

# Wastewater-based genomic and epidemiological monitoring of SARS-CoV-2, South Africa, 2020-2021

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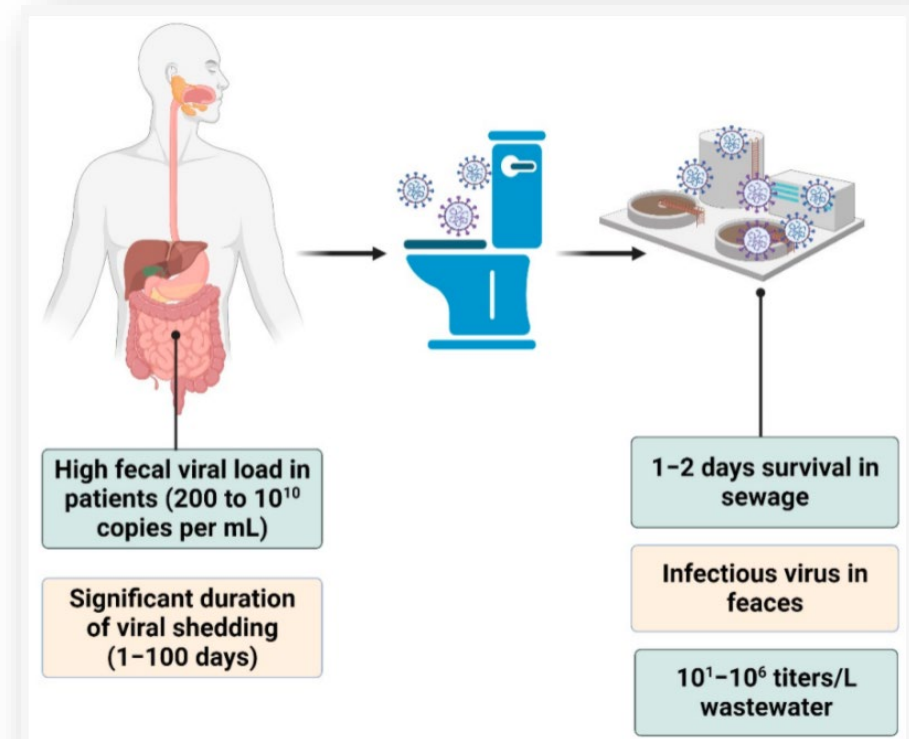
# Outline

- Background
  - Principles and rationale for using wastewater-based epidemiology (WBE) for SARS-CoV-2 surveillance
  - The SACCESS network partners and sites
- Methodology
  - PCR detection and quantitation methodology
  - RNA sequencing methodology
- Results
- Conclusion

# WBE for COVID-19

## *Principles and global experience*

- SARS-CoV-2 replicates in cells lining respiratory and gastro-enteric systems
- Whilst there is no evidence for faeco-oral transmission of SARS-CoV-2, the virus is shed in faeces
  - Viral load  $10^3$ - $10^7$  copies/ml stool
  - 0-45 days after symptom onset,
  - 23% of persons with negative RT-PCR on throat swab will have positive stool

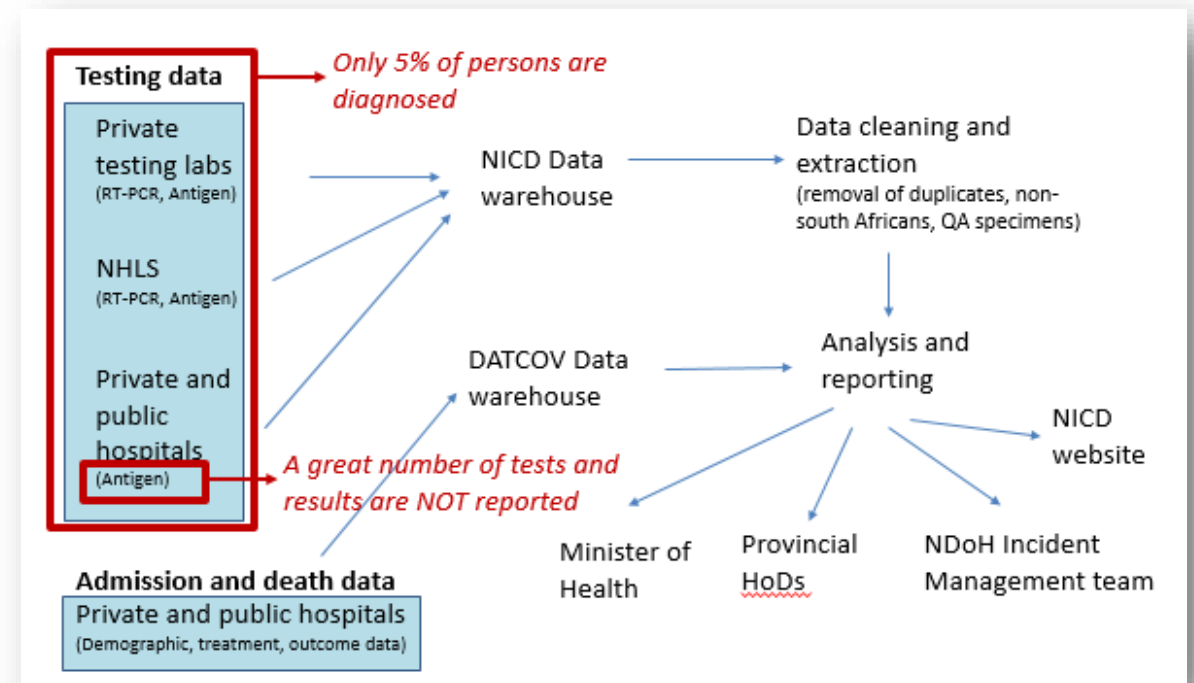


Amereh et al. Sewage Systems Surveillance for SARS-CoV-2: Identification of Knowledge Gaps, Emerging Threats, and Future Research Needs  
*Pathogens* 2021, 10(8), 946; <https://doi.org/10.3390/pathogens10080946>

# WBE for COVID-19

## *Principles and global experience*

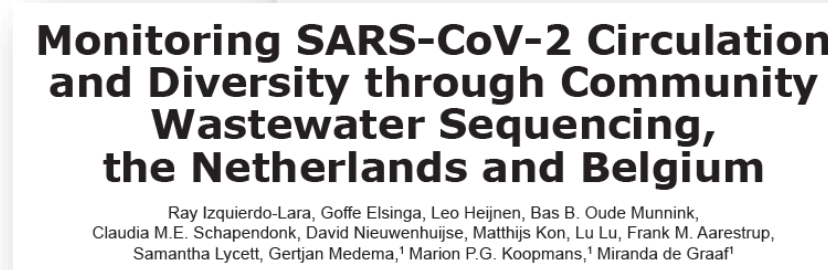
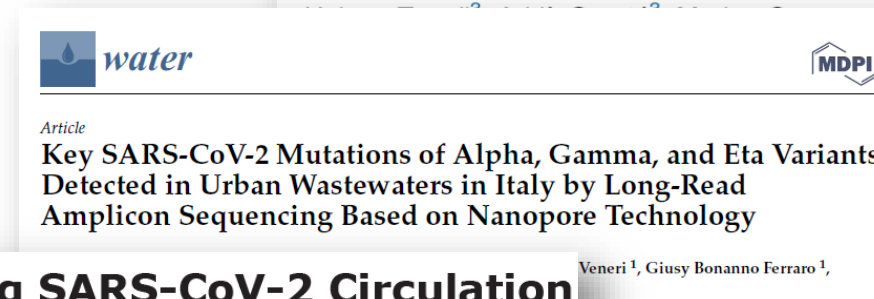
- Wastewater testing
  - overcomes the limitations of clinical surveillance
  - allows epidemiologists to infer the burden and distribution of SARS-CoV-2 in populations without the need for clinical testing,
  - Is much cheaper than clinical testing



# WBE for COVID-19

## *Principles and global experience*

- Wastewater testing
  - Can be used to identify SARS-CoV-2 variants that are circulating in populations under surveillance



# WBE for COVID-19

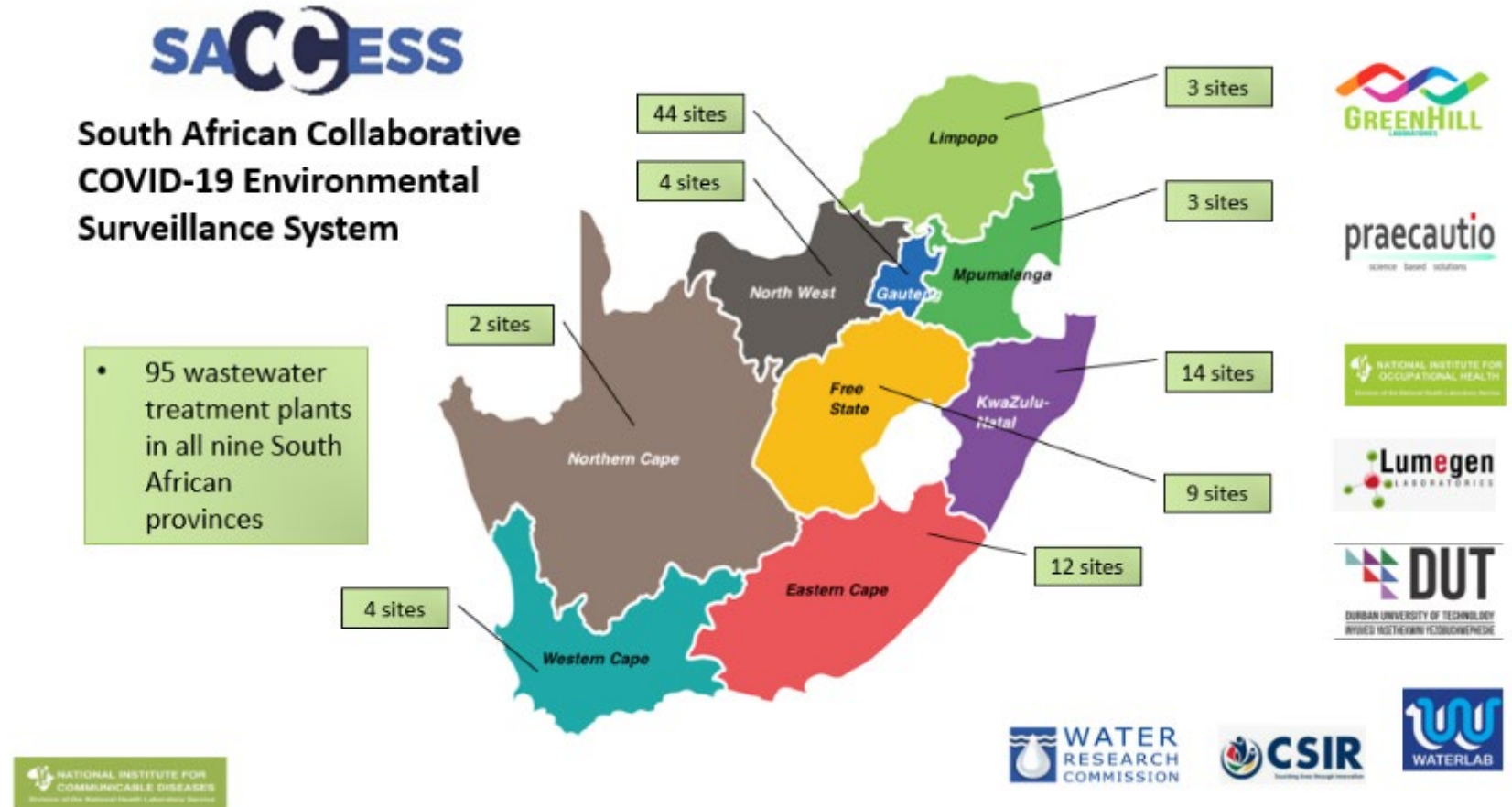
## *Principles and global experience*

- We aimed to show
  - temporal and geographical trends in SARS-CoV-2 levels and sequences from wastewater
  - and compare these with case loads and clinical SARS-CoV-2 sequences
  - to demonstrate the usefulness of wastewater-based epidemiology for SARS-CoV-2 as a surveillance tool.

# Wastewater based epidemiology in South Africa

## Methodology

- 7 laboratories
- 95 wastewater treatment plants
- All doing
  - Concentration
  - Qualitative PCR detection
- Genome sequencing (NICD only)
  - Proof of concept established on results from 17 specimens



# Wastewater based epidemiology in South Africa

## Methodology

'Grab sample' of influent



concentration



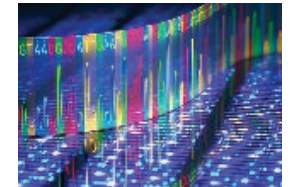
RNA extraction



RT-PCR detection and quantitation



Sequencing



Name of Laboratory partner	Method for virus concentration	Method for nucleic acid extraction	RT-PCR assay	Quantification
NICD	Centricon® Plus-70 centrifugal	QIAamp® viral RNA mini kit	Allplex™ 2019-nCoV Assay	EDX SARS-CoV-2 Standard including RNA transcripts of E, N and RdRP genes
GreenHill / Praecautio	Amicon® Ultra-15 Centrifugal Filter)	Omega Bio-Tek Mag-Bind® Viral DNA/RNA 96 Kit	CDC 2019-Novel Coronavirus (2019-nCoV) Real-Time RT-PCR Diagnostic Panel	Relative quantification based on CDC 2019-Novel Coronavirus (2019-nCoV) Real-Time RT-PCR Diagnostic Panel
NIOH	Skim milk flocculation	MagMAX Viral and Pathogen Nucleic Acid Isolation Kit	TaqPath COVID-19 CE-IVD RT-PCR Kit Thermo Fisher	Standard curve method using TaqPath kit positive control
WaterLab/UP	Skim milk flocculation	QIAamp® Ultrasens® Virus kit	Allplex™ 2019-nCoV Assay	Standard curves using the 2019_nCoV_N positive control plasmid (Integrated DNA Technologies, Inc, Coralville, IA)
SAMRC-TB PLATFORM	Centrifugation	ZymoBiomixs RNA Extraction Kit	AllPlex 2019-nCoV Assay	EDX SARS-CoV-2 Standard including RNA transcripts of E, N and RdRP genes
Lumegen	Passive sampling + resuspension in PBS	MN DNA/RNA pathogen extraction Kit	TaqPath COVID-19 CE-IVD RT-PCR Kit (Thermo Fisher)	5-point standard curve of the TaqPath positive control
CSIR	Polyethylene Glycol	Omega Bio-tek ENZA total RNA Kit II	2019-nCoV CDC EUA Kit	Relative quantification based on the 2019-nCoV CDC positive control



# Wastewater-based Epidemiology for COVID-19

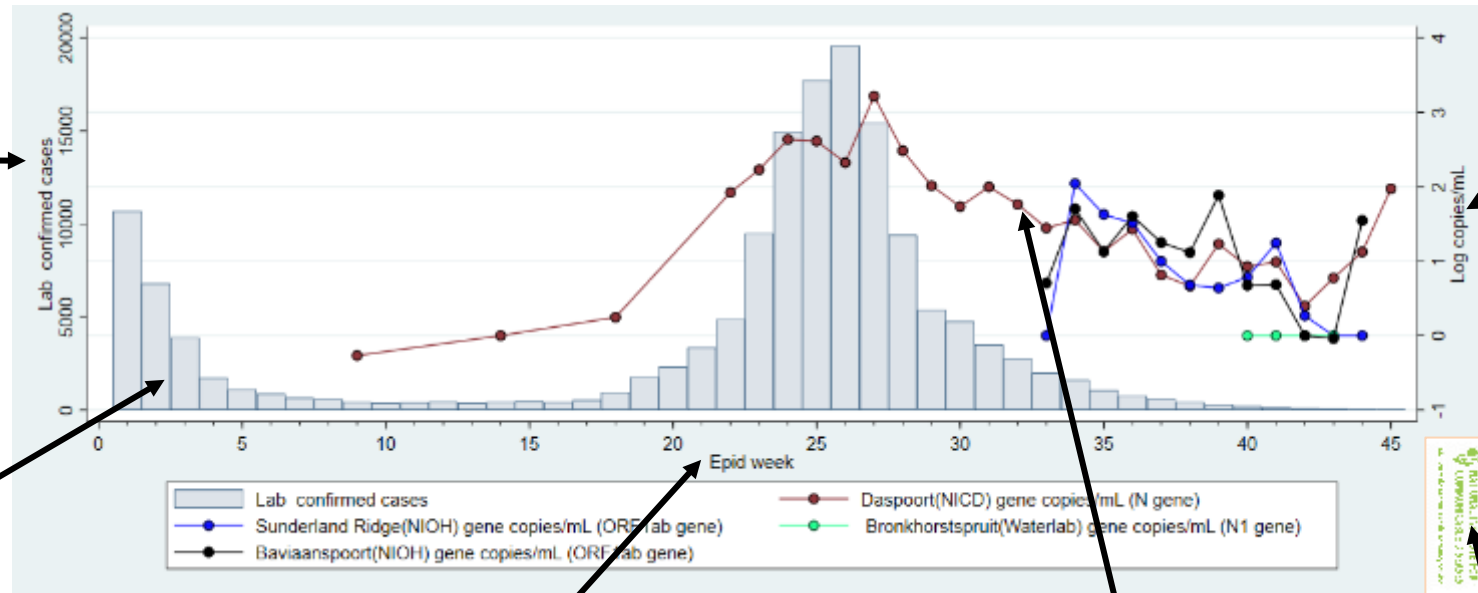
## Interpretation of quantitative results

**Left vertical axis:**

Number of lab confirmed cases in *the metro or district where the water treatment plant is located*

**Bars:**

Number of lab confirmed clinical cases in specimens submitted to NICD from persons in the metro/subdistrict where the plant is located



**Horizontal axis:**  
Epidemiological weeks

**Coloured lines:**

Changes in wastewater SARS-CoV-2 results over time for different treatment facilities

**Right vertical axis:**

Log (ie 10 to the power x) copies of SARS-CoV-2 genome per ml of wastewater. So log 2=10<sup>2</sup> =100 copies per millilitre, log 3=10<sup>3</sup>=1000 copies per millilitre

**Laboratory logo:**

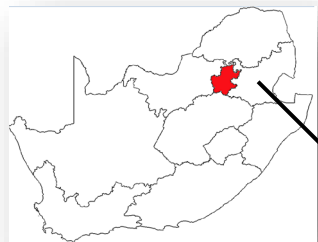
Laboratory producing the graphs for SARS-CoV-2 quantification

# SARS-CoV-2 detection in wastewater in South Africa

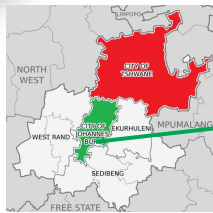
## Gauteng wastewater treatment plants

- Current trends in SARS-CoV-2 levels across Gauteng

South Africa  
Gauteng Province

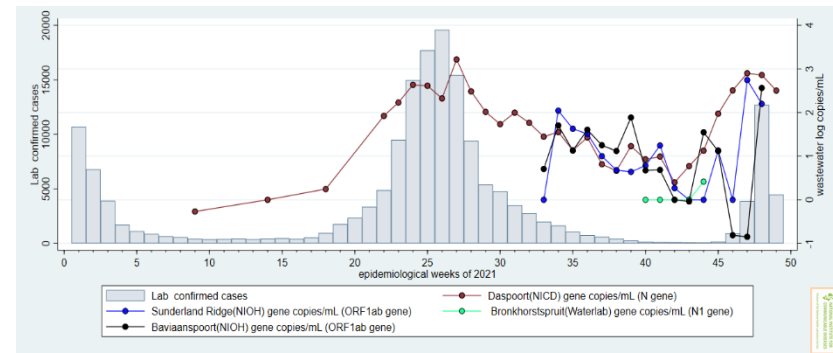


City of Tshwane

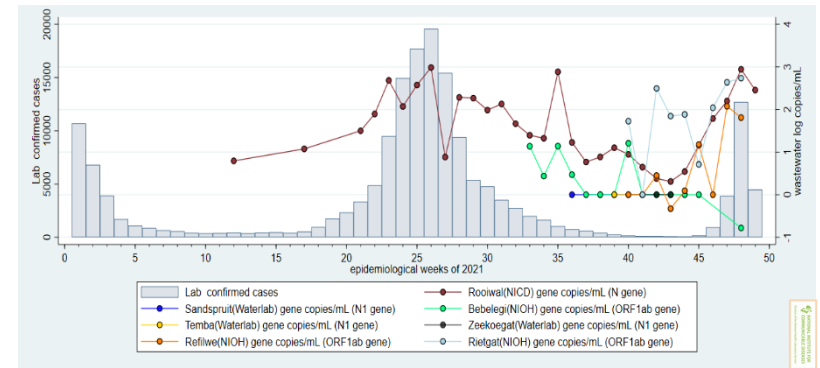


City of Johannesburg

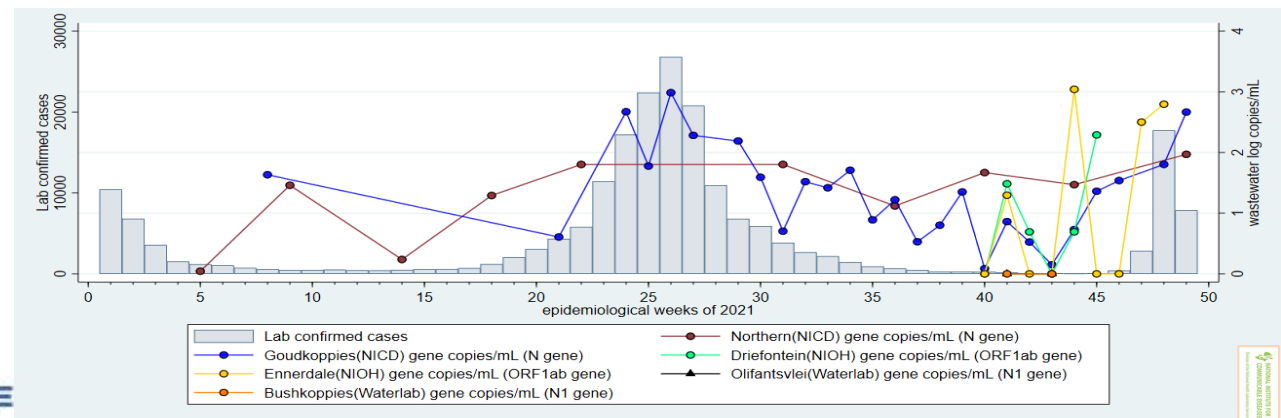
City of Tshwane - South



City of Tshwane - North



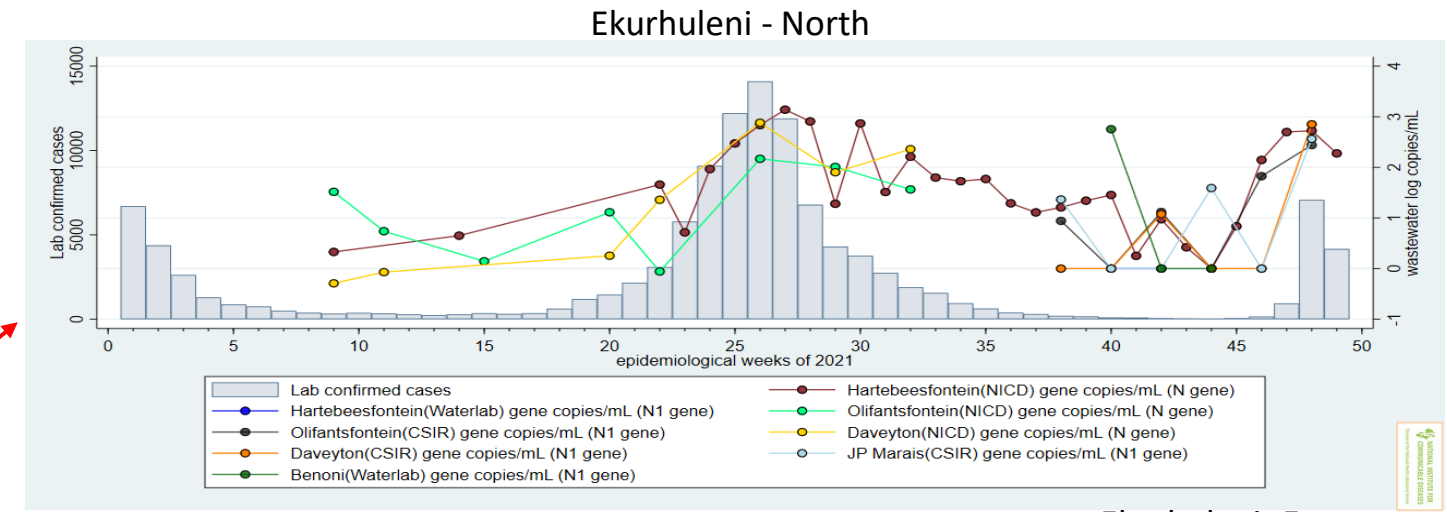
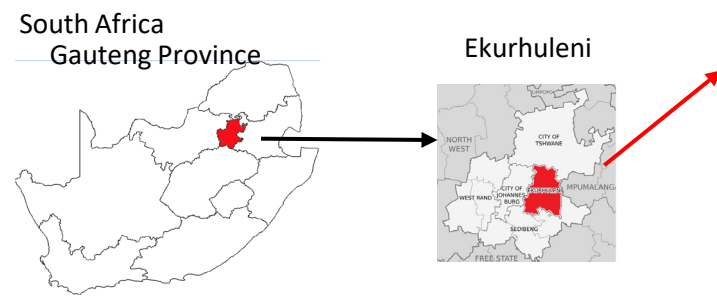
City of Johannesburg



# SARS-CoV-2 detection in wastewater in South Africa

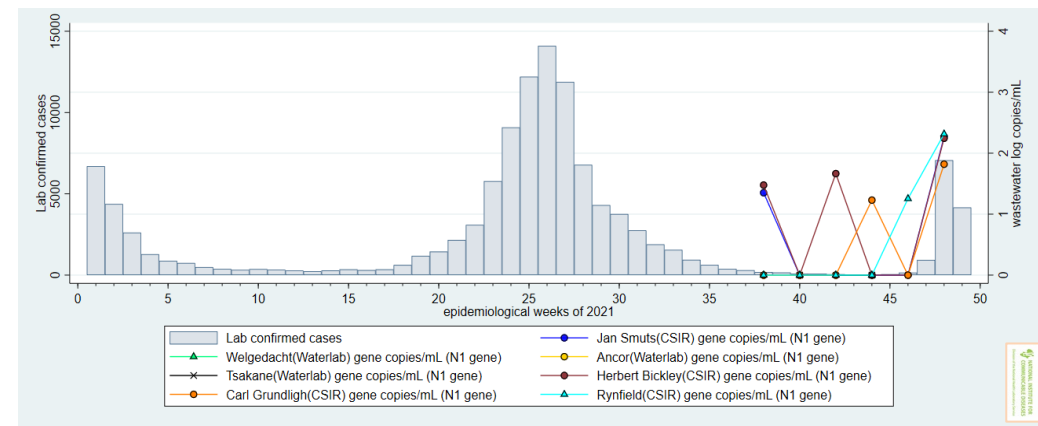
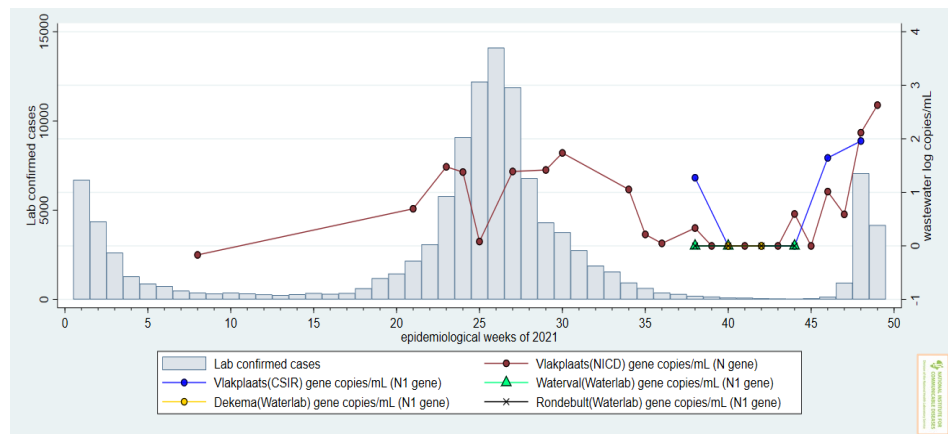
## Gauteng wastewater treatment plants

- Current trends in SARS-CoV-2 levels across Gauteng

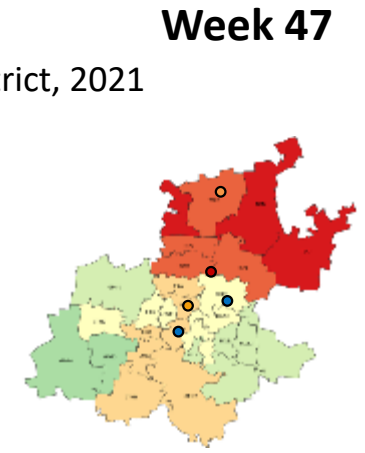
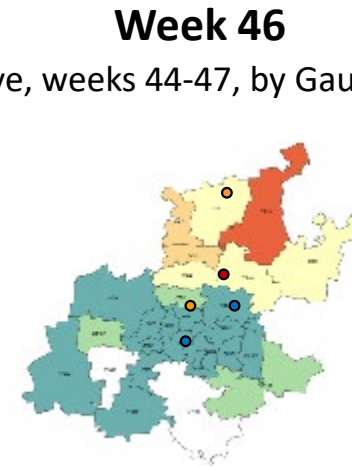
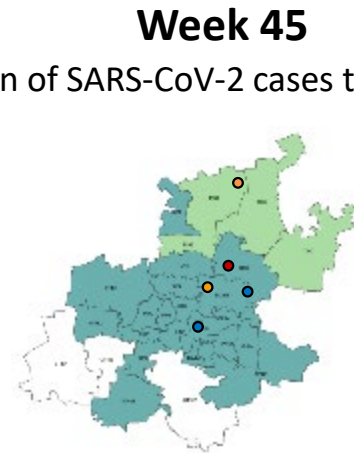
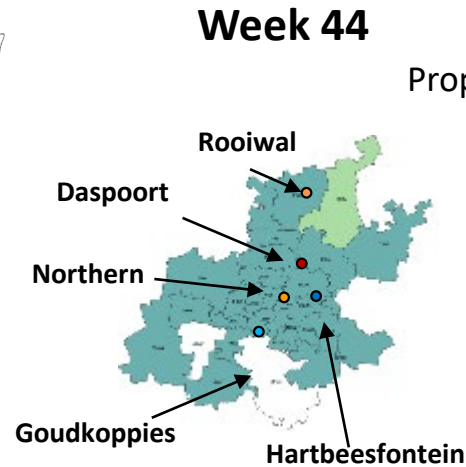
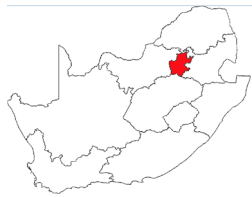


Ekurhuleni - South

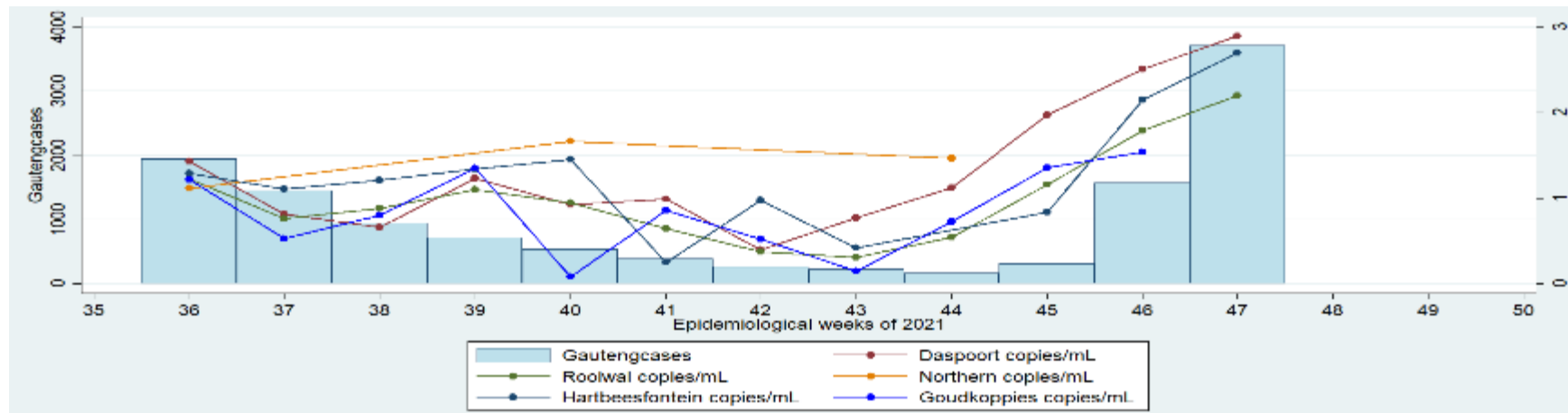
Ekurhuleni- East



# SARS-CoV-2 detection in wastewater in South Africa



Proportion of SARS-CoV-2 cases testing positive, weeks 44-47, by Gauteng subdistrict, 2021



Wastewater levels (lines) at selected treatment plants, by total Gauteng cases, epi weeks 36-47, 2021

# SARS-CoV-2 detection in wastewater in South Africa

Week 44

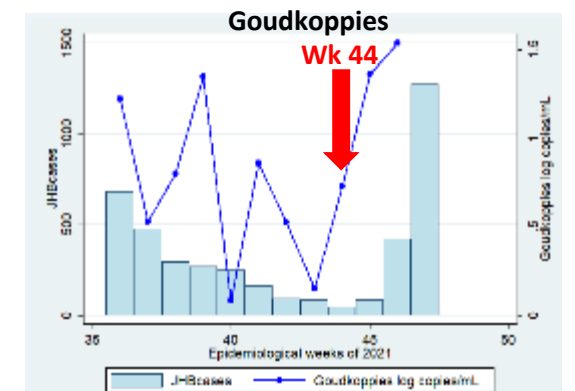
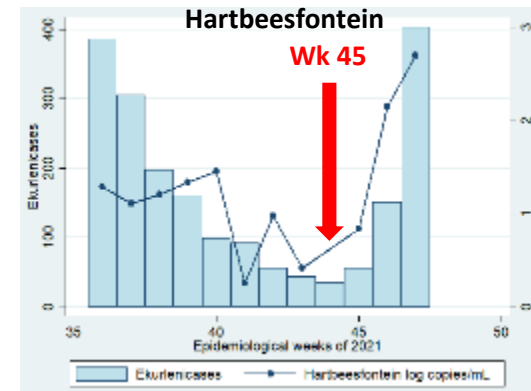
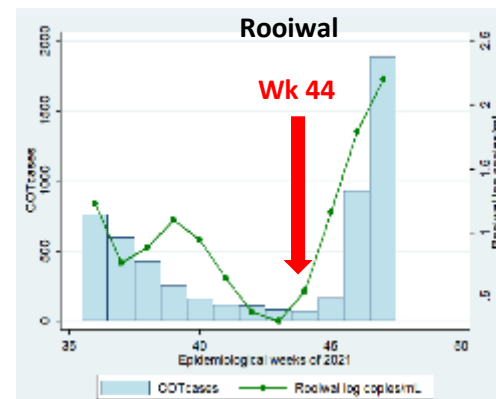
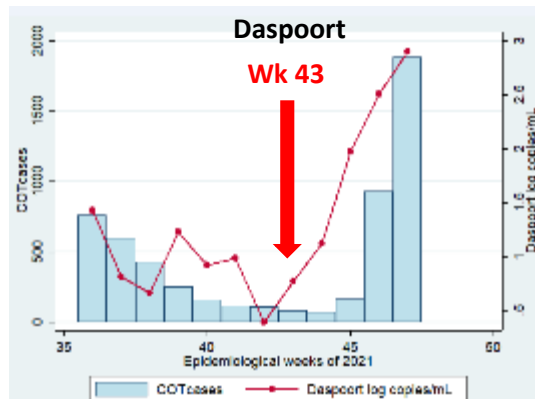
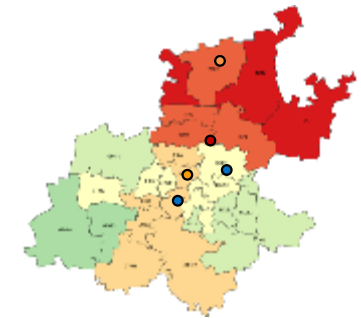
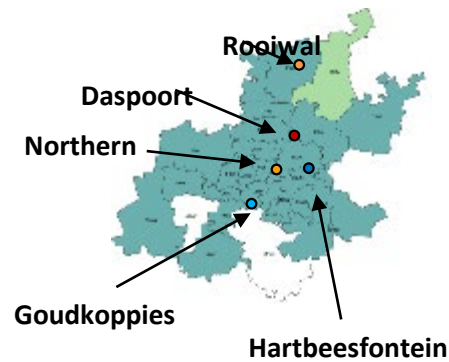
Week 45

Week 46

Week 47

Proportion of SARS-CoV-2 cases testing positive, weeks 44-47, by Gauteng subdistrict, 2021, data courtesy NICD Centre for Respiratory Disease and Meningitis

Prop. test +ve



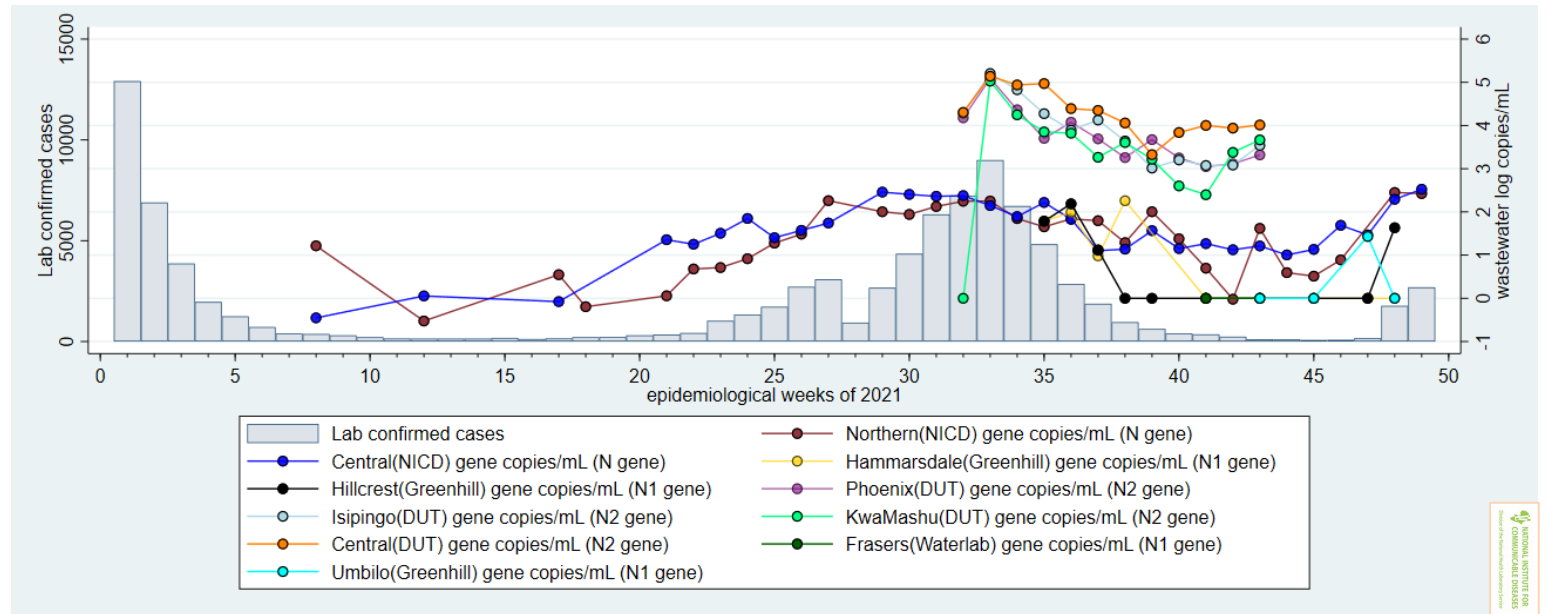
Wastewater levels (lines) at selected treatment plants, **by total cases in each Gauteng district where the treatment plant is situated**, epi weeks 36-47, 2021

Data courtesy NICD Centre for Vaccines and Immunology

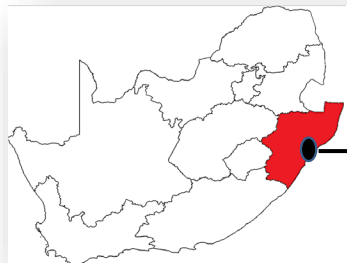
# SARS-CoV-2 detection in wastewater in South Africa

## Ethekwini wastewater treatment plants

- Current trends in SARS-CoV-2 levels across eThekwini wastewater plants
  - 2 plants
  - Ongoing upward trend which is levelling



South Africa  
KwaZulu-Natal Province

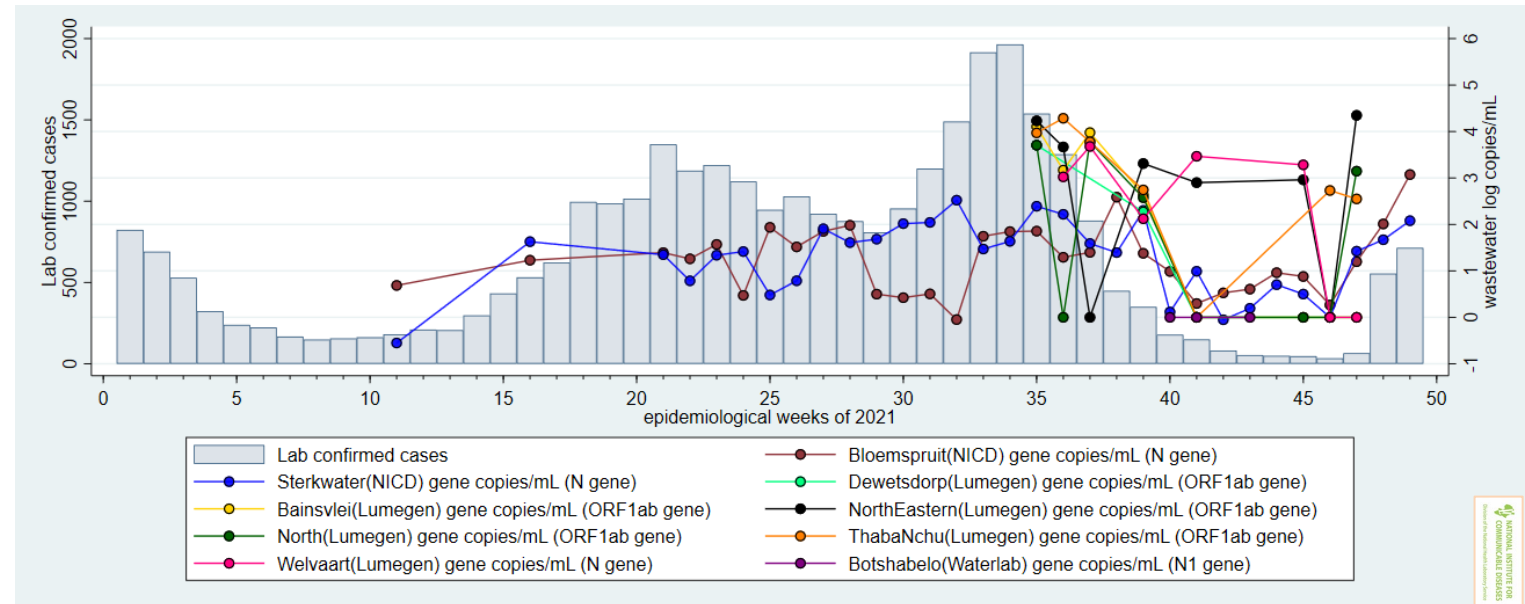
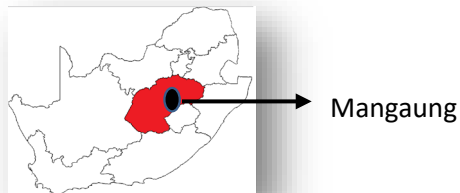


eThekwini

# SARS-CoV-2 detection in wastewater in South Africa

## Mangaung waste water treatment plants

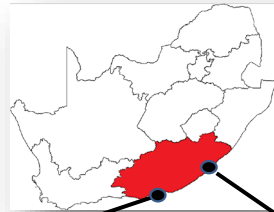
- Current trends in SARS-CoV-2 levels across Mangaung wastewater plants
  - 2 plants
  - One plant with increasing levels
  - One plant with decreasing levels



# SARS-CoV-2 detection in wastewater in South Africa

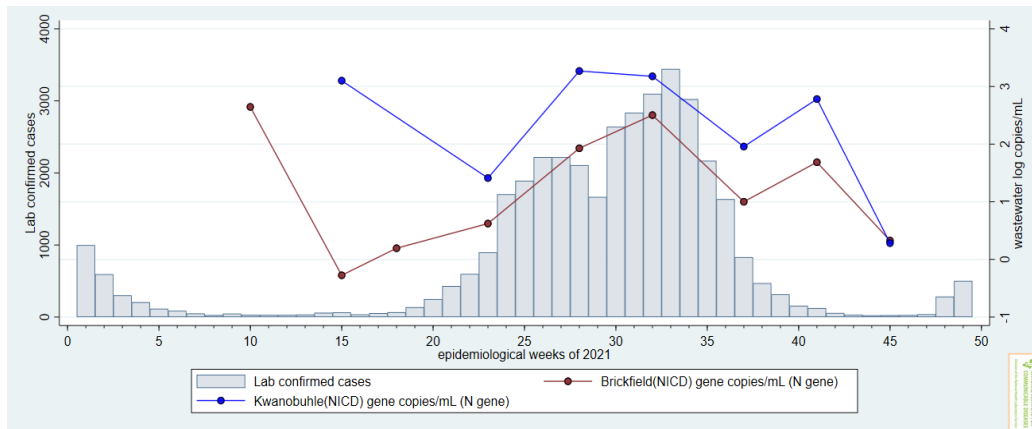
## Eastern Cape wastewater treatment plants

- Current trends in SARS-CoV-2 levels across NMMB wastewater plants
  - 2 plants
  - Increasing levels

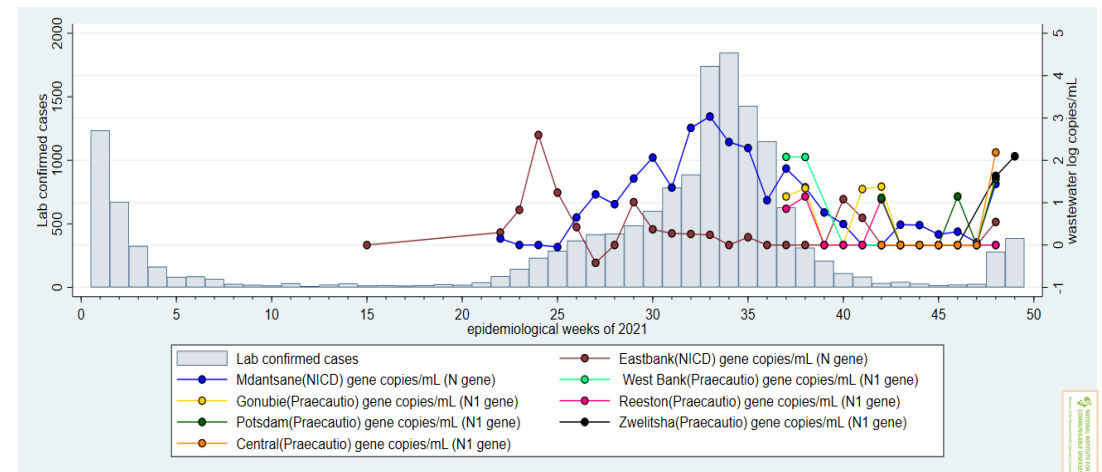


- Current trends in SARS-CoV-2 levels across BCM wastewater plants
  - 2 plants
  - Increasing levels

Nelson Mandela Metro area



Buffalo City Metropolitan area

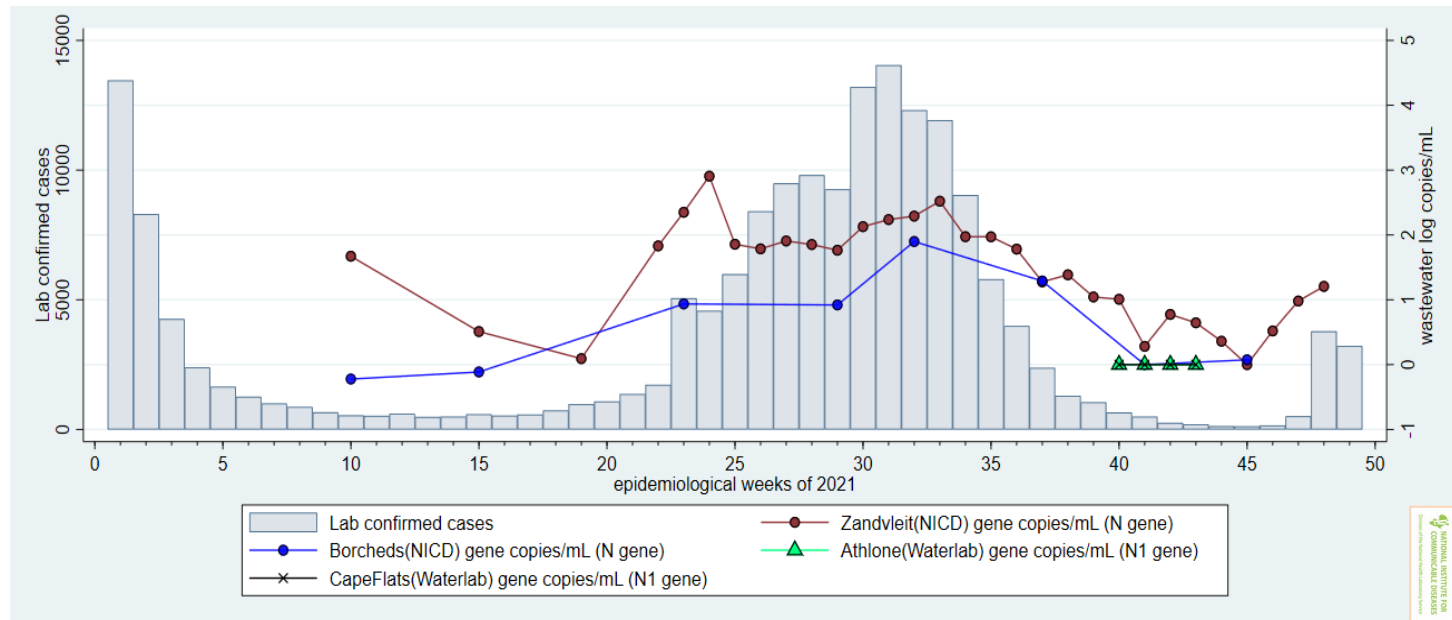




# SARS-CoV-2 detection in wastewater in South Africa

## City of Cape Town wastewater treatment plants

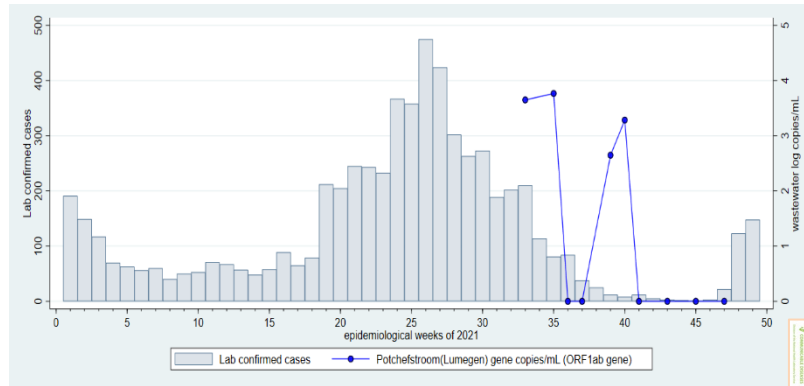
- Current trends in SARS-CoV-2 levels across City of Cape Town wastewater plants
  - 2 plants
  - Stable/increasing levels



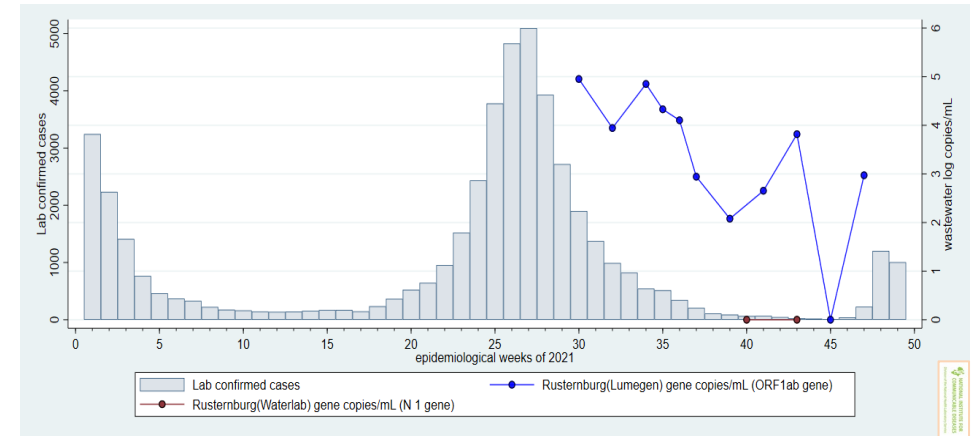
# SARS-CoV-2 detection in wastewater in South Africa

## North west wastewater treatment plants

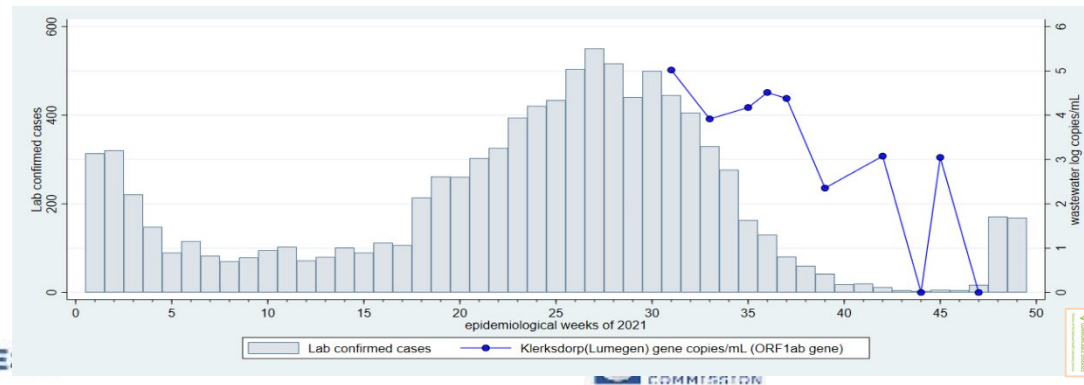
JB Marks - Potchefstroom



Bojanala - Rustenberg



Matlosana - Klerksdorp



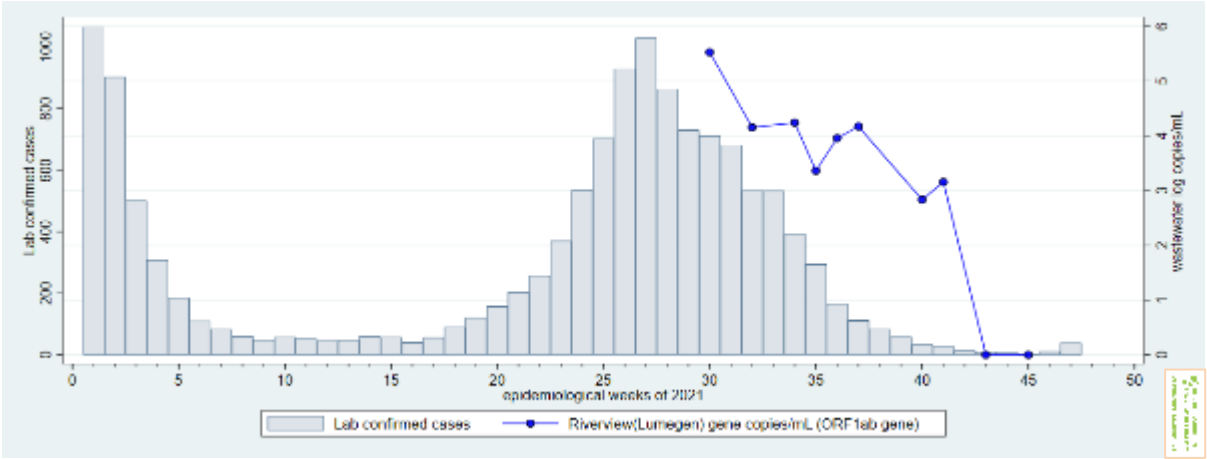
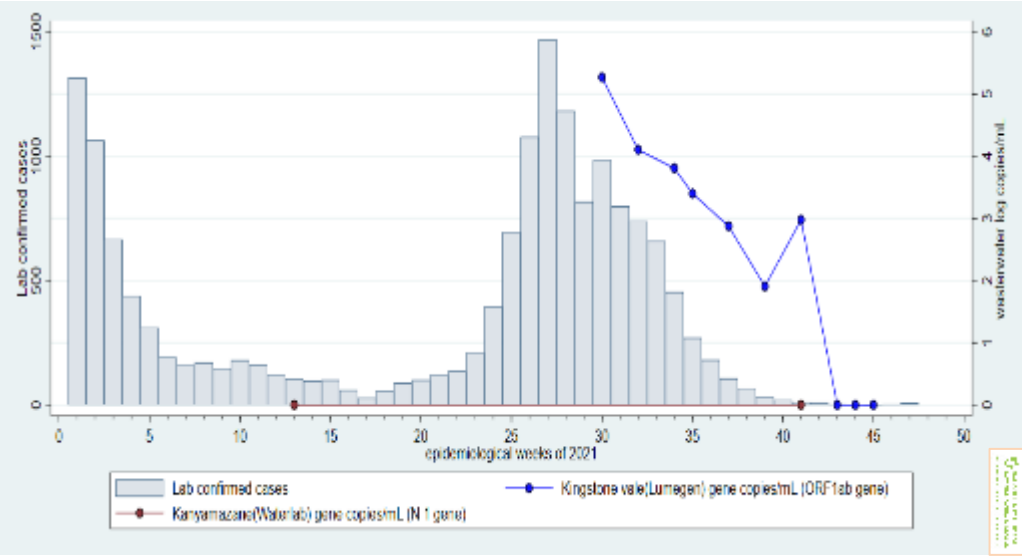
- Current trends in SARS-CoV-2 levels across North West Province

- 3 plants
- Increasing levels

# SARS-CoV-2 detection in wastewater in South Africa

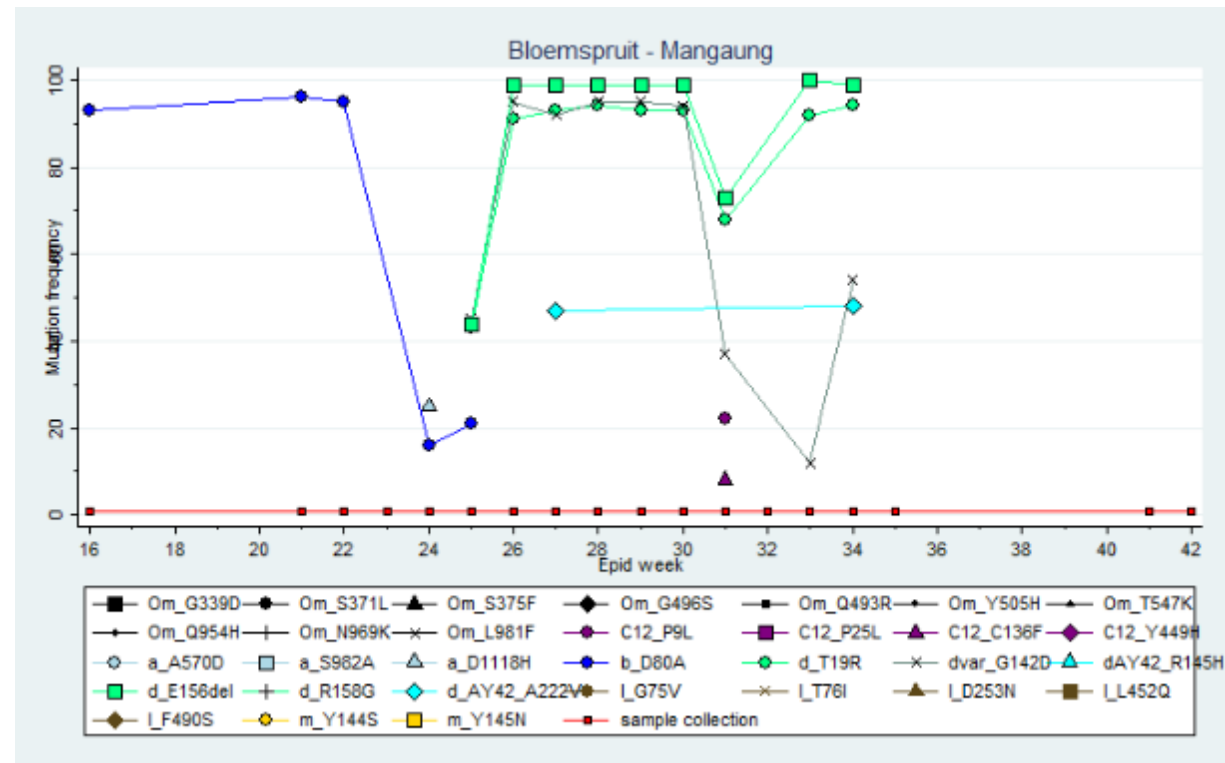
## Mpumalanga wastewater treatment plants

- Mbombela
- Emalaheni



# SARS-CoV-2 detection in wastewater in South Africa

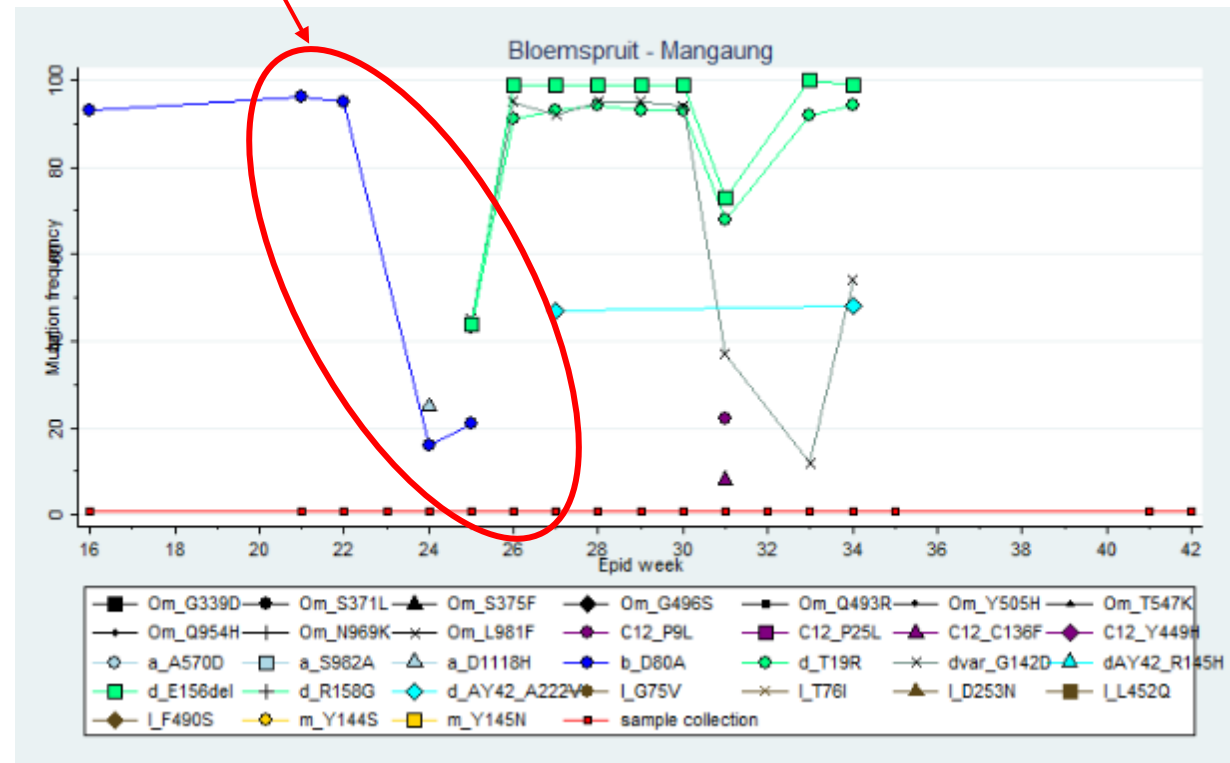
- Full genome sequencing of RNA fragments present in wastewater
  - Allows for detection of single nucleotide polymorphisms (SNPs compatible with different variants)
  - Tracking of variants and read frequency of mutations over time



# SARS-CoV-2 detection in wastewater in South Africa

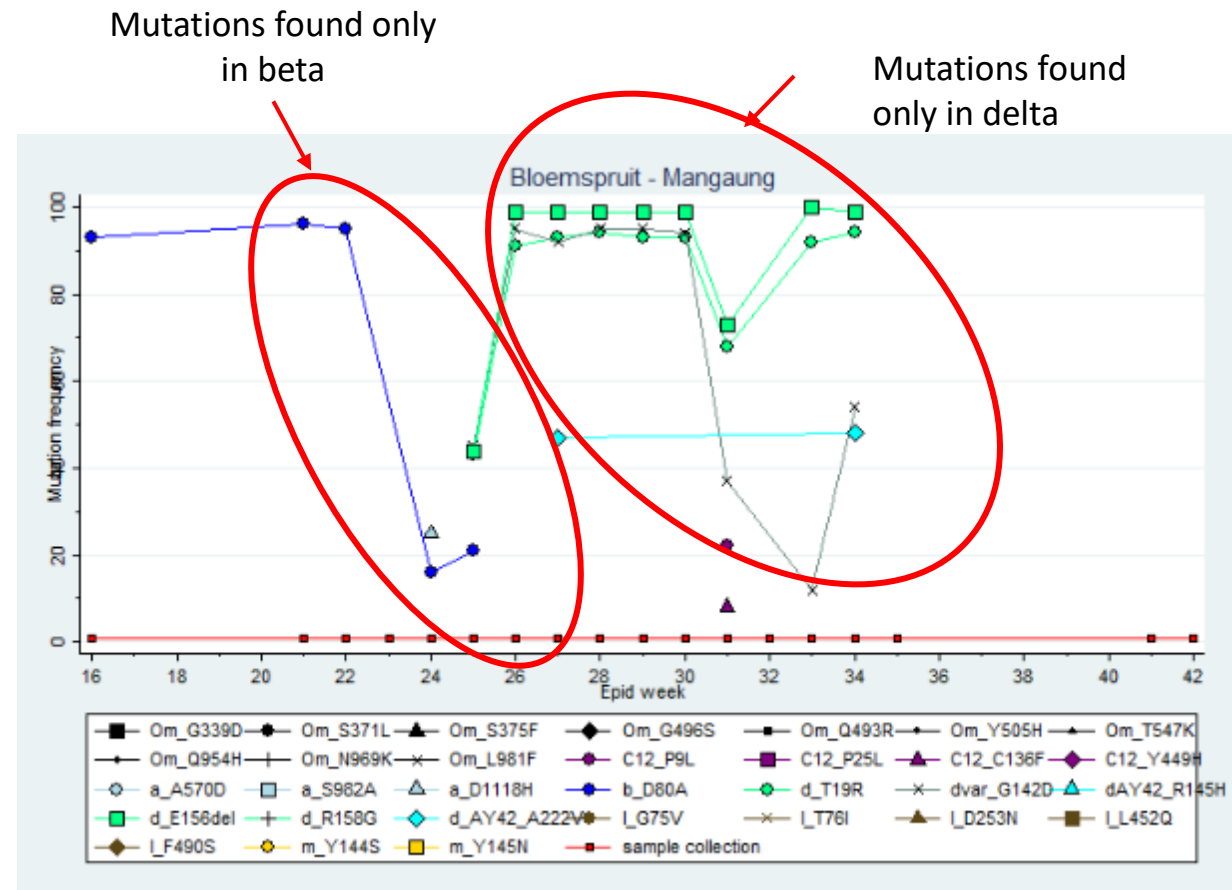
- Mangaung
  - Week 16,
    - Beta dominance
    - C.1.2
  - Week 22
    - Delta appeared and became dominant

Mutations found only  
in beta



# SARS-CoV-2 detection in wastewater in South Africa

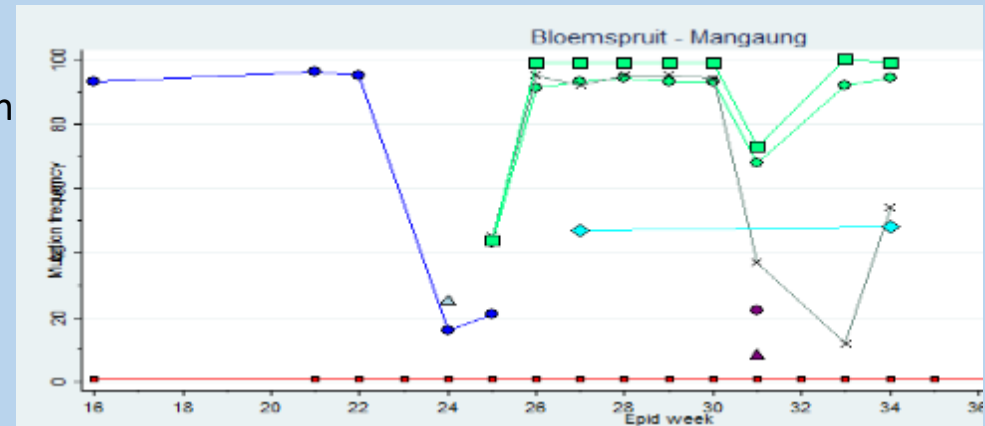
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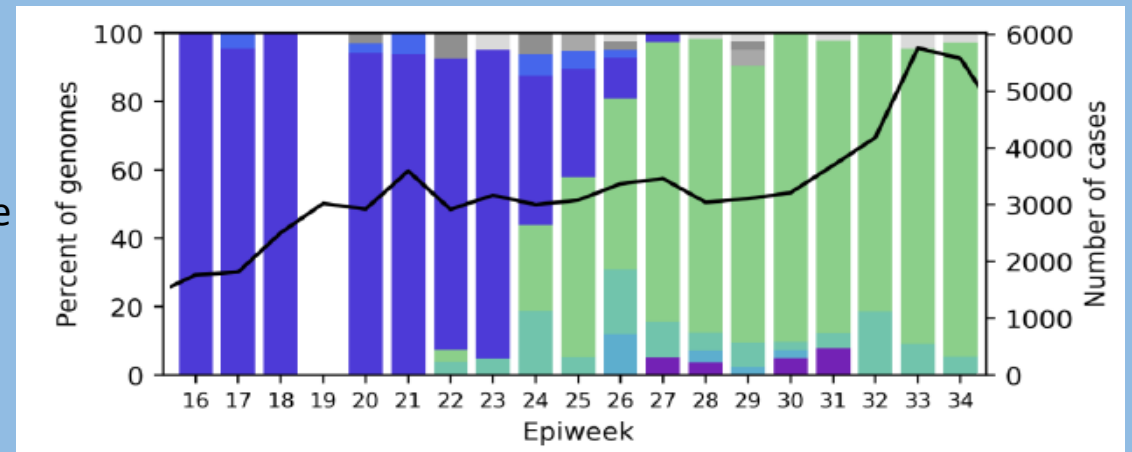
# SARS-CoV-2 detection in wastewater in South Africa

- Mangaung – Bloemspruit WWTP
  - Week 16,
    - Beta dominance
    - C.1.2
  - Week 22
    - Delta appeared and became dominant
- **Good correspondence** with clinical genomics and lineages

SNP mutations corresponding with specific variants identified in 12 wastewater samples



SARS-CoV-2 variants from **clinical isolates** obtained from Free State province (n=c.800)



Clinical isolates figure courtesy CRDM, NICD Jinal Bihman

# Conclusion

- Amongst sentinel wastewater treatment plants in South Africa
  - Quantitative SARS-CoV-2 viral load in genome copies/ml demonstrates concordance with burden of disease as determined by clinical testing
  - Sequencing of SARS-CoV-2 RNA in wastewater demonstrates the presence of single nucleotide polymorphisms compatible with variants detected in clinical specimens over time



# Conclusion

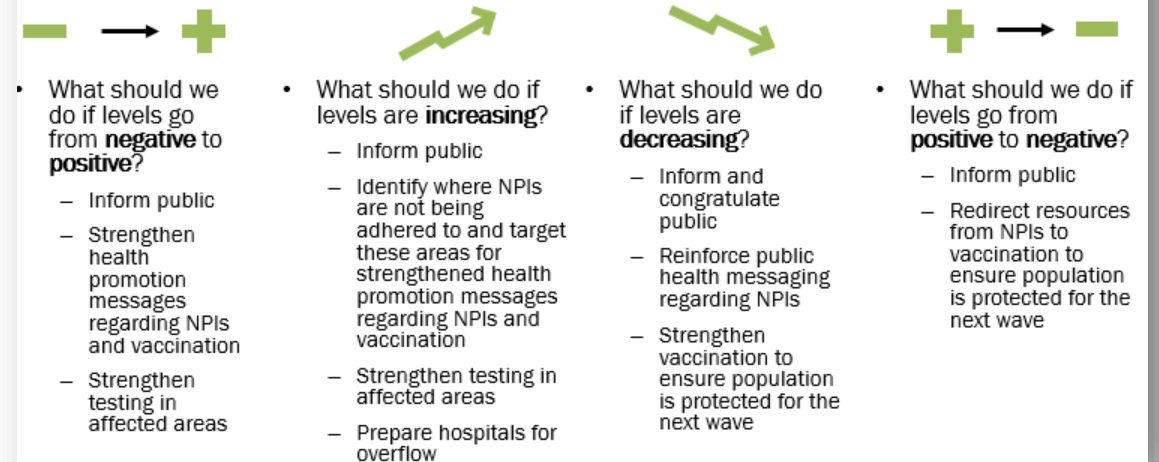
- Amongst sentinel wastewater treatment plants in South Africa
  - Quantitative SARS-CoV-2 viral load in genome copies/ml demonstrates concordance with burden of disease as determined by clinical testing
  - Sequencing of SARS-CoV-2 RNA in wastewater demonstrates the presence of single nucleotide polymorphisms compatible with variants detected in clinical specimens over time
- Wastewater-based epidemiology methods successfully detected the 4<sup>th</sup> wave in Gauteng
- Determination of the presence of omicron variant of concern in wastewater in SACCESS network laboratories is awaited, but has been identified in Cape Town wastewater treatment plants

# Way forward

- Integrate wastewater-based epidemiological surveillance findings into clinical surveillance networks
- Make results accessible through web-based dashboard
- Determine thresholds and triggers for action based on wastewater levels
- Improve turn-around times for genomic sequencing

## Wastewater-based Epidemiology for COVID-19

How can we use wastewater testing for strengthening public health responses



# Acknowledgements

- Water Research Commission
- NICD- Centre for Vaccines and Immunology, Admin, finance and procurement
- Partner labs

