Proof of Concept Studies on the Detection of SARS-CoV-2 in Sanitation Samples in South Africa

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Background: COVID-19

- COVID-19 caused by *Severe Acute Respiratory Syndrome Coronavirus 2* (SARS-CoV-2)
- Recognized as a pandemic by WHO on 11 March 2020
- Main transmission route of virus is direct and indirect contact through patient respiratory droplets
- Science is continuously progressing with regards to other possible detection and transmission routes for SARS-CoV-2:
  - Virus detection and persistence (does not infectious) in patients stool samples after pharyngeal swabs became negative
  - Detected in faeces and urine of infected patients
  - Aerosol transmission potential needs to be validated
  - The genetic material of viral SARS-CoV-2 can be detected in wastewater – this does not mean infectivity
  - Viral RNA detected in wastewater around the world: Netherlands, U.S.A, Australia, Italy, Israel, France
  - Lesson learned from 2002–2004 SARS outbreak: SARS-COV-1

COVID-19 Surveillance – Hotspot Detection

TIONAL POLITICS CORONAVIRUS COMPANIES ECONOMY BUSINESS W

Covid-19 was already in Italy in December, sewage study suggests

Covid-19 may have been circulating before China reported the first cases of a new disease on December 31, scientists say

19 JUNE 2020 - 12:35 *by* DEENA BEASLEY, KATE KELLAND AND EMILIO PARODI

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2:05

SARS-CoV-2 RNA concentrations in primary municipal sewage sludge as a leading indicator of COVID-19 outbreak dynamics

Jordan Peccia[1,‡,*], Alessandro Zulli[1,‡], Doug E. Brackney[2,‡], Nathan D. Grubaugh[3], Edward H. Kaplan[8,4,1], Arnau Casanovas-Massana[3], Albert I. Ko[3], Amyn A. Malik[5,6], Dennis Wang[5], Mike Wang[5], Daniel M. Weinberger[3], Saad B. Omer[3,5,6,7,*]

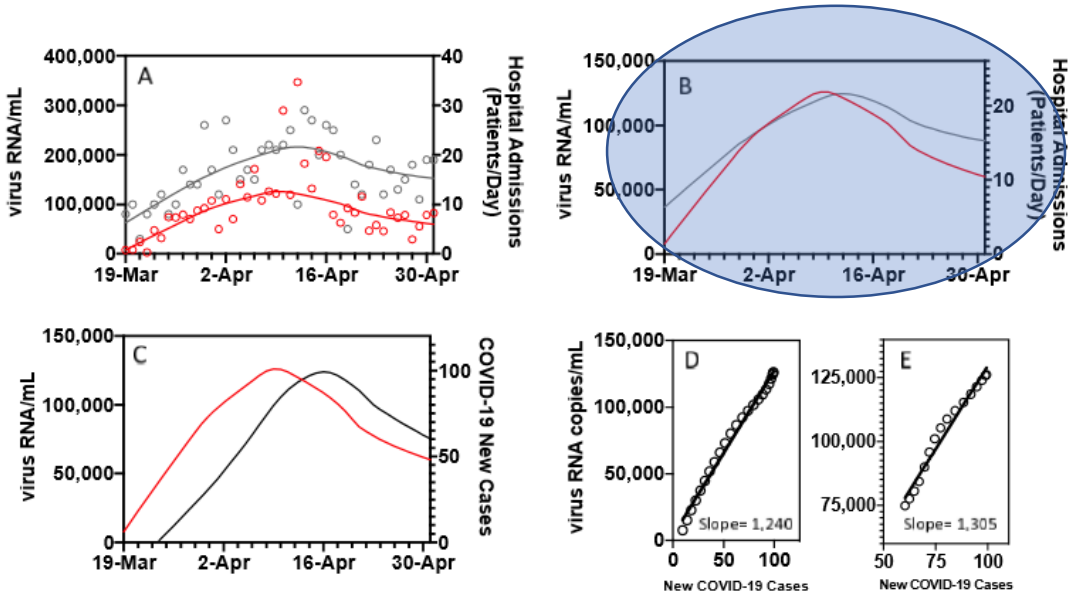


Figure 2. (A) Average sludge SARS-CoV-2 RNA concentration time course data (o) and average hospital admissions data (o) with LOWESS smoothing; (**B**) rescaled smoothed SARS-CoV-2 virus RNA concentrations (---) and hospital admissions (---); **(C)** smoothed sludge SARS-CoV-2 virus RNA concentration (---) with smoothed COVID-19 epidemiology curve (---); (**D**) regression between smoothed virus RNA and new COVID-19 cases (ascending), slope=1,240 virus RNA copies/new case, R^2=0.99; (**E**) regression between smoothed virus RNA and new COVID-19 cases (descending), slope=1,305 virus RNA copies/new case, R^2=0.97.

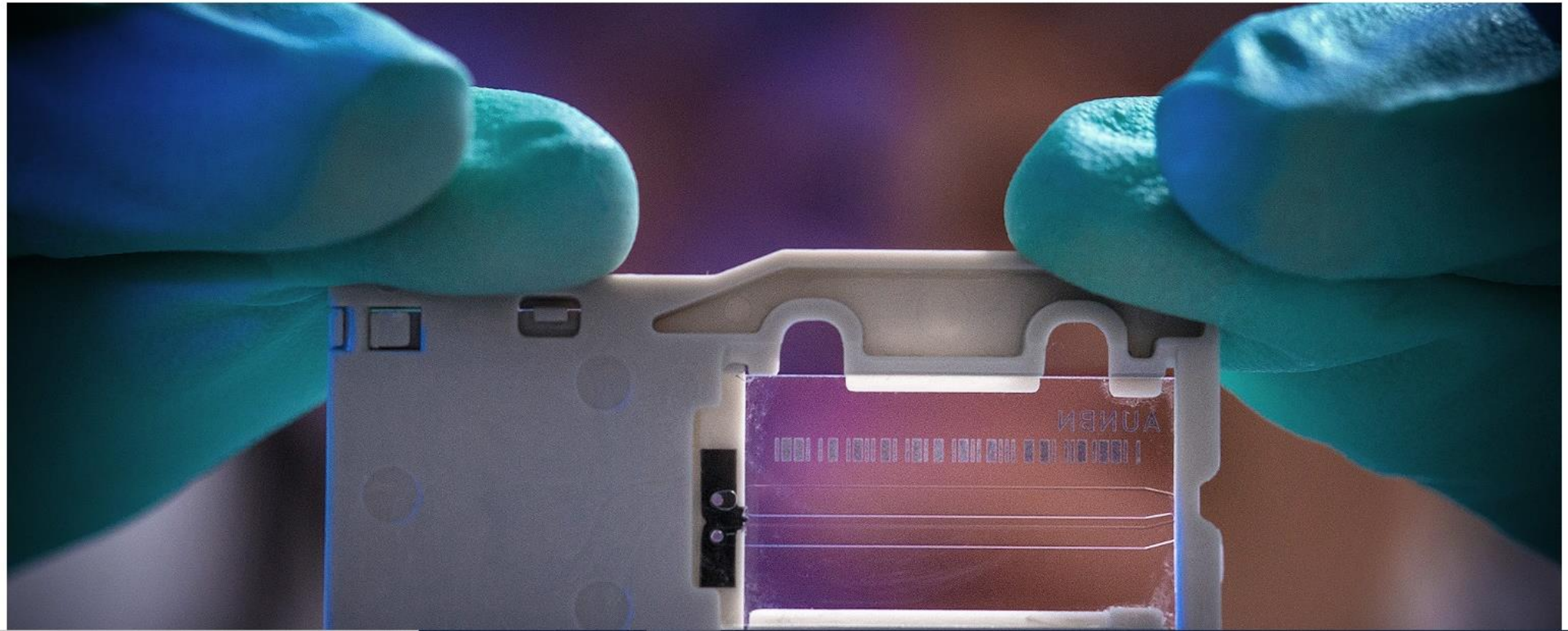
Real Time PCR (RT-PCR)

- Laboratory technique of molecular biology based on the polymerase chain reaction (PCR).
- Monitors the amplification of a targeted DNA molecule during the PCR
- Cycle Threshold (Ct): number of cycles required for the fluorescent signal to cross the threshold (background level)
- Higher the Ct level the lesser the amount of target nucleic acid in the sample
- **Cts < 29** are strong positive reactions indicative of high target nucleic acid in the sample
- **Cts of 30-37** are positive reactions indicative of moderate amounts of target nucleic acid
- **Cts of 38-40** are weak reactions indicative of minimal amounts of target nucleic acid which could represent an infection state or environmental contamination

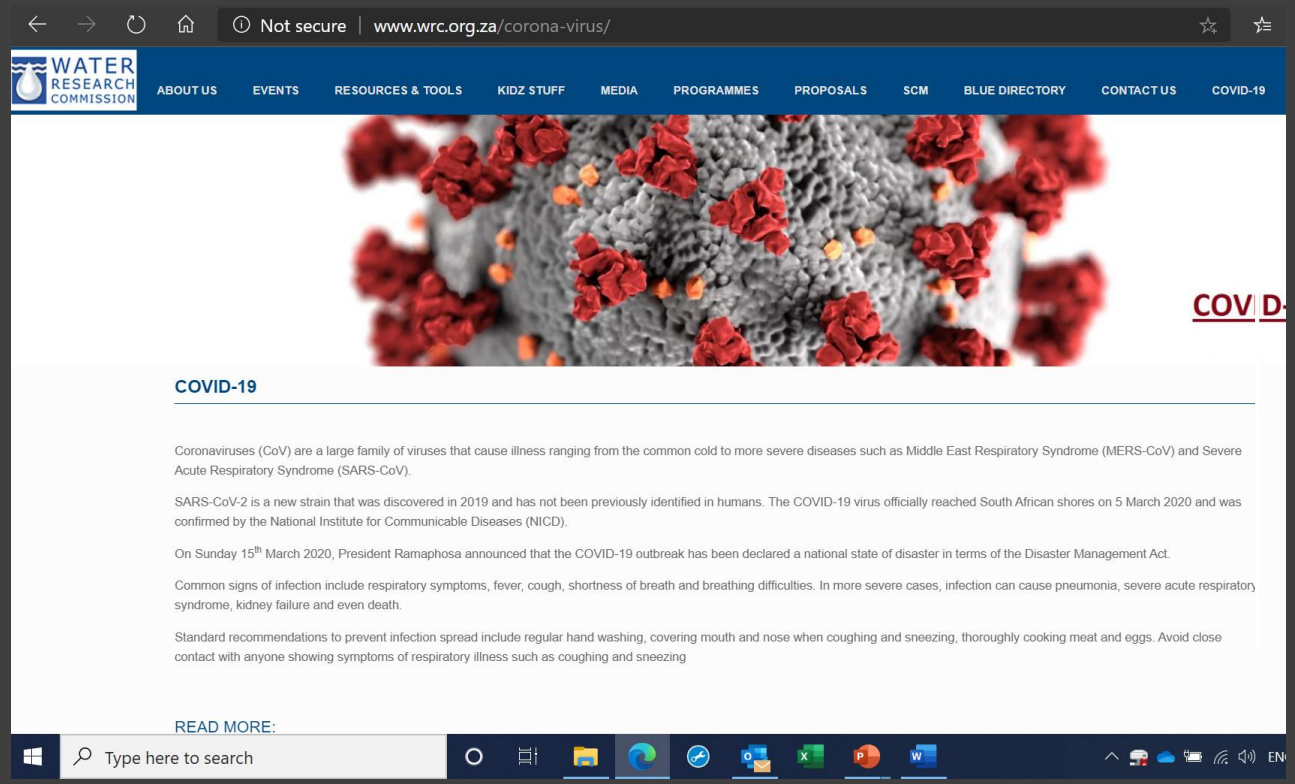
OP-ED

SA needs a surveillance programme as Covid-19 RNA signals show up in wastewater samples

By Jay Bhagwan, Nonhlanhla Kalebaila and Dhesigen Naidoo • 14 July 2020



WRC COVID-19 Website



Guidelines

- Stop-the-spread-of-germs
- A Booklet with South African Guidelines, Manuals, & Literature on WWT – 1985 – 2010
- W2RAP Guideline
- Selection of Management Options
- Requirements for thermal sludge management practices and for commercial products containing sludge
- Requirements for the beneficial use of sludge at high loading rates
- Requirements for the Agricultural Use of Wastewater Sludge
- Guidelines on using W2DST_Final
- Guidelines on using W2DST
- A Booklet with South African Guidelines, Manuals, & Literature on WWT – 1985 – 2010

Fact Sheets

- Fact Sheet 1_Water Quality , Sanitation and Hygiene Management in light of Covid-19
- Fact Sheet 2_Maintaining Good Hand Hygiene
- Fact Sheet 4_Death and burial in the time of covid-19 environmental and health risks

Infographics

English Infographics:

Infographic 1_ Water Quality , Sanitation and Hygiene Management in light of Covid-19

Infographic 2_ Maintaining Good Hand Hygiene

Vernacular Infographics:

Infographic WRC_COVID-19_Infographic 1_Xhosa

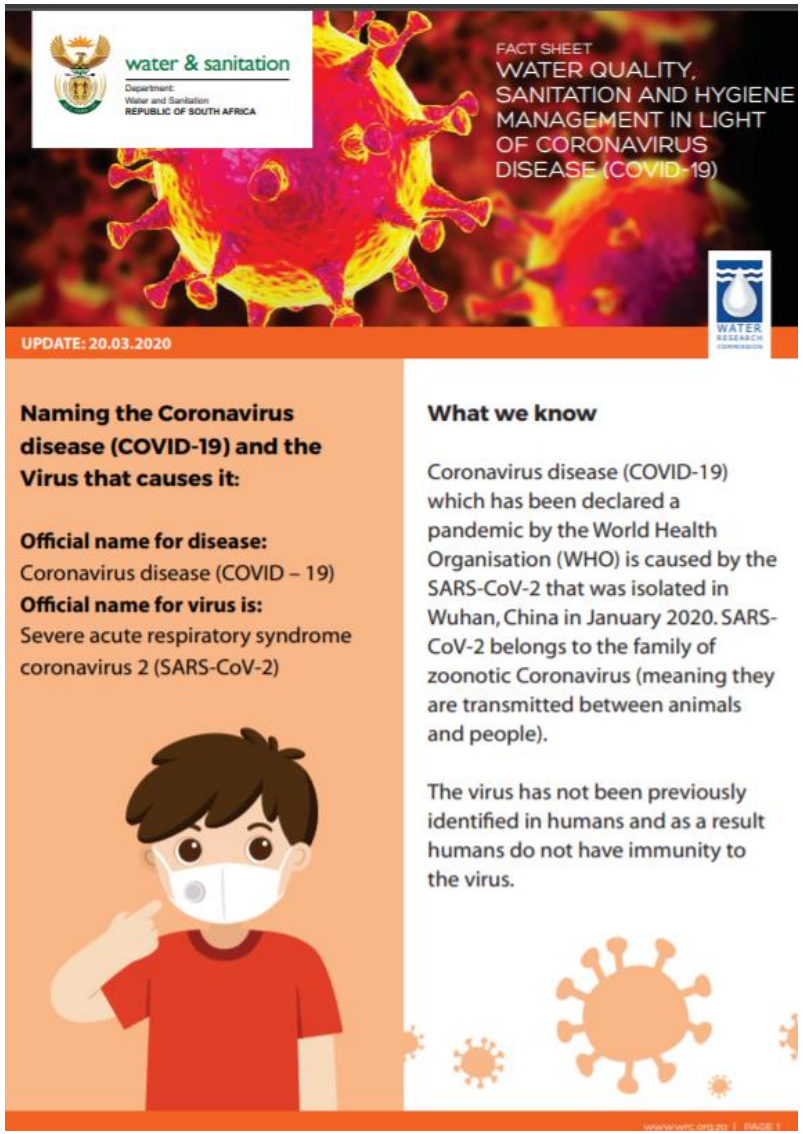
Infographic WRC_COVID-19_Infographic 1 Isizulu

Infographic WRC_COVID-19_Infographic 1 Sepedi

General Information

- Water filter brochure2
- COVID19-symptoms
- Persistence of coronaviruses on inanimate surfaces and

Knowledge Products



South African COVID-19 Sanitation-based Surveillance Programme

- National programme to monitor the country's water and sanitation streams for remnants of the virus
- National surveillance programme could assist health officials in tracking Covid-19 cases and mapping hotspot areas in communities
- Programme is being undertaken in Phases:
  1. Wastewater-based epidemiology – Proof-of-concept
  2. Pilot-scale monitoring using established sampling protocols and design
  3. Full-scale national sewershed surveillance
- Wastewater programme is being completed by Non-Sewered Sanitation Surveillance programme
  - No developing country is 100% sewered
  - 2 Billion people rely on NSS – Could we develop a similar surveillance tool for NSS areas?

Proof-of-Concept – Establishing Methods

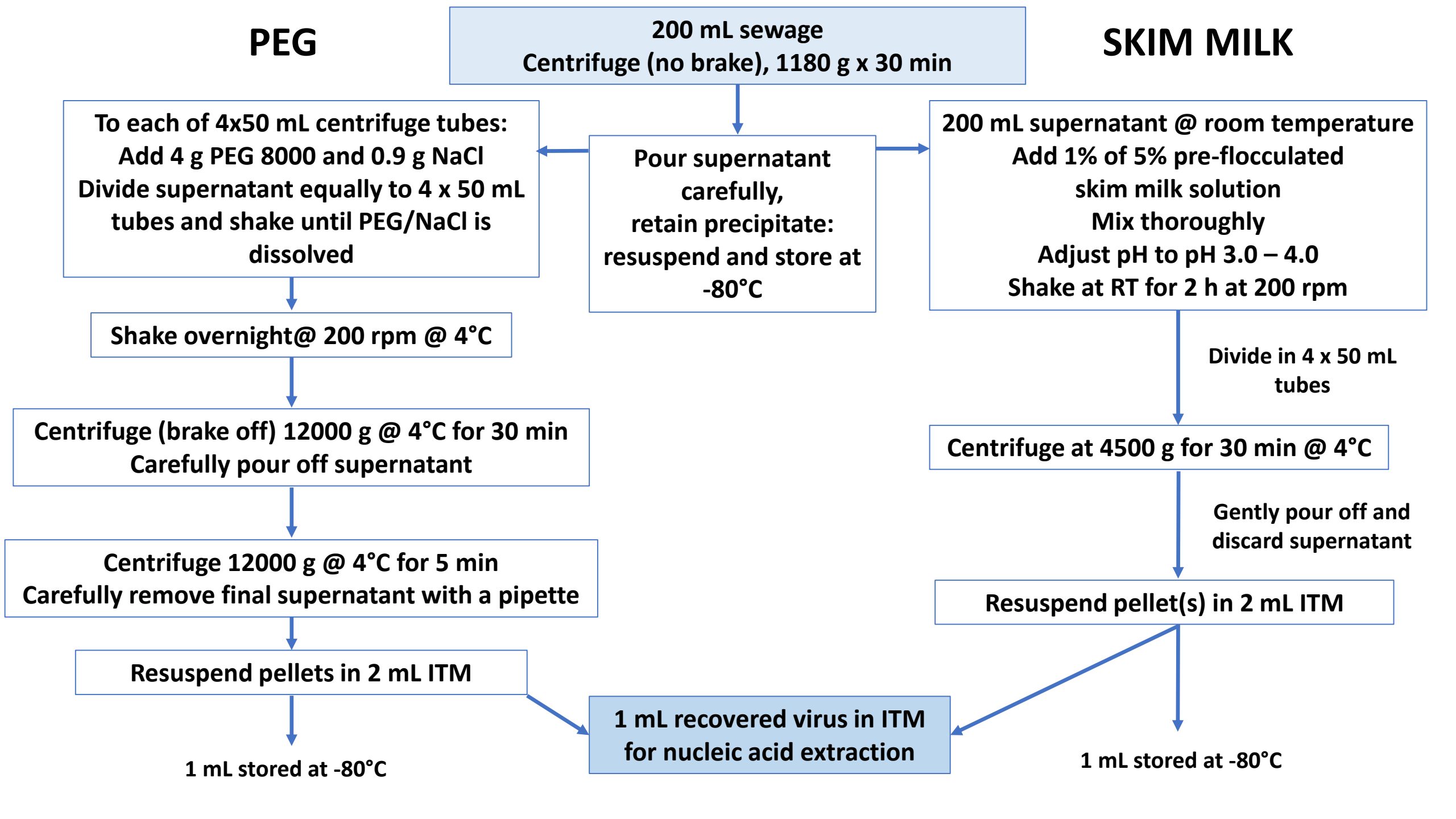
- Testing and validation of a sampling protocol for raw sewage and settled primary sludge
- Testing and validation of the virus extraction and analysis protocol
- Testing and validation of a sampling and extraction protocol for surface and groundwater
- Development of preliminary methodology for quantification of viral load as an indicator of number of infected individuals in a community
- Guidance on data analysis/interpretation
- Recommendations for data communication and integration into national reporting platforms

Sampling Methodology

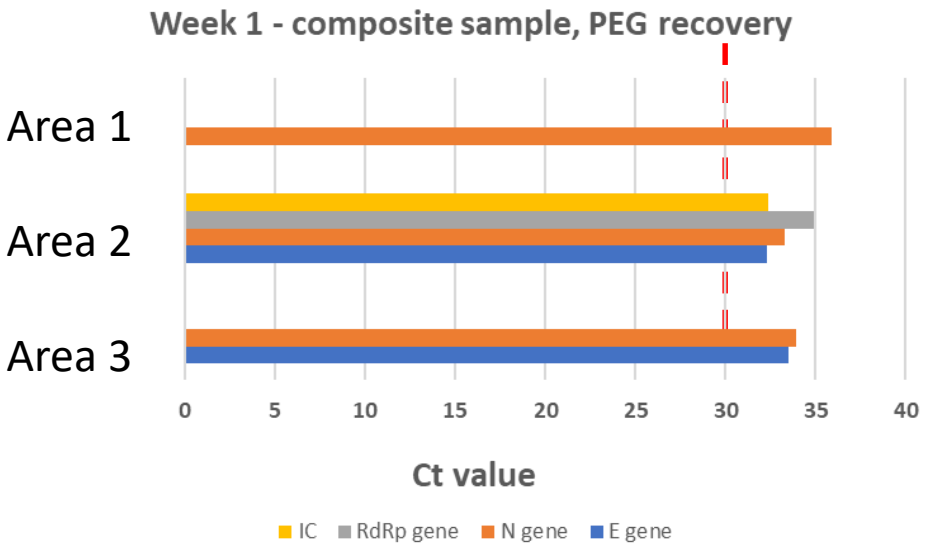
- Three (3) geolocations (provinces)
- Grab and composite samples:
  - 24-hour WWTW influent composite samples from hotspot Metros
  - Grab sampling of influent during peak flow periods to determine if better detection is observed
- Primary sludge grab samples will be taken from selected sites for comparison in recovery
- Weekly samples for 4 weeks – obtain a picture of prevalence of the virus in sewage
- Sewage from hospitals in each hotspot metro will be sampled on a weekly basis
- Surface water sampling: grab samples from rivers, groundwater and surface run-off
- Composite samples from industrial site sewage treatment plants: Eskom power stations

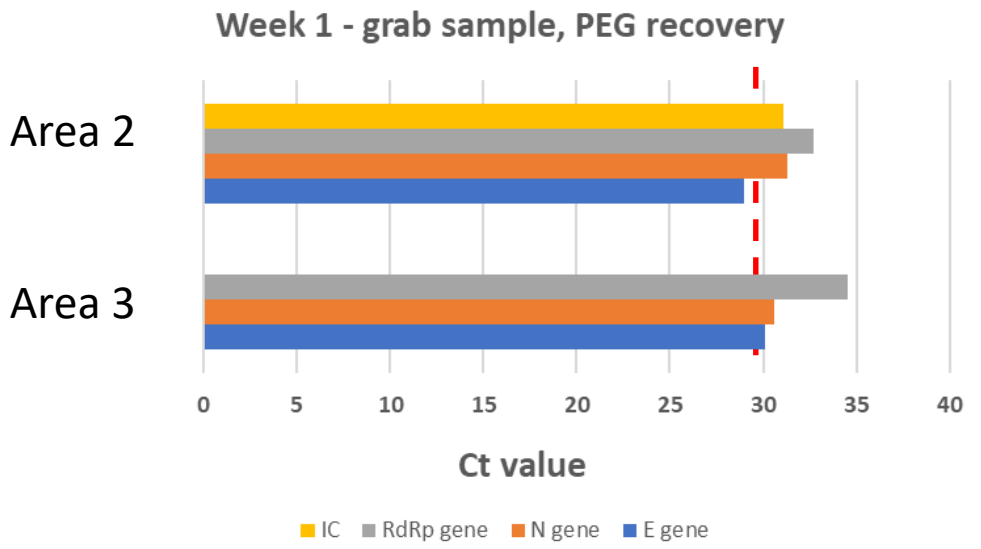
Virus Recovery Methods

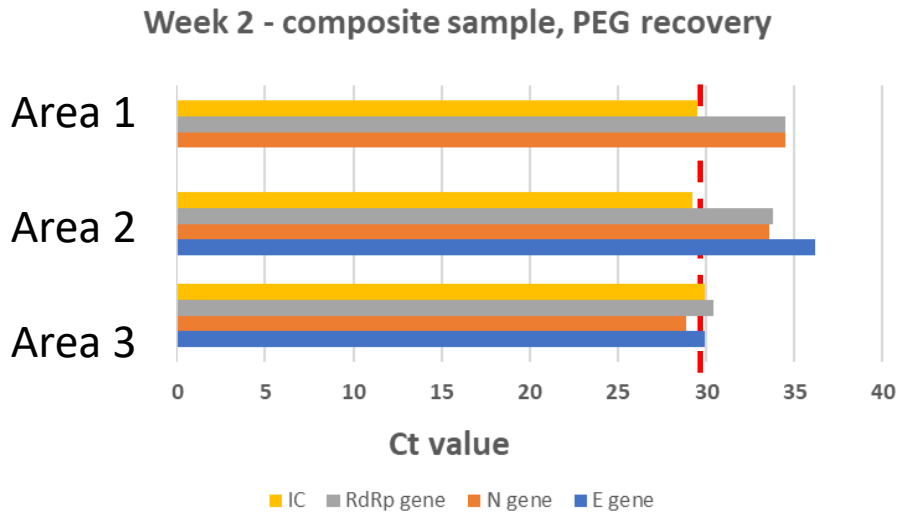
- Comparison between PEG 8000/NaCl and skim milk virus recovery methods
- Mengovirus recovery efficiency – varied from 0.5% to 8% for PEG8000/NaCl method
- 1-2 L sewage samples were received and stored at 4°C until processing
- Samples were mixed thoroughly and a 200 mL aliquot was used for each recovery method

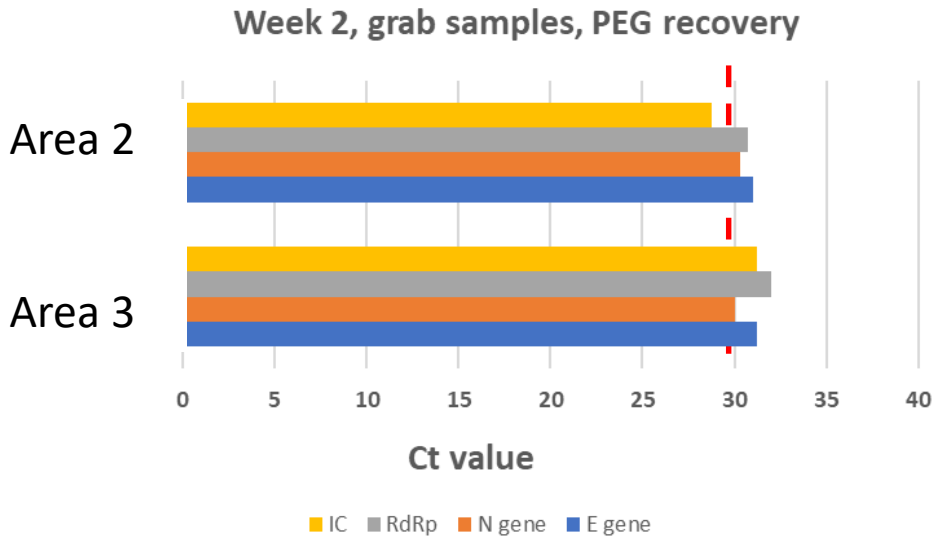

PEG
200 mL sewage
Centrifuge (no brake), 1180 g x 30 min
SKIM MILK
To each of 4x50 mL centrifuge tubes:
Add 4 g PEG 8000 and 0.9 g NaCl
Divide supernatant equally to 4 x 50 mL tubes and shake until PEG/NaCl is dissolved
Pour supernatant carefully,
retain precipitate:
resuspend and store at -80°C
200 mL supernatant @ room temperature
Add 1% of 5% pre-flocculated skim milk solution
Mix thoroughly
Adjust pH to pH 3.0 – 4.0
Shake at RT for 2 h at 200 rpm
Shake overnight@ 200 rpm @ 4°C
Divide in 4 x 50 mL tubes
Centrifuge (brake off) 12000 g @ 4°C for 30 min
Carefully pour off supernatant
Centrifuge at 4500 g for 30 min @ 4°C
Gently pour off and discard supernatant
Centrifuge 12000 g @ 4°C for 5 min
Carefully remove final supernatant with a pipette
Resuspend pellet(s) in 2 mL ITM
Resuspend pellets in 2 mL ITM
1 mL recovered virus in ITM for nucleic acid extraction
1 mL stored at -80°C
1 mL stored at -80°C

SARS-CoV-2 Detection: Grab vs Composite

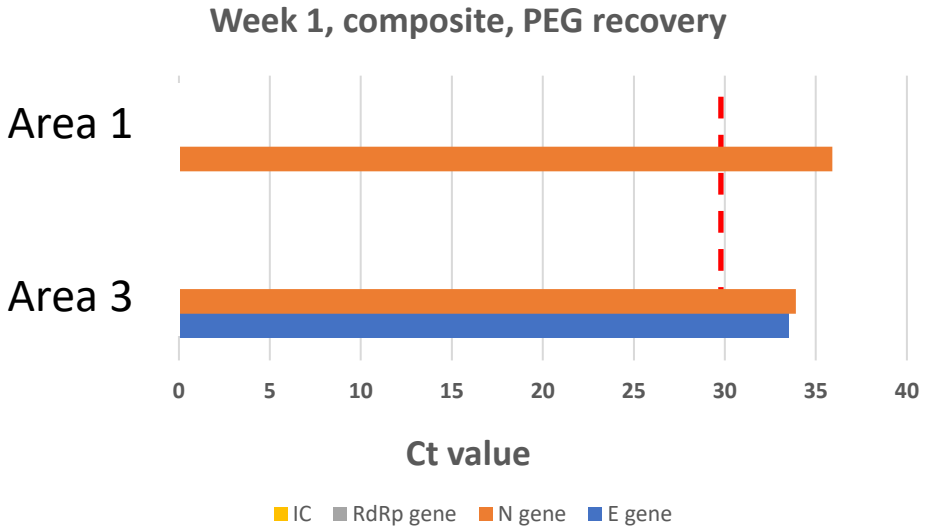


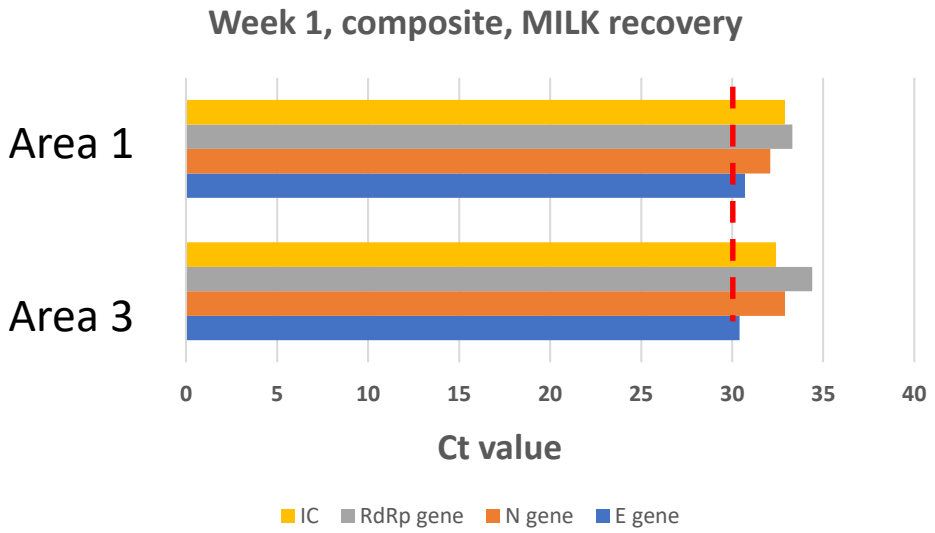






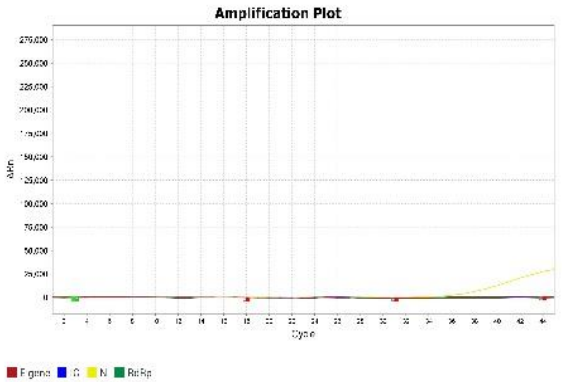
Comparison between PEG and SKIM MILK recovery



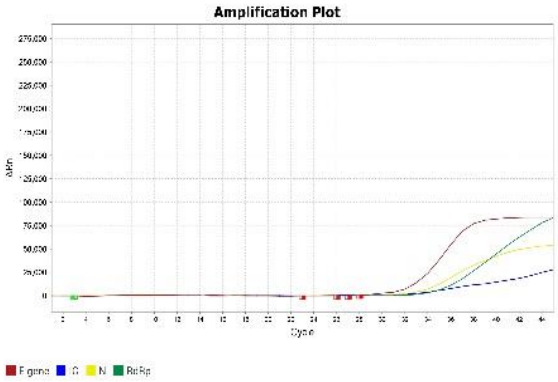


Area 1

PEG

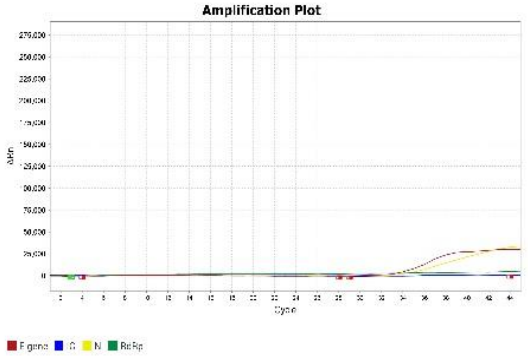


MILK



Area 3

PEG



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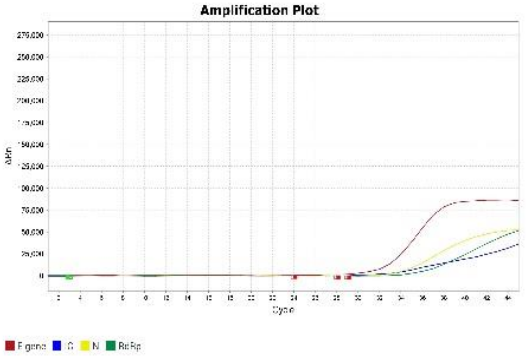
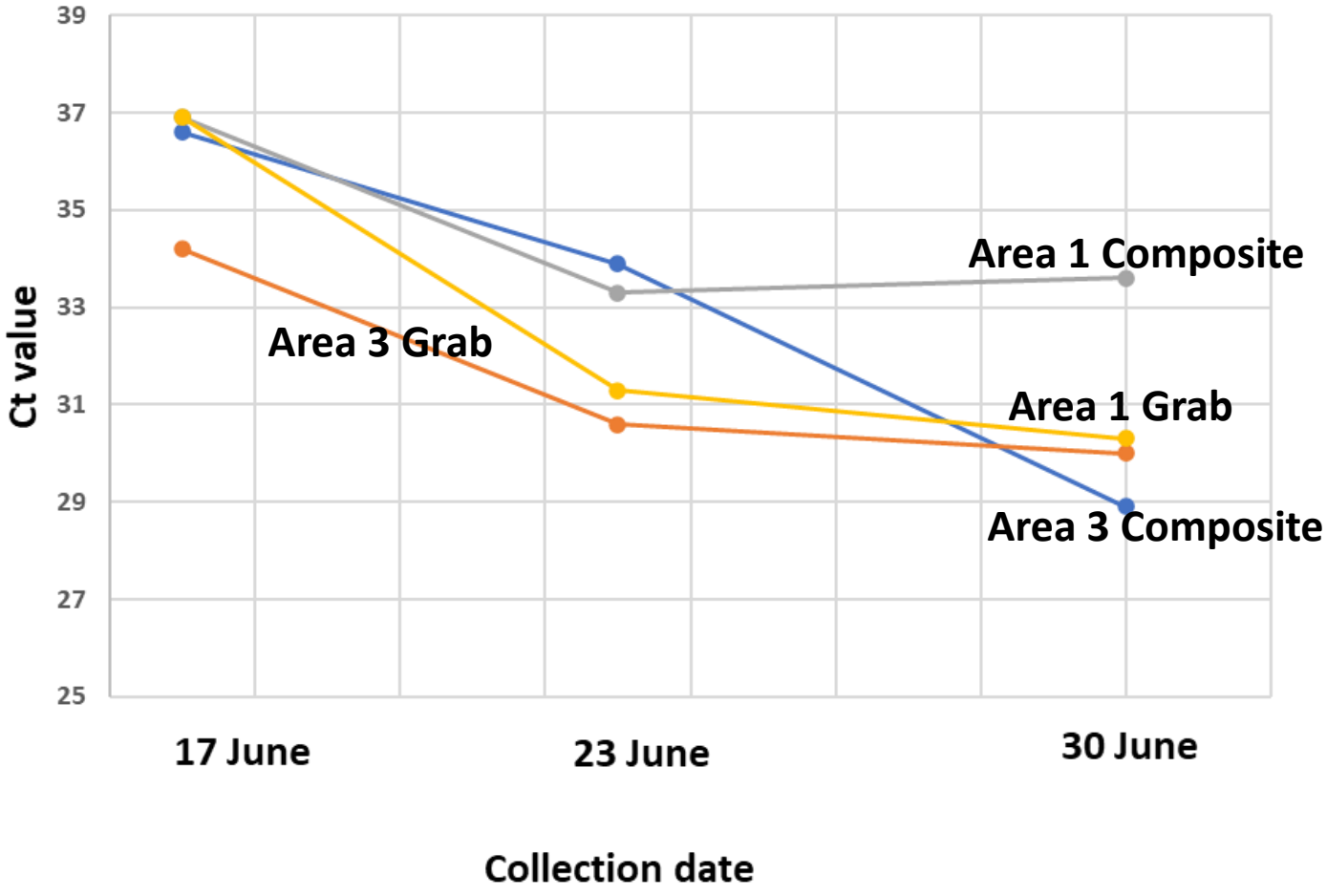
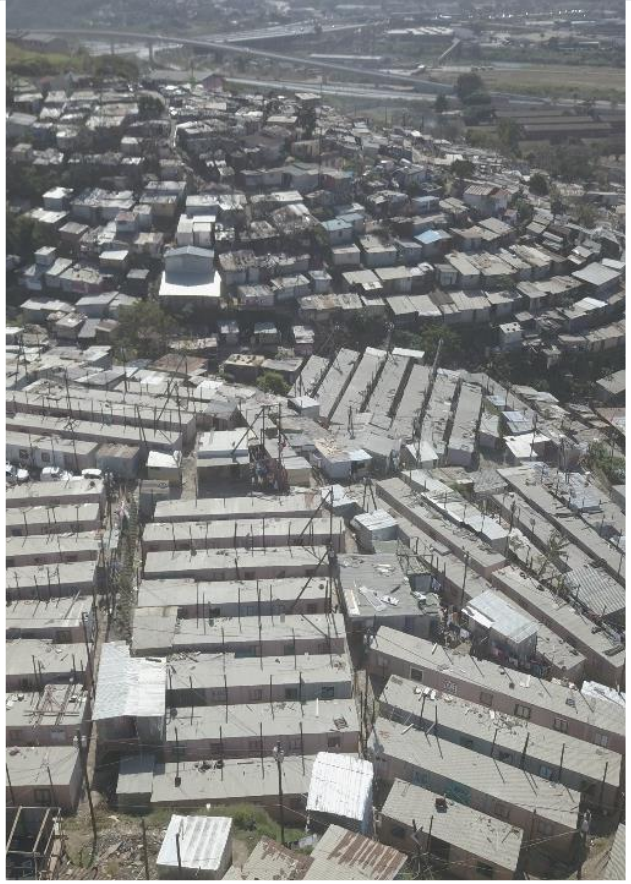
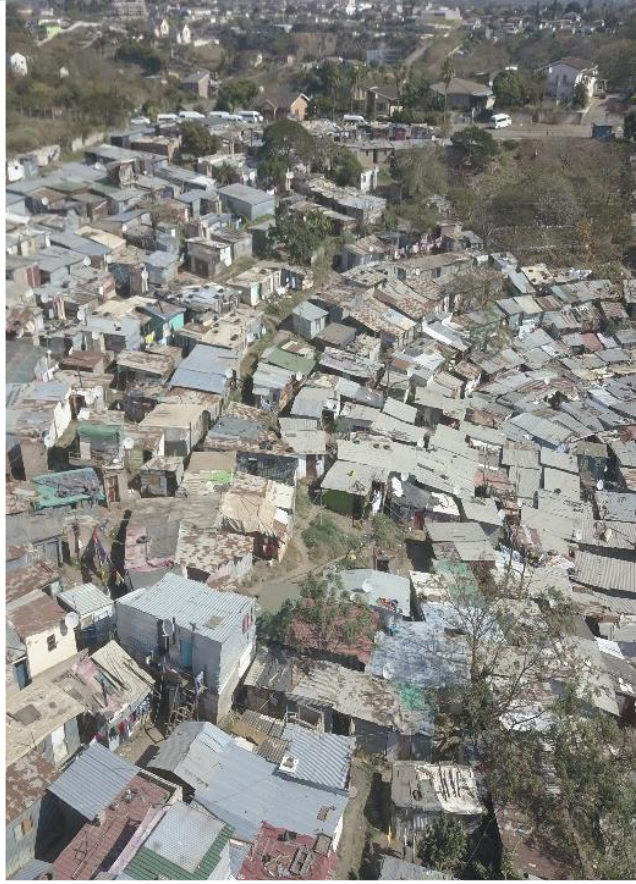


Trends in Ct values in composite and grab samples



Sewer vs Non-Sewer



Non-Sewered Sanitation – 2 Billion People

Possible Hotspots

- Densely populated settlements
- Space constraints
- Frequent person-to-person contact
- Accessibility and usage
- Limited human resources for monitoring
- OHS and maintenance / cleaning / disinfection challenge

A tool for the most vulnerable

Concluding Remarks

- Preliminary results show proof of concept in terms of virus extraction method and positive gene amplification of SARS-CoV-2 at three WWTW sampled
- Samples from remaining WWTW as well as hospitals and surface water samples to be analysed in soon
- Standard curves will be generated for SARS-CoV-2 N1 and N3 using a commercial SARS-CoV-2 N gene plasmid to validate a method for quantification of the virus, as the ultimate aim of the study is to develop infection trends within communities
- As the epidemiological data from this proof of concept study becomes available, it will be processed and mapped to show incidences of infection in communities linked to the selected WWTW to visualise trends
- Based on this, recommendations will be made for data integration into national reporting

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