



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



UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA



Dr Sudhir Pillay

Research Manager: Sanitation

Water Research Commission

South Africa

Email: sudhirp@wrc.org.za



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

ATIONAL POLITICS CORONAVIRUS COMPANIES ECONOMY BUSINESS WC

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



Listen to this article

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. Grubaugh³, Edward H. Kaplan^{8,4,1}, Arnau Casanovas-Massana³, Albert I. Ko³, Aryn A. Malik^{5,6}, Dennis Wang⁵, Mike Wang⁵, Daniel M. Weinberger³, Saad B. Omer^{3,5,6,7,‡}

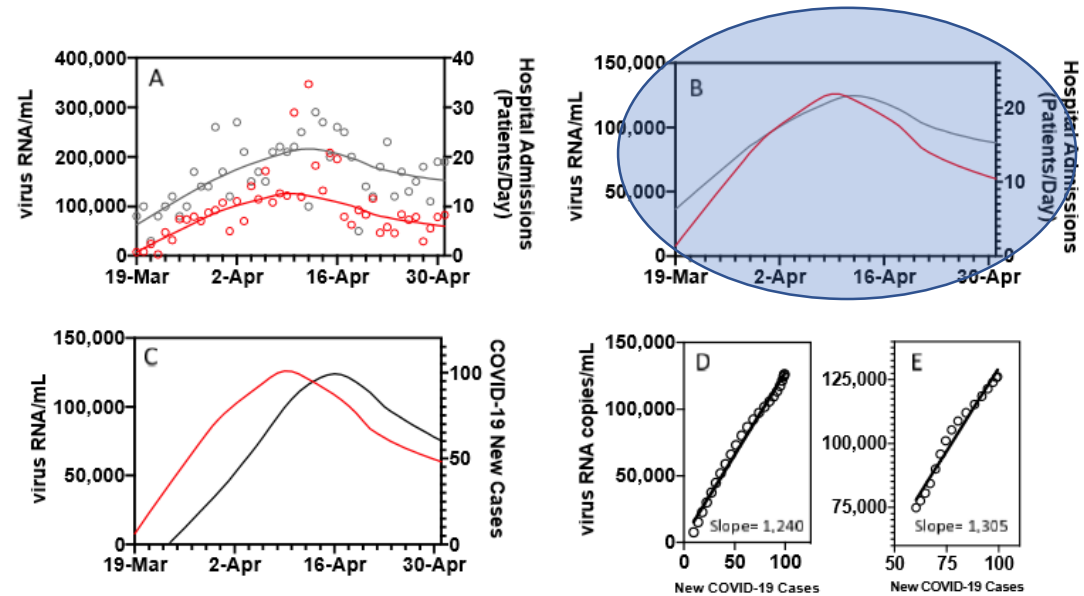


Figure 2. (A) Average sludge SARS-CoV-2 RNA concentration time course data (○) and average hospital admissions data (○) with LOWESS smoothing; (B) rescaled smoothed SARS-CoV-2 virus RNA concentrations (---) and hospital admissions (---); (C) smoothed sludge SARS-CoV-2 virus RNA concentration (---) with 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)

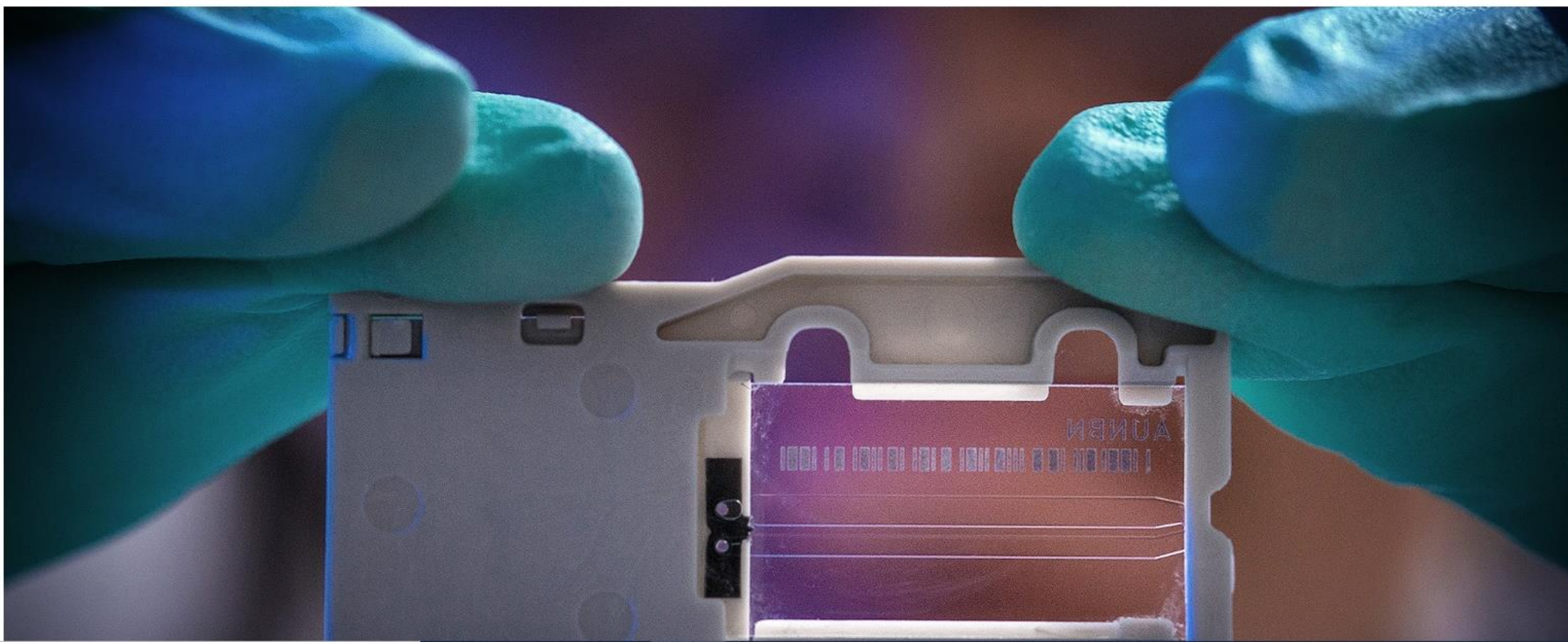
- Laboratory technique of molecular biology based on the polymerase chain reaction (PCR).
- Monitors the amplification of a targeted DNA molecule during the PCR
- Cycle Threshold (Ct): number of cycles required for the fluorescent signal to cross the threshold (background level)
- Higher the Ct level 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



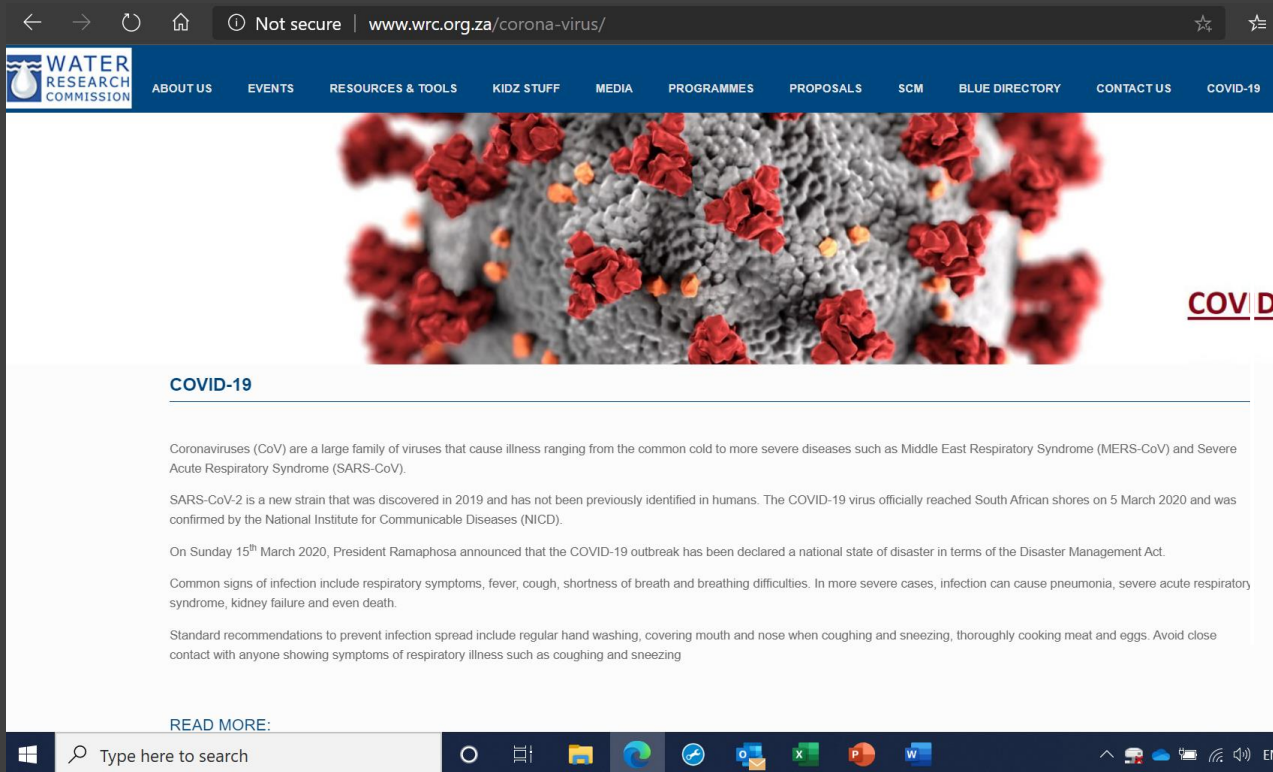
OP-ED

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



← → ↻ 🏠 🔒 Not secure | www.wrc.org.za/corona-virus/ ☆ ⌵

WATER RESEARCH COMMISSION ABOUT US EVENTS RESOURCES & TOOLS KIDZ STUFF MEDIA PROGRAMMES PROPOSALS SCM BLUE DIRECTORY CONTACT US COVID-19

COVID-19

Coronaviruses (CoV) are a large family of viruses that cause illness ranging from the common cold to more severe diseases such as Middle East Respiratory Syndrome (MERS-CoV) and Severe Acute Respiratory Syndrome (SARS-CoV).

SARS-CoV-2 is a new strain that was discovered in 2019 and has not been previously identified in humans. The COVID-19 virus officially reached South African shores on 5 March 2020 and was confirmed by the National Institute for Communicable Diseases (NICD).

On Sunday 15th March 2020, President Ramaphosa announced that the COVID-19 outbreak has been declared a national state of disaster in terms of the Disaster Management Act.

Common signs of infection include respiratory symptoms, fever, cough, shortness of breath and breathing difficulties. In more severe cases, infection can cause pneumonia, severe acute respiratory syndrome, kidney failure and even death.

Standard recommendations to prevent infection spread include regular hand washing, covering mouth and nose when coughing and sneezing, thoroughly cooking meat and eggs. Avoid close contact with anyone showing symptoms of respiratory illness such as coughing and sneezing

[READ MORE:](#)

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



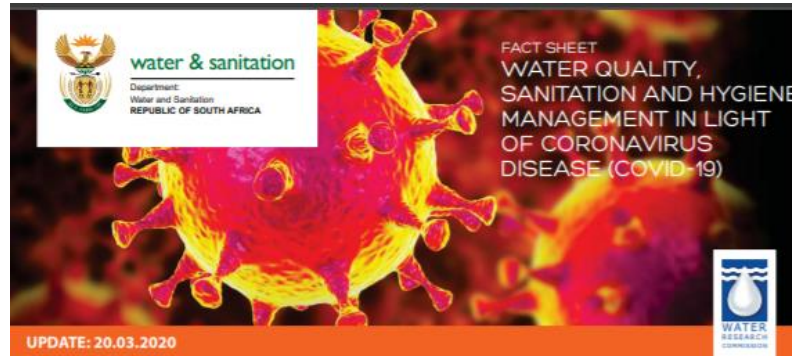
Why must we wash our hands?

Washing our hands with soap and water and cleaning our hands with a sanitiser can help stop the spread of COVID-19, the deadly new disease caused by the new Coronavirus. Since we use our hands to eat and touch our faces, they are one of the main ways in which the new Coronavirus can enter our bodies. So, keeping our hands clean by maintaining good hand hygiene is one of the most effective ways to prevent the spread of the disease.



When must we wash our hands?

- After fetching water and before handling drinking water
- Before and after preparing food
- Before we eat any food or feed children
- After using the toilet
- After changing nappies or coming into contact with urine or faeces
- After handling animals
- Before and after giving care to a sick person
- After wiping or blowing nose
- After contact with blood or any bodily fluid.



Naming the Coronavirus disease (COVID-19) and the Virus that causes it:

Official name for disease:

Coronavirus disease (COVID – 19)

Official name for virus is:

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)



What we know

Coronavirus disease (COVID-19) which has been declared a pandemic by the World Health Organisation (WHO) is caused by the SARS-CoV-2 that was isolated in Wuhan, China in January 2020. SARS-CoV-2 belongs to the family of zoonotic Coronavirus (meaning they are transmitted between animals and people).

The virus has not been previously identified in humans and as a result humans do not have immunity to the virus.



Background

As the Coronavirus disease (COVID-19) pandemic continues its scourge across the world, South African municipalities have been asked to prepare for the possibility of increased fatalities which might exceed current burial and crematoria facilities. Apart from ensuring there are enough facilities, an equally important consideration is to ensure that death and burial occur safely given the highly infectious nature of the SARS-CoV-2 virus (the virus responsible for COVID-19). As little is generally known about SARS-CoV-2, clarity is being sought around the risk to environmental and human health as a result of impending mass burial of COVID-19 victims.

Irrespective of a pandemic, due to the sensitive

nature of death and burial practices very limited information is available on possible contaminants and their associated risks during the death and subsequent burial process. However, South Africa is among a handful of countries that has sought to determine possible adverse impacts of cemeteries. A completed 2018 Water Research Commission study (WRC Report No. 2449/1/18) provides a thorough environmental risk assessment of cemeteries based on different case studies and also provides guidance on contaminant monitoring and management of cemeteries¹.

Based on WRC Report No 2449/1/18 and local and international sources, which make reference to burial and death in the context of a viral pandemic, this factsheet addresses some pertinent questions.

The factsheet referred to as "DEATH AND BURIAL IN THE TIME OF COVID-19: ENVIRONMENTAL AND HEALTH RISKS" was developed in accordance with the World Health Organisation's (WHO) interim guidance on infection prevention and control for the safe management of a dead body in the context of COVID-19.



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% sewerred
 - 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

Pour supernatant
carefully,
retain precipitate:
resuspend and store at
-80°C

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

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

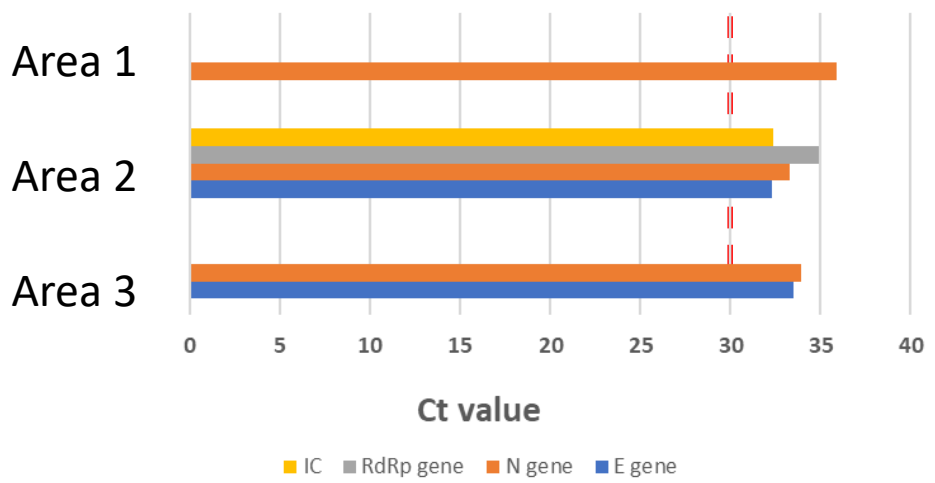
1 mL stored at -80°C

1 mL stored at -80°C

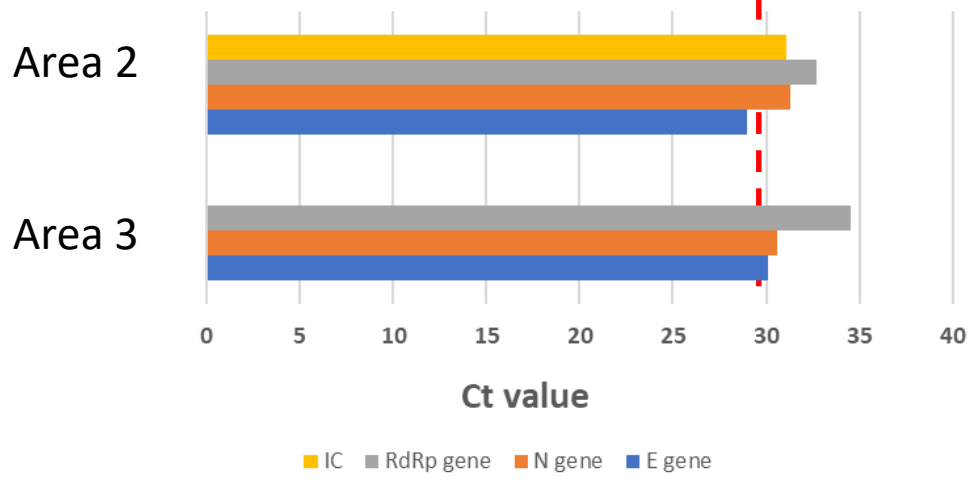


SARS-CoV-2 Detection: Grab vs Composite

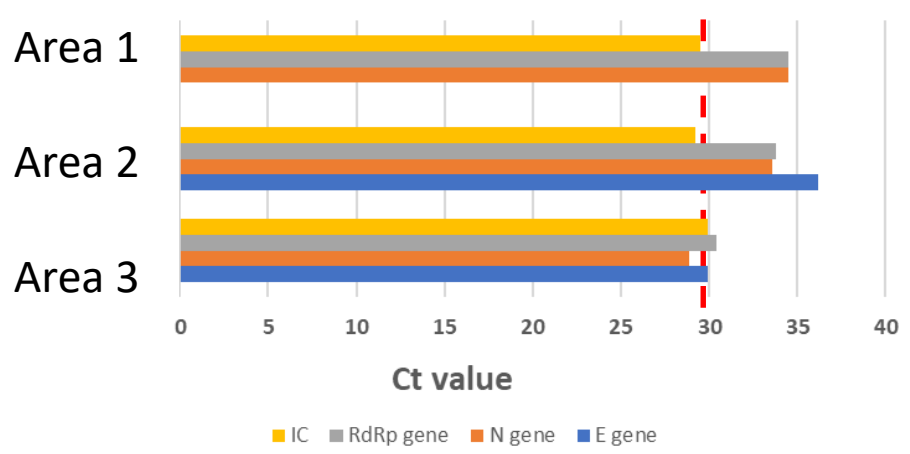
Week 1 - composite sample, PEG recovery



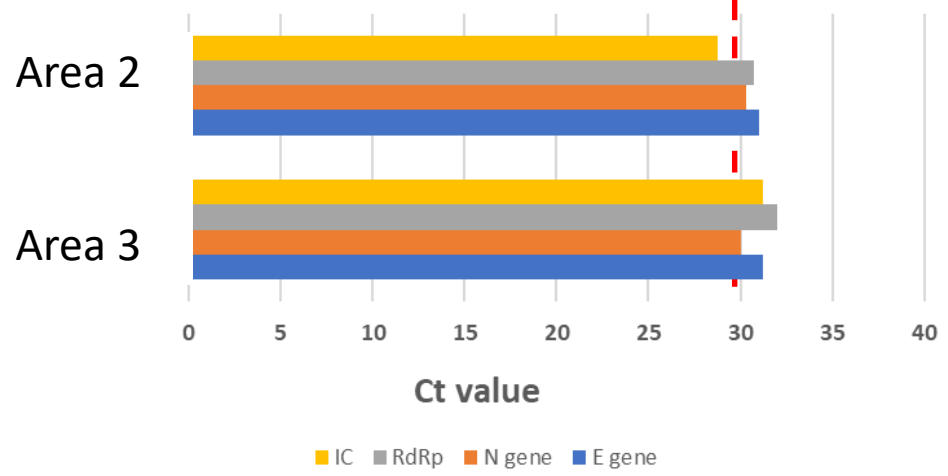
Week 1 - grab sample, PEG recovery



Week 2 - composite sample, PEG recovery



Week 2, grab samples, PEG recovery

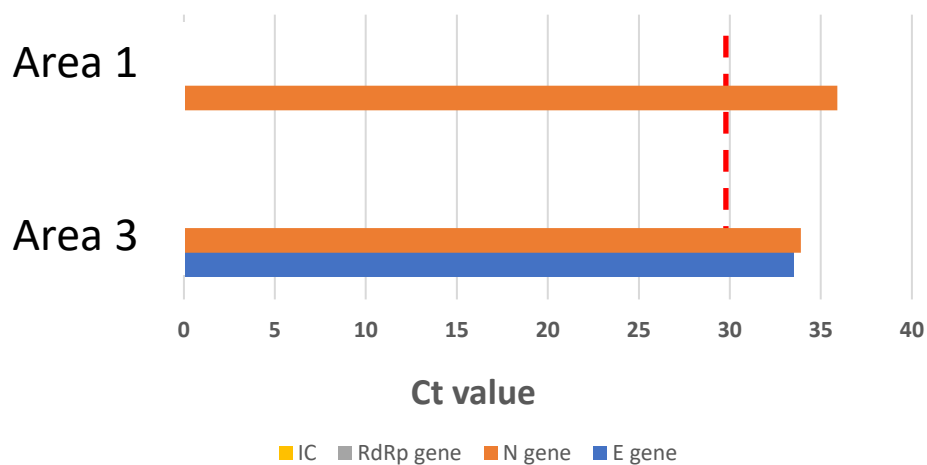




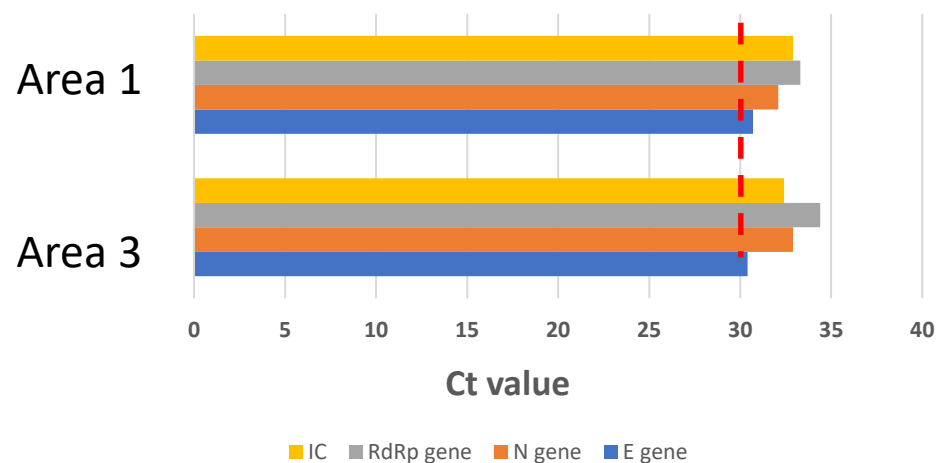
WATER
RESEARCH
COMMISSION

Comparison between PEG and SKIM MILK recovery

Week 1, composite, PEG recovery

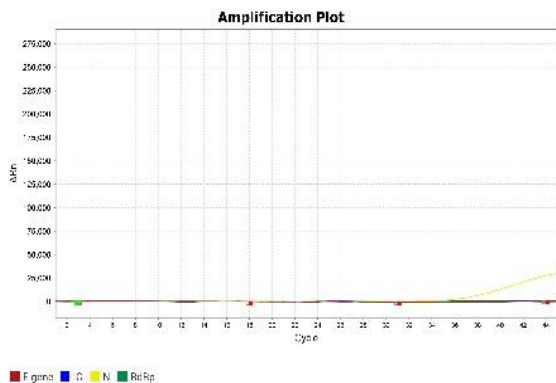


Week 1, composite, MILK recovery

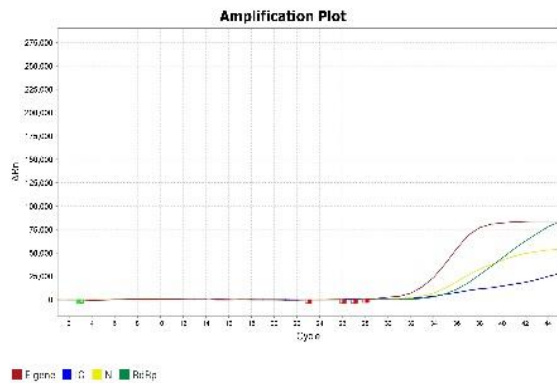


Area 1

PEG

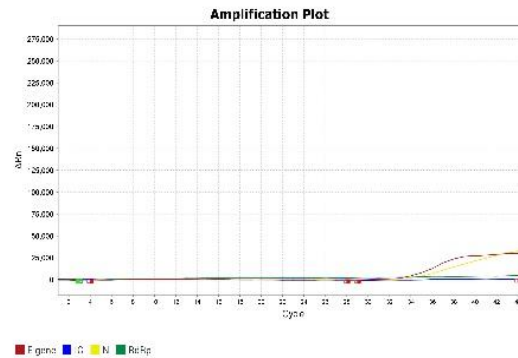


MILK

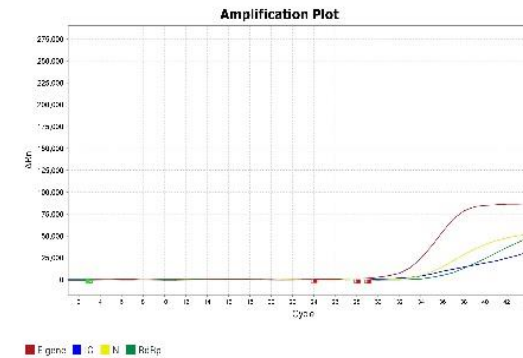


Area 3

PEG

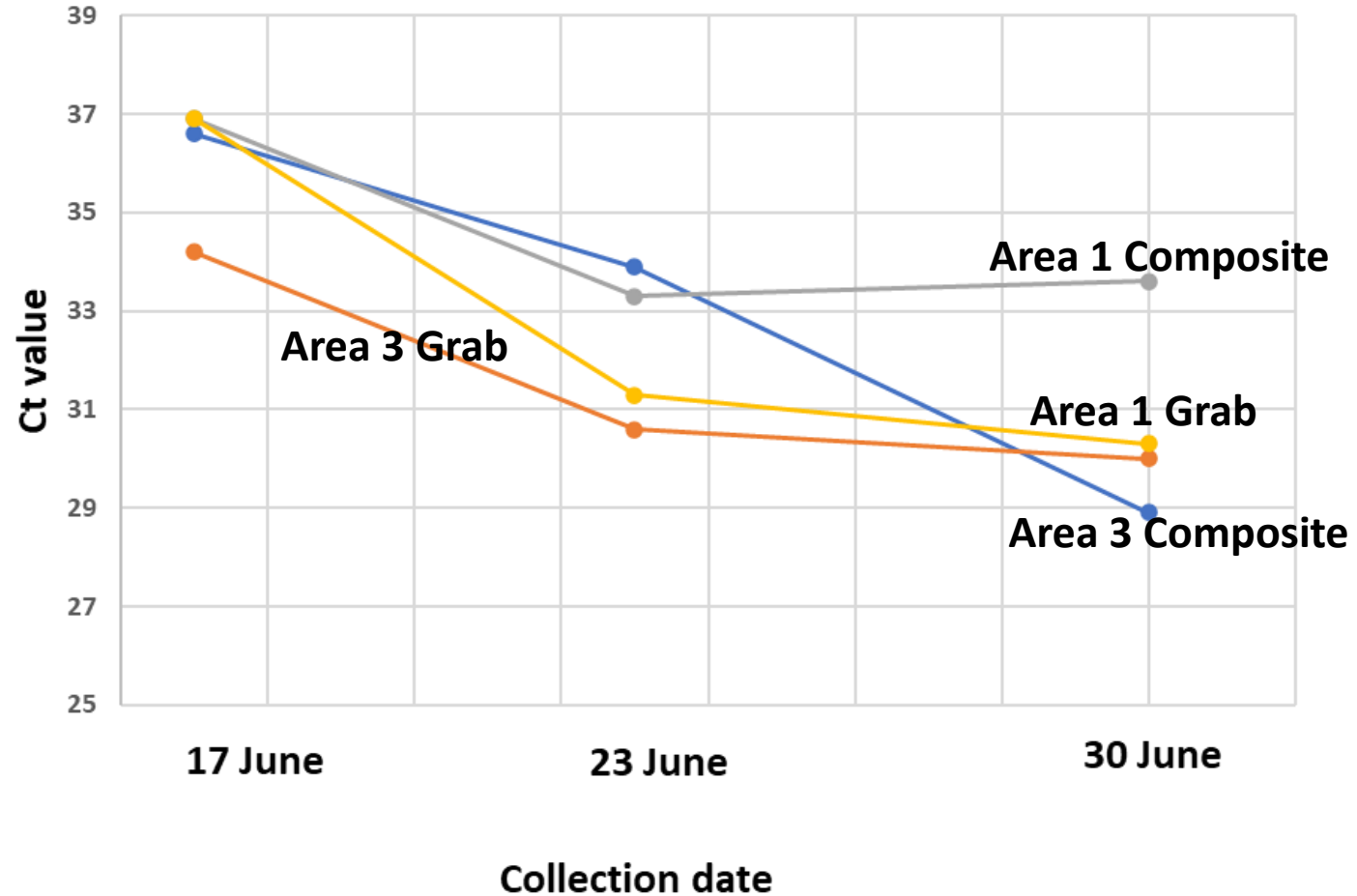


MILK



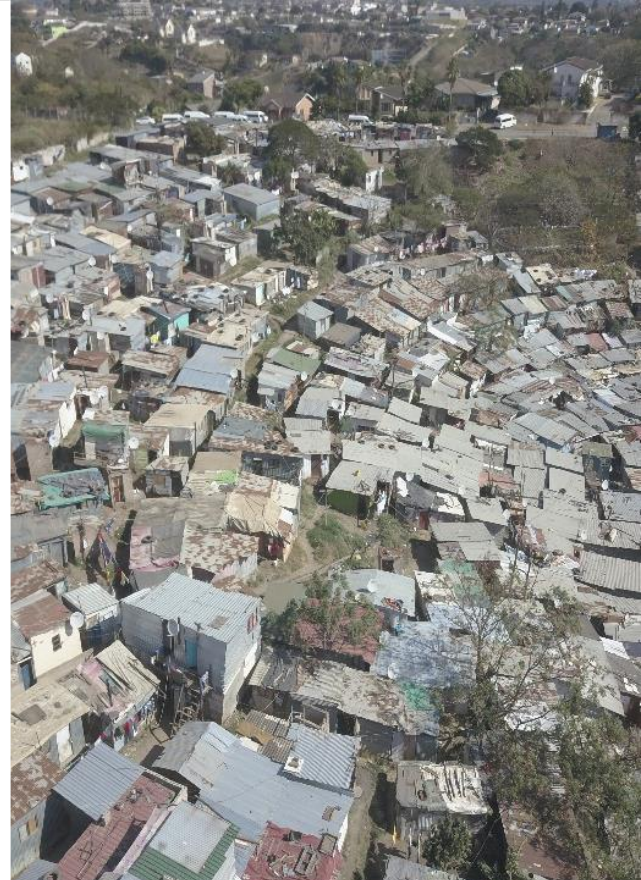


Trends in Ct values in composite and grab samples





Sewer vs Non-Sewer



Non-Sewered Sanitation – 2 Billion People

Possible Hotspots

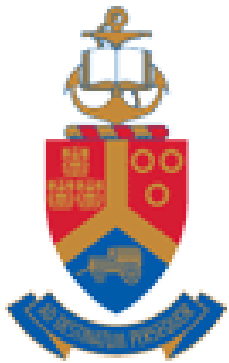
- Densely populated settlements
- Space constraints
- Frequent person-to-person contact
- Accessibility and usage
- Limited human resources for monitoring
- OHS and maintenance / cleaning / disinfection challenge



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



UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA



WATERLAB



our future through science



WATER
RESEARCH
COMMISSION