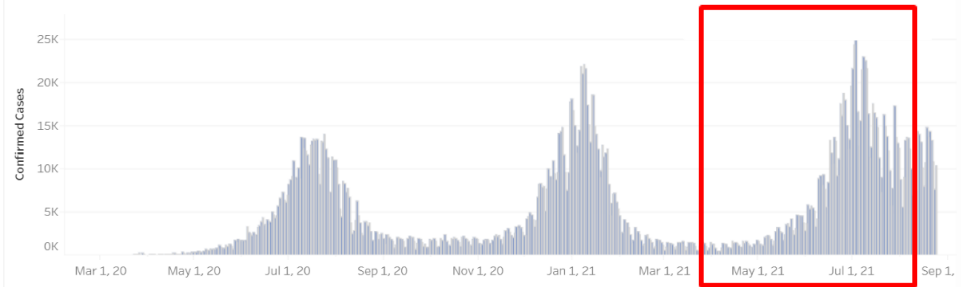
KwaZulu Natal: eThekweni Grab sampling from Johanna Road Informal Settlement and uMhlangane River



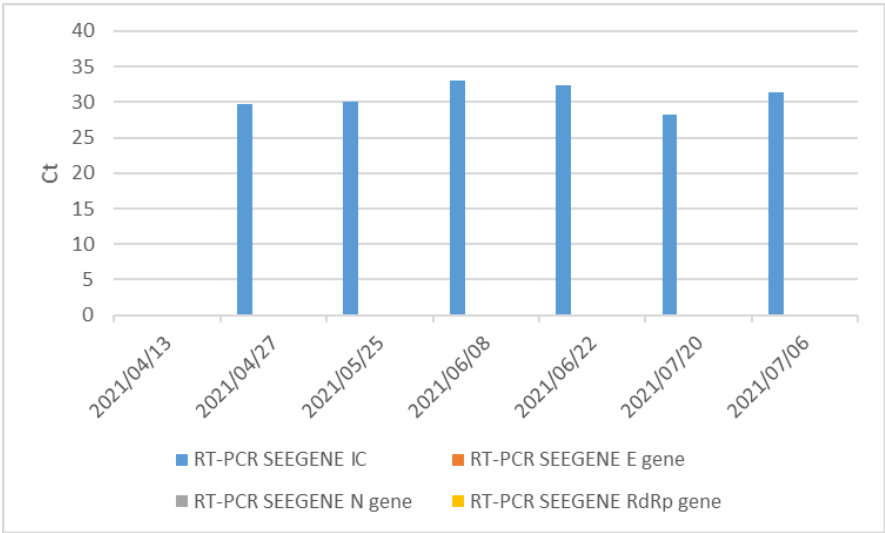
Western Cape: Plankenbrug River Downstream Informal Settlements in Kayamandi



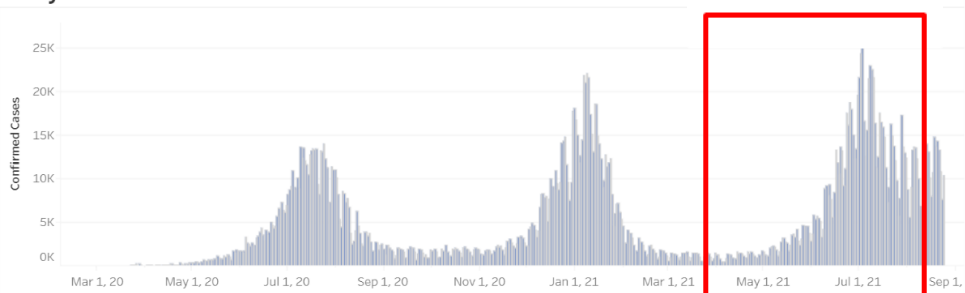
Daily Confirmed Cases



Western Cape: Franschoek River Downstream Informal Settlement



Daily Confirmed Cases



Western Cape: Sampling toilet contents from non-sewered areas



PORTABLE FLUSH TOILETS (PFTs)

- *Supplementary service*
- Encumbered land
- Capital outlay per unit
- Serviced 3 x per week
- Disinfectant added at service



CHEMICAL

- Encumbered land
- Vehicular access
- No capital outlay
- Serviced 3 x per week
- Costly
- 25% of shared toilets

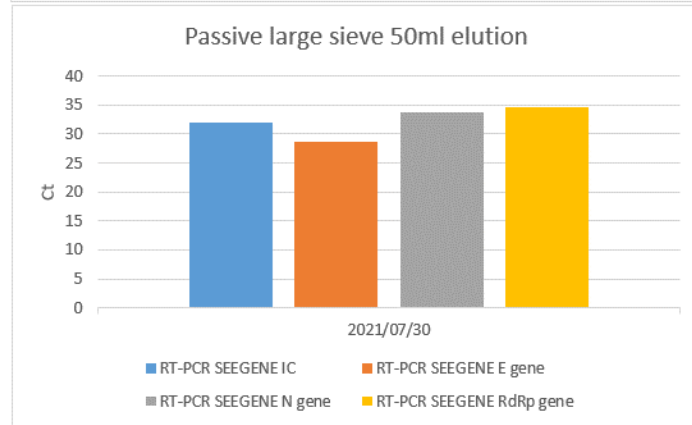
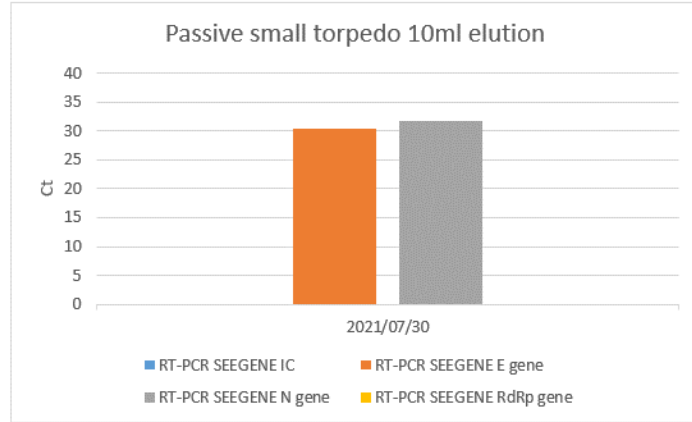




Each truck keeps record of where they collected waste



Passive samplers inserted as the waste is being emptied into WWTW by tanker



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
- Sampling from on-site sanitation is costly and impractical
- Difficult to correlate viral loads to clinical cases. However, monitoring trends in viral load can be used successfully to implement an early warning system and assess community infections

- Opportunity for training and capacity building, and development of community “champions”
- 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.
- 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



Thank you



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