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## **OVERVIEW**

This report summarises and interprets findings from detection and quantification of SARS-CoV-2 levels by the NICD Centre for Vaccines and Immunology in influent (untreated) wastewater in 17 wastewater treatment plants (WWTP) across five provinces tested by the NICD, and 70 additional plants tested by SACCESS partners including the National Institute for Occupational Health, Lumegen Laboratories, GreenHill Laboratories, SAMRC-TB Platform and Praecautio to cover all provinces. Levels of SARS-CoV-2 in wastewater correlate with population levels of SARS-CoV-2 over time and indicate the geographic distribution of disease. SARS-CoV-2 is shed from symptomatic and asymptomatic persons in stool but is not transmitted by faecal-oral route nor via wastewater. This report is based on data collected from June 2021 until 25<sup>th</sup> February 2022 (epidemiological week 8). Generally, levels of SARS-CoV-2 continue to decrease across the country corresponding to the decrease in clinical cases and the exit from the 4<sup>th</sup> wave. Detailed analyses are described below.

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## HIGHLIGHTS

- The wastewater levels of SARS-CoV-2 continue to decrease or remain low, in majority of plants across the country
- However, in some wastewater plants in Gauteng, we have noted marginal increases or stable levels of SARS-CoV-2, suggestive of ongoing transmission among asymptomatic population. These plants include:
  - ♦ Godrich and Daspoort wastewater plants in Tshwane South
  - ◊ Rooiwal and Klipgat plants Tshwane North
  - Vlakplats in Ekurhuleni South
  - ♦ Hartebeesfontein and JP Marais in Ekurhuleni North
- SARS-CoV-2 may also be circulating in areas served by Bloemspruit and Sterkwater in Mangaung, Free State, requiring close monitoring.
- There are delays in submission of results from partner laboratories from non-metro plants due to reagent supply problems.
- The public health authorities are urged to continue to strengthen surveillance for clinical cases in these areas, promote vaccination and non-pharmaceutical interventions in all areas.



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## DETECTION AND QUANTITATION OF SARS-COV-2 AT SENTINEL WASTEWATER TREATMENT SITES IN SOUTH AFRICAN URBAN AREAS, MARCH 2021- JANUARY 2022

### **CO-FUNDED BY THE WATER RESEARCH COMMISSION AND THE NICD**

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- 7. National Institute for Occupational Health, a division of the National Health Laboratory Service, Johannesburg
- 8. Lumegen Laboratories, (Pty) Ltd, Potchefstroom
- 9. Greenhill Laboratories
- 10. Praecautio
- 11. Tuberculosis Platform, South African Medical Research Council, Pretoria.
- 12. Institute of Wastewater Management, Durban University of Technology
- 13. City of Cape Town Health Department
- 14. Water Research Commission, Pretoria
- 15. Department of Chemical Pathology, School of Pathology, University of the Witwatersrand, Johannesburg

## BACKGROUND

The detection and monitoring of SARS-CoV-2 through wastewater was first proposed in April 2020. Initial reports describing the feasibility and practical usefulness of this approach emerged simultaneously from several countries during August 2020. Recent evidence has shown that SARS-CoV-2 can be detected in wastewater prior to the appearance of clinical cases, and longitudinal tracking of SARS-CoV-2 viral load in wastewater correlates with the burden of clinically diagnosed cases. Furthermore, the sequencing of SARS-CoV-2 RNA fragments in wastewater has identified variants of concern as well as mutations not detected in clinical cases.

In South Africa, SARS-CoV-2 epidemiology is monitored through laboratory testing of clinical cases using reverse-transcriptase polymerase chain reaction (RT-PCR) tests and rapid antigen tests, COVID-19 hospital admissions and COVID-19 - related deaths. Laboratory testing data is relayed by testing laboratories to the National Institute for Communicable Diseases (NICD) via the DATCOV system. From these data sources,

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epidemiological indicators including incidence rates of testing and case detection, hospitalisation and death rates are made available to key stakeholders and the general public.

Clinical epidemiology based on reporting of laboratory-confirmed cases of SARS-CoV-2 has limitations. Household transmission studies in South African urban and rural settings have demonstrated that a large proportion of cases are asymptomatic or so mild as not to elicit health-seeking, and that laboratoryconfirmed cases likely represent less than 10% of SARS-CoV-2 cases prevalent in a community at any given time. Secondly, there is increasing use of rapid antigen detection tests in clinical settings. Results of these tests may not be reported to surveillance networks. Consequently, laboratory diagnosis is increasingly less representative of the burden of disease.

In November 2020, a network of testing laboratories, which became known as the South African Collaborative COVID-19 Environmental Surveillance System (SACCESS) network, was established in order to support the development of a common testing methodology, identify and address challenges, and share best practices related to qualitative, quantitative and RNA sequencing of SARS-CoV-2 in wastewater. Treatment of wastewater in South Africa is the responsibility of local government. Approximately 1050 wastewater treatment works (WWTPs) are administered by metropolitan councils and local government and treat industrial and domestic waste. SACCESS partners and the NICD have engaged with local government to support sample collection, interpretation and utilisation of the results for public health purposes.

The SACCESS network aims to detect and quantify SARS-CoV-2 in wastewater in urban settings in South Africa, to compare trends, temporal and geographic distribution of SARS-CoV-2 levels in wastewater with trends in clinical epidemiology so as to support the use of wastewater-based epidemiology for COVID-19 outbreak prevention and response activities.

## **METHODS**

#### Outbreak context and clinical case epidemiology

Since the first case of SARS-CoV-2 in South Africa was detected on 3<sup>rd</sup> March 2020, laboratories in the country have conducted over 22 million RT-PCR and antigen tests. Four distinct waves of SARS-CoV-2 infection occurred, peaking in June 2020, December 2020, July 2021, and December 2021 respectively. The current de-duplicated and geospatially allocated national line list of laboratory-confirmed cases of SARS-CoV-2 (identified by RT-PCR or antigen test) is provided by the NICD for comparison with results from SARS-CoV-2 testing of wastewater.

#### Establishment of the laboratory testing network

Commencing in 2018, the NICD had been conducting testing of wastewater for poliovirus as part of the National Department of Health's polio surveillance programme. In 2020, the NICD commenced testing of influent wastewater samples from these 18 sites, including eight in Gauteng Province, two in the City of Cape Town (Western Cape Province), two in Mangaung (Free State Province), two in eThekwini (KwaZulu-Natal Province) and four in Eastern Cape Province (two in Buffalo City Metro and two in Nelson Mandela Metro). Quantitative testing results for these sites are available from week 8 of 2021, onwards.

Additional plants across all metropolitan areas as well as sentinel site plants in smaller provinces were included from February 2021. From August 2021, quantitative testing was conducted on all specimens submitted to partner laboratories for testing. Presently, samples from 87 WWTPs are being tested for SARS-CoV-2. The supplementary Table 1 at the bottom of the page shows all the data for these plants, including their geographical location, the surrounding suburbs, water service authority, the testing laboratory, and dates testing began in these sites.

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#### SARS-CoV-2 detection and quantitation methodology

The general approach of SARS-CoV-2 detection in wastewater used at all network laboratories is virus concentration, followed by nucleic acid extraction and molecular detection. At the identified wastewater treatment facilities grab or passive samples of influent are collected and transported at <5°C to the testing facility. Table 1 summarises the sample collection, processing and detection methodology used by laboratories involved in the surveillance project. The levels of SARS-CoV-2 in wastewater are reported in copies/mL of wastewater. These values are log-transformed before constructing the graphs.

 Table 1. Sampling and methodology used by laboratories involved in the NICD-WRC led COVID-19 wastewater surveillance project.

Name of laboratory	Sampling	Virus concentration	Nucleic acid extraction	Molecular analysis	Molecular analysis platform
National Institute for Communicable Diseases (NICD)	Grab	Ultrafiltration (Centricon® Plus-70 centrifugal ultra-filter device)	QlAamp® viral RNA mini kit	RT-qPCRª using the Allplex™ 2019-nCoV Assay and the EDX SARS-CoV-2 standard	7500 Real-Time PCR System (Applied Biosystems
GreenHill Laboratories / Praecautio	Grab	Ultrafiltration (Amicon® Ultra-15 Centrifugal Filter Unit)	Omega Bio-Tek Mag- Bind® Viral DNA/RNA 96 Kit	RT-qPCR using the CDC 2019-Novel Coronavirus (2019- nCoV) Real-Time RT-PCR Diagnostic Panel	Rotor-Gene Q (Qiagen)
National Institute for Occupational Health (NIOH)	Grab	Skim milk flocculation	MagMAX Viral and Pathogen Nucleic Acid Isolation Kit	RT-qPCR using the TaqPath COVID-19 CE-IVD RT-PCR Kit (Thermo Fisher)	QuantStudio™ 5 Real-Time PCR System 96-well, 0.1 mL, desktop (Applied Biosystems)
Waterlab/University of Pretoria	Grab	Skim milk flocculation	QlAamp® Ultrasens® Virus kit	RT-qPCR using the Allplex <sup>™</sup> 2019-nCoV Assay and the using the 2019_ nCoV_N positive control plasmid (Integrated DNA Technologies, Inc, Coralville, IA)	QuantStudio™ 5 Real-Time PCR System (Applied Biosystems)
South African Medical Research Council – Tuberculosis platform (SAMRC-TB Platform)	Grab	None – sample is centrifuged then supernatant analysed	ZymoBiomics RNA Extraction Kit	RT-qPCRª using the Allplex™ 2019-nCoV Assay and the EDX SARS-CoV-2 standard	QuantStudio 5 (Applied Biosystems)
Lumegen	Passive	Passive sampler and resuspension in phosphate buffered saline	MN DNA/RNA pathogen extraction Kit	RT-qPCR using the TaqPath COVID-19 CE-IVD RT-PCR Kit (Thermo Fisher)	QuantStudio 5 (Applied Biosystems)
Council for Scientific and Industrial Research (CSIR)	Grab	Polyethylene Glycol precipitation	Omega Bio-tek ENZA total RNA Kit II	RT-qPCR using the 2019-nCoV CDC EUA Kit	Qiagen Rotor- Gene 6000 (5-plex) (Qiagen)
Durban University of Technology – Institute of Wastewater Management	Grab	Ultrafiltration (Centricon® Plus-70 centrifugal ultra-filter device)	QlAamp® viral RNA mini kit	RT-ddPCR <sup>b</sup> using CDC 2019-nCoV_N2 Primers, Fam Labelled, double quenched probes	QX200 AutoDG Droplet Digital PCR System (Bio-rad)

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#### Interpretation of SARS-CoV-2 levels in wastewater

Interpretation of SARS-CoV-2 wastewater levels is evolving. We have elected to use interpretive principles outlined in Table 2 to support public health preparedness and response activities. In general, increasing or decreasing trends in levels are reported based on two or more results, as a single sample that increases or decreases compared with the result from the previous week may represent an outlier. Small changes (up to 0.5log copies/ml) are not regarded as significant changes unless they form part of a general upward or downward trend. Comparison of results over time when quantification is done by the same laboratory using the same quantitative methodology is meaningful. The use of different methodologies by different laboratories precludes comparison of quantitative results across laboratories.

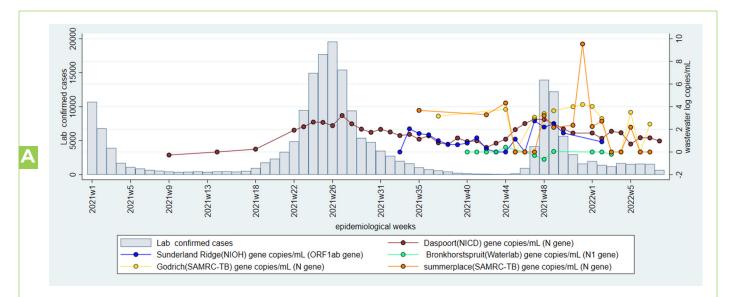
**Table 2.** Principles of SARS-CoV-2 detection and quantification on influent samples from wastewater treatment plants andinterpretive principles to guide application of test results to support COVID-19 public health responses, South Africa.

Testing modality	Interpretive principles to support public health responses
	When a test result changes from
Detection of SARS-CoV-2	<ul> <li>positive to negative, this signifies fewer/no cases in population</li> <li>negative to positive, this indicates the need for increased population awareness and action</li> <li>Qualitative results (presence or absence) are comparable between laboratories</li> </ul>
Quantification of SARS- CoV-2	*The concentration of SARS-CoV-2 at a particular facility may be used to infer the burden of SARS- CoV-2 in the population served by the wastewater treatment facility. *Changes in the concentration of SARS-CoV-2 give an indication of whether the burden of disease is increasing or decreasing *Quantitative results between laboratories are not comparable. *Quantitative results should be interpreted for a single wastewater treatment plant tested by the same laboratory using the same methodology over time

## RESULTS

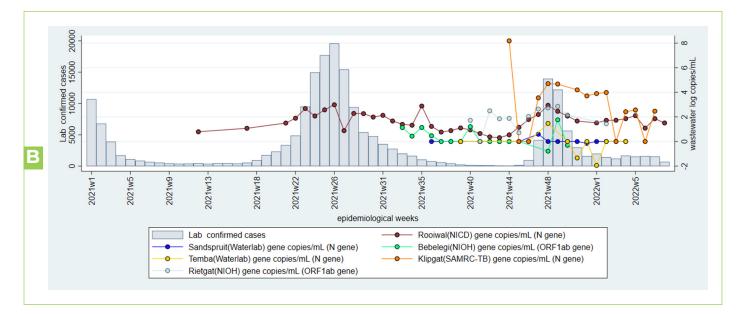
**Gauteng Province** 

A: City of Tshwane South (sub-districts 3, 4, 6, and 7)

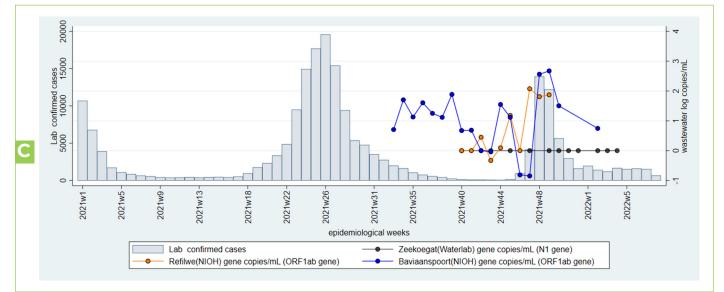


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#### B: City of Tshwane North (sub-districts 1 & 2)

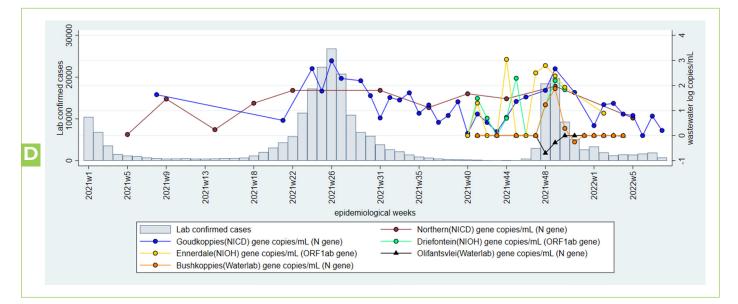


### C: City of Tshwane North (sub-district 5)



**Figure 1 A-C.** Laboratory confirmed cases of SARS-CoV-2 (bars) and levels of SARS-CoV-2 in log copies/ml of wastewater (coloured lines) for selected wastewater treatment plants (WWTP) and metropolitan areas in Tshwane District Municipality (Tshwane South and North), Gauteng Province during epidemiological weeks 1 of 2021 to week 7 of 2022. The testing laboratory and quantified SARS-CoV-2 gene is named in brackets after the name of the WWTP. Note that comparisons of levels over time should only be done for specimens tested in the same laboratory.

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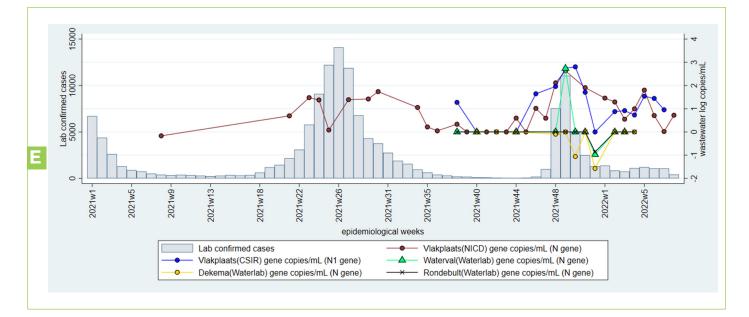


D: City of Johannesburg Metropolitan Municipality

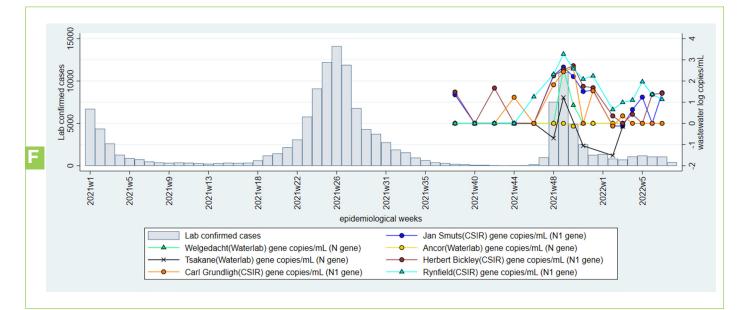
**Figure 1 D.** Laboratory confirmed cases of SARS-CoV-2 (bars) and levels of SARS-CoV-2 in log copies/ml of wastewater (coloured lines) for selected wastewater treatment plants (WWTPs) in the City of Johannesburg Metropolitan Municipality, Gauteng Province during epidemiological weeks 1 of 2021 to week 7 of 2022. The testing laboratory and quantified SARS-CoV-2 gene are named in brackets after the name of the WWTP. Note that comparisons of levels overtime should only be made for specimens tested in the same laboratory.

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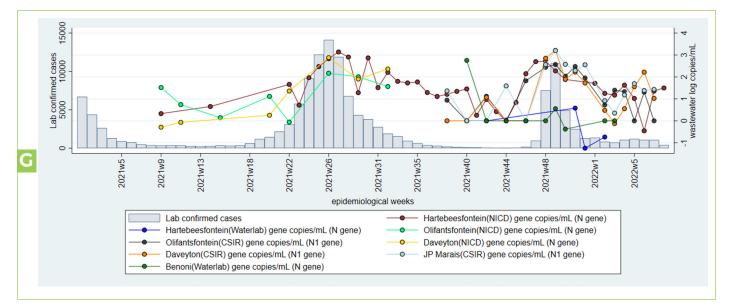
### E: Ekurhuleni East (sub-districts D, E or E1, E2)



### F: Ekurhuleni South (sub-districts A, F or S1, S2)



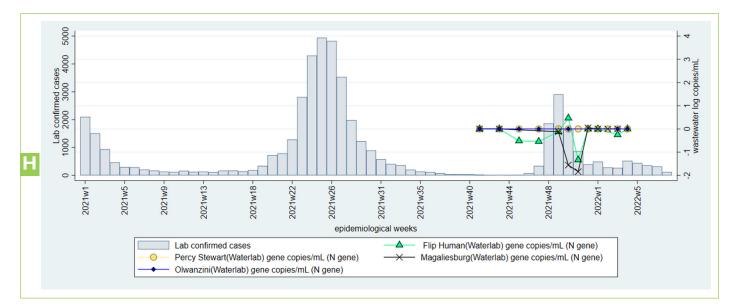
### WEEK 8 2022



G: Ekurhuleni North (sub-districts B, C or N1,N2)

**Figure 1 E-G.** Laboratory confirmed cases of SARS-CoV-2 (bars) and levels of SARS-CoV-2 in log copies/ml of wastewater (coloured lines) for selected wastewater treatment plants (WWTP) in Ekurhuleni Metropolitan Municipality, Gauteng Province during epidemiological weeks 1 of 2021 to week 8 of 2022. The testing laboratory and quantified SARS-CoV-2 gene are named in brackets after the name of the WWTP. Note that comparisons of levels over time should only be made for specimens tested in the same laboratory.

### **H: West Rand District Municipality**



**Figure 1H.** Laboratory confirmed cases of SARS-CoV-2 (bars) and levels of SARS-CoV-2 in log copies/ml of wastewater (coloured lines) for selected wastewater treatment plants (WWTP) in West Rand District Municipality, Gauteng Province during epidemiological weeks 1 of 2021 to week 4 of 2022. The testing laboratory and quantified SARS-CoV-2 gene are named in brackets after the name of the WWTP. Note that comparisons of levels over time should only be made for specimens tested in the same laboratory.

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In Tshwane South, the SARS-CoV-2 levels are increasing in Godrich but will require more recent samples. In addition, SARS-CoV-2 continues to circulate in Daspoort as the levels remain consistently at llogcpopy/ml of wastewater over three weeks.

In Tshwane North (sub-district 1&2), the SARS-CoV-2 levels in Klipgat WWTP increased in week 7 but will require recent samples. In Rooiwal the SARS-CoV-2 levels continues to circulate in Daspoort as the levels remain consistently at approximately 2logcopies/ml of wastewater over two weeks- week 7 and 8.

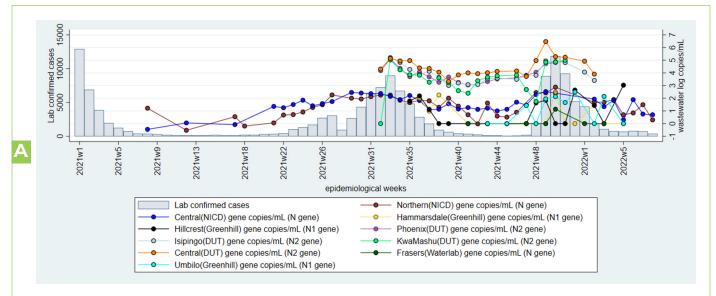
The SARS-CoV-2 levels all WWTPs in Johannesburg have decreased corresponding to a decrease in clinical case load.

In Ekurhuleni East (sub-districts D, E or E1, E2) the SARS-CoV-2 levels in Jan Smuts, increased in week 7 but will require recent samples for confirmation. The SARS-CoV-2 levels in areas served by the Vlakplaats WWTP in Ekurhuleni South are seen to be declining corresponding to declining number of clinical cases. Also, in Ekurhuleni North, the levels in Hartebeesfontein and JP Marais are still high suggesting continued community transmission within areas served by these WWTPs

The public health authorities should continue to strengthen surveillance for clinical cases in these areas, promote vaccination and non-pharmaceutical interventions in all areas.

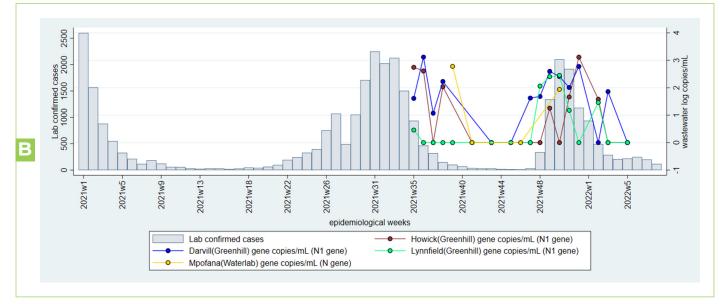
#### KwaZulu-Natal Province

#### 2A: eThekwini Metropolitan Municipality



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### **B: uMgungundlovu District Municipality**



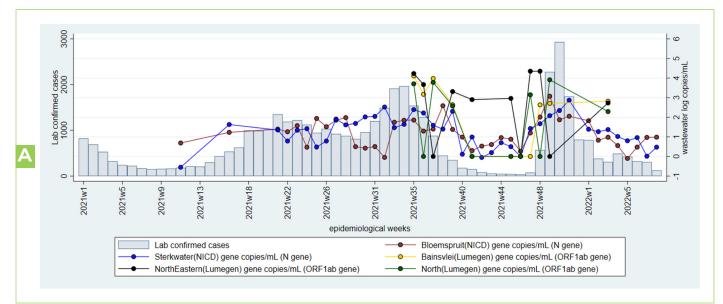
**Figure 2A-B.** Laboratory confirmed cases of SARS-CoV-2 (bars) and levels of SARS-CoV-2 in log copies/ml of wastewater (coloured lines) from wastewater treatment plants (WWTP) in eThekwini, (A-B) and uMgungundlovu Metro (C), KwaZulu Natal Province during epidemiological weeks 1-51, 2021 and week 8, 2022. The testing laboratory and quantified SARS-CoV-2 gene are named in brackets after the name of the WWTP. Note that comparisons of levels over time should only be made for specimens tested in the same laboratory.

The SARS-CoV-2 levels are decreasing in majority of the plants in eThekwini and uMgungundlovu, corresponding to the decrease in clinical cases. These SARS-CoV-2 levels may be attributed to the Omicron subvariant. Therefore, the authorities should continue to strengthen surveillance for clinical cases in this area and promote vaccination and non-pharmaceutical interventions in all areas.

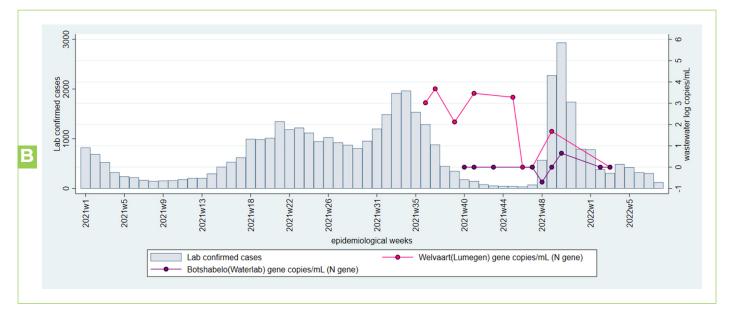
### WEEK 8 2022

### Free State Province- Mangaung

#### A: Bloemfontein sub-district

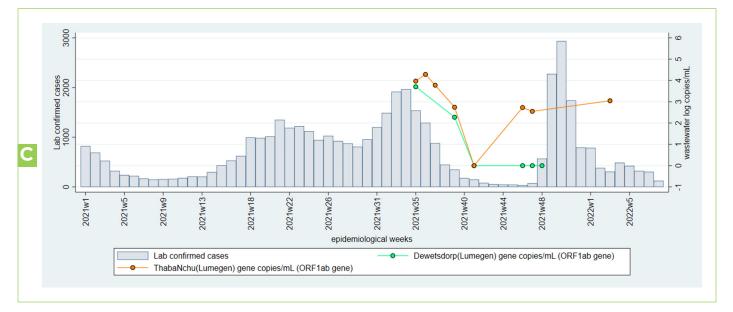


### B. Botshabelo sub-district



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#### C. Neledi & ThabaNchu sub-districts



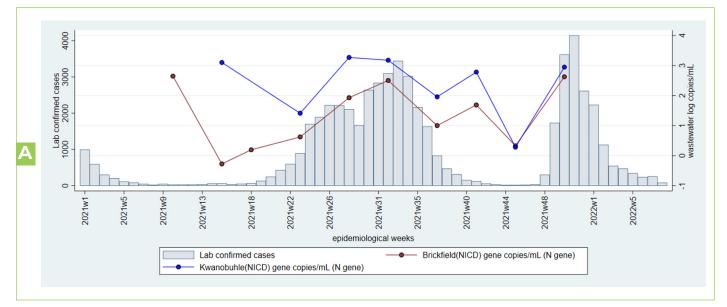
**Figure 3 A-C.** Laboratory confirmed cases of SARS-CoV-2 (bars) and levels of SARS-CoV-2 in log copies/ml of wastewater (coloured lines) from wastewater treatment plants (WWTPs) in Mangaung, Free State Province (Bloemfontein, Botshabelo, Naledi and ThabaNchu) during epidemiological weeks 1, 2021 to 8, 2022. The testing laboratory and quantified SARS-CoV-2 gene are named in brackets after the name of the WWTP. Note that comparisons of levels over time should only be made for specimens tested in the same laboratory.

The recent SARS-CoV-2 levels in all the plants in the three sub-districts in Free State continue to decline or remain stable at low levels, corresponding to the decreases in clinical cases. However, the SARS-CoV-2 may still be circulating in areas served by Bloemspruit and Sterkwater in Mangaung, requiring close monitoring. The public health authorities should continue surveillance for cases, promote vaccination and non-pharmaceutical interventions.

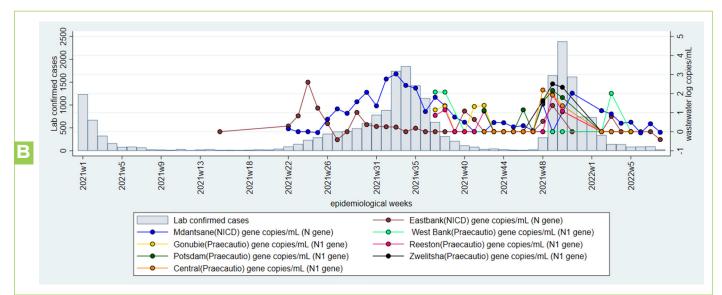
### WEEK 8 2022

#### **Eastern Cape Province**

A: Nelson Mandela Metropolitan Municipality



**Figure 4A.** Laboratory confirmed cases of SARS-CoV-2 (bars) and levels of SARS-CoV-2 in log copies/ml of wastewater (coloured lines) from wastewater treatment plants (WWTPs) in Nelson Mandela Metro, Eastern Cape Province during epidemiological weeks 1-45, 2021. The testing laboratory and quantified SARS-CoV-2 gene are named in brackets after the name of the WWTP. Note that comparisons of levels over time should only be made for specimens tested in the same laboratory.



#### **B: Buffalo City Metropolitan Municipality**

**Figure 4B.** Laboratory confirmed cases of SARS-CoV-2 (bars) and levels of SARS-CoV-2 in log copies/ml of wastewater (coloured lines) from wastewater treatment plants (WWTPs) in Nelson Mandela Metro, Eastern Cape Province during epidemiological weeks 1, 2021 to 8, 2022. The testing laboratory and quantified SARS-CoV-2 gene are named in brackets after the name of the WWTP. Note that comparisons of levels over time should only be made for specimens tested in the same laboratory.

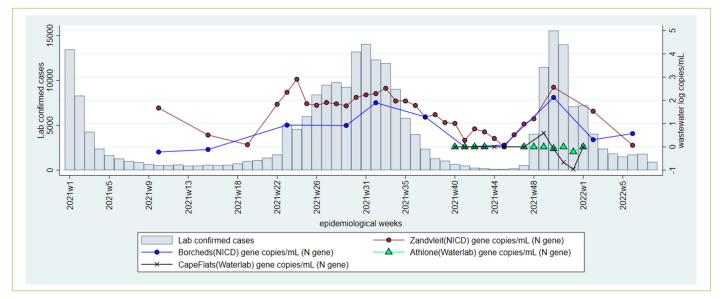
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In Buffalo City Metro, results show that SARS-CoV-2 levels in all WWTPs have steadily decreased and remained at zero levels, corresponding to the decline in clinical cases. The public health authorities should continue to promote vaccination and non-pharmaceutical interventions. In Nelson Mandela Metro, assessment cannot be made due to absence of recent results. Readers are referred to the SAMRC wastewater dashboard for more in-depth data regarding levels of SARS-CoV-2 in wastewater plants in Nelson Mandela Metro (https://www.samrc.ac.za/wbe/).

#### Western Cape Province

#### **City of Cape Town:**



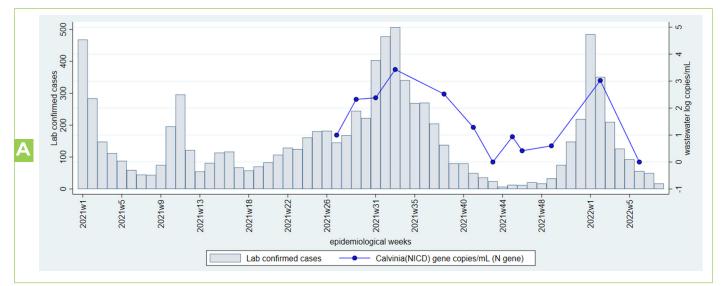
**Figure 5.** Laboratory confirmed cases of SARS-CoV-2 (bars) and levels of SARS-CoV-2 in log copies/ml of wastewater (coloured lines) from wastewater treatment plants (WWTPs) in the City of Cape Town, Western Cape Province during epidemiological weeks 1, 2021 to 6, 2022. The testing laboratory and quantified SARS-CoV-2 gene are named in brackets after the name of the WWTP.

The SARS-CoV-2 levels in Borcherds Quarry and Zandvleit have also declined to below 1 gene copy/ ml of wastewater, corresponding to the decline in clinical case load. Readers are referred to the MRC website, which provides data from additional wastewater treatment plants in the City of Cape Town and other Western Cape districts (https://www.samrc.ac.za/wbe/) to contextualise the results. The public health authorities should continue surveillance for cases, promote vaccination and non-pharmaceutical interventions.

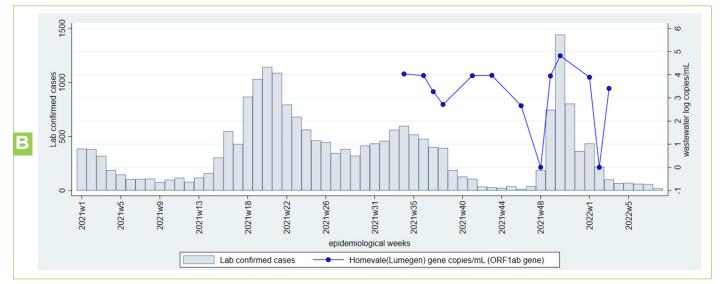
### WEEK 8 2022

## Northern Cape Province

A: Namakwa District Municipality



### **B:** Frances Baard District Municipality



**Figure 6A-B.** Laboratory confirmed cases of SARS-CoV-2 (bars) and levels of SARS-CoV-2 in log copies/ml of wastewater (coloured lines) in wastewater treatment plants (WWTPs) from Calvinia in Namakwa Metro (a) and Kimberly in Frances Baard District (b), Northern Cape Province during epidemiological weeks 1, 2021 to week 2, 2022. The testing laboratory and quantified SARS-CoV-2 gene are named in brackets after the name of the WWTP. Note that comparisons of levels over time should only be made for specimens tested in the same laboratory.

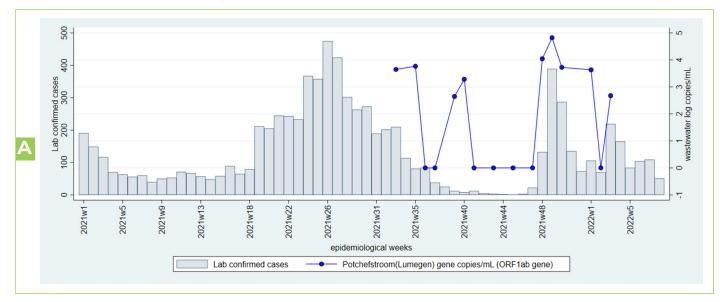
In Calvina WWTP in Namakwa subdistrict the SARS-CoV-2 levels were seen to decrease in week 5, 2022, corresponding to decline in clinical cases. More recent results are required. On the other hand, a current assessment cannot be made for France Baard due to the absence of recent results. The public health authorities should continue surveillance for cases, promote vaccination and non-pharmaceutical interventions.



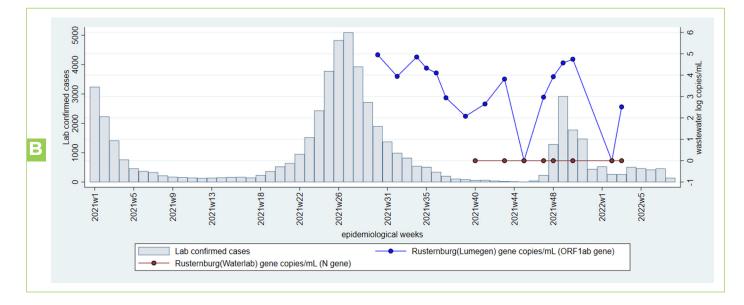
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#### **North West Province**

### A: JB Marks Local Municipality

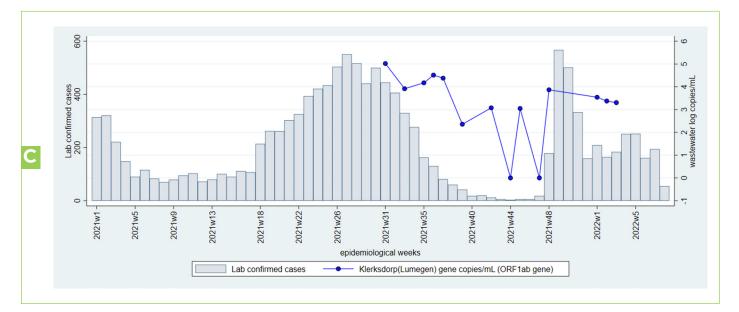


### **B: Bojanala District Municipality**



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#### **C: City of Matlosana Municipality**



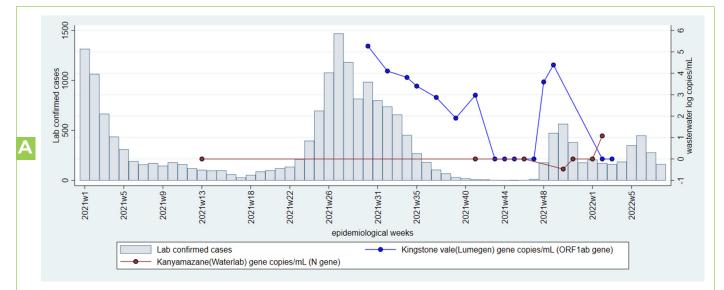
**Figure 7A-C.** Laboratory confirmed cases of SARS-CoV-2 (bars) and levels of SARS-CoV-2 in log copies/ml of wastewater (coloured lines) in wastewater treatment plants (WWTPs) from Potchefstroom, JB Marks District (A) Rustenberg, Bojanala District (B), and City of Matlosana, Northwest Province during epidemiological weeks 1, 2021 to 3, 2022. The testing laboratory and quantified SARS-CoV-2 gene are named in brackets after the name of the WWTP. Note that comparisons of levels over time should only be made for specimens tested in the same laboratory.

The SARS-CoV-2 levels in Potchefstroom (JB Marks), Rustenburg (Bojanala) and Klerksdorp (Matlosana) as of week 3,2022 were around 3log copies/ml of wastewater suggesting ongoing population transmission by the Omicron subvariant. However, more recent and consistent results are required in this area. The public health authorities should continue surveillance for cases, promote vaccination and non-pharmaceutical interventions.

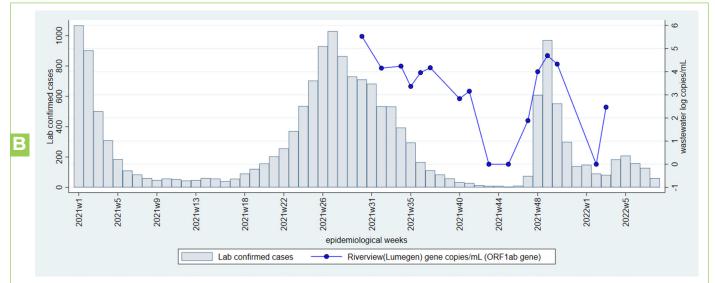
### WEEK 8 2022

### Mpumalanga Province

### A: Mbombela Local Municipality



### **B: Emalahleni Local Municipality**



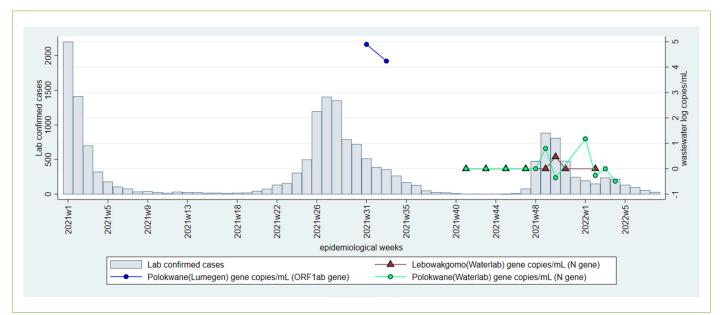
**Figure 8A-B.** Laboratory confirmed cases of SARS-CoV-2 (bars) and levels of SARS-CoV-2 in log copies/ml of wastewater (coloured lines) in wastewater treatment plants (WWTPs) from Mbombela and Emalahleni Local Municipality, Mpumalanga Province during epidemiological weeks 30-45, 2021. The testing laboratory and quantified SARS-CoV-2 gene are named in brackets after the name of the WWTP. Note that comparisons of levels over time should only be made for specimens tested in the same laboratory.

The SARS-CoV-2 levels in Mbombela (Kingstonvale) have consistently decreased corresponding to the decrease in clinical cases. Although the levels in Riverview increased in week 3 suggesting ongoing population transmission by the Omicron subvariant, more recent and consistent results are required in this area The public health authorities should continue surveillance for cases, promote vaccination and non-pharmaceutical interventions.

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#### **Limpopo Province**

#### **Polokwane Local Municipality**



**Figure 9.** Laboratory confirmed cases of SARS-CoV-2 (bars) and levels of SARS-CoV-2 in log copies/ml of wastewater (coloured lines) in wastewater treatment plants (WWTPs) from Polokwane Local Municipality, Limpopo Province during epidemiological weeks 31-33, 2021.

Results are difficult to interpret, but levels are declining in Polokwane corresponding to decline in clinical case load. Current samples are needed for this area.



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## LIMITATIONS

It is not possible to estimate population burden of disease using wastewater testing of SARS-CoV-2 as sources of variability are multiple, including variation in length and concentration of SARS-CoV-2 excretion by infected persons, variation in degradation rate of viral RNA in wastewater and sampling error. Interpretation of results from quantitative testing of SARS-CoV-2 in wastewater is enhanced when the population served by the wastewater treatment plants is well characterised in terms of SARS-CoV-2 testing rates, health seeking behaviour, hospital admissions and deaths due to SARS-CoV-2, as well as other general indicators of health. Further exploration of the relationship between quantitative SARS-CoV-2 results, local trends in clinical case burden, environmental factors, and test methodology will support the interpretation of observed fluctuations in RNA levels. Quality assessment and inter-laboratory comparisons are underway to ensure participating laboratories are providing consistent and comparable results.

## CONCLUSION

SARS-CoV-2 data from wastewater at South African sentinel sites show concordance with clinical, epidemiologic curves in the respective locations, illustrating the potential of the SACCESS network to provide descriptive epidemiological data pertaining to geographic variation and burden of SARS-CoV-2.

## ACKNOWLEDGEMENTS

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- The NICD Centre for Respiratory Disease and Meningitis are thanked for their assistance in setting up and troubleshooting PCR testing, and ongoing supportive collaboration.
- Staff of SACCESS network laboratories are thanked for their assistance in generating these results.



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Supplementary Table: Data for all wastewater treatment plants tested by SACCESS network

S/ No	Wastewater plant name	Province	Metro or District	Official subdistrict SD or Local municipality	Subdistrict as represented on the graphs	Water service authority	Suburbs in drainage reticulation	Testing laboratory	Date quantitative testing started
1		Eastern Cape			No subdistrict	Buffalo City Local Municipality	Wisonia, Dawn, Summer Pride, Amalinda Forest, Haven Hills, Buffalo flats ext, Scenery Park		20-10-2021
2		Eastern Cape			No subdistrict	Buffalo City Local Municipality			
3	Gonubie	Eastern Cape			No subdistrict	Buffalo City Local Municipality	Sunrise-on-Sea, Conubie Manor, Thorn Ridge, Cyprus Dale, Bay View, Donny-brook, Gonubie, Gonubie Park		15-09-2021
4		Eastern Cape			No subdistrict	Buffalo City Local Municipality	Mdantsane Newlands		01-06-2021
5		Eastern Cape			No subdistrict	Buffalo City Local Municipality	Zone 12 to Zone 18, Unit P, Potsdam, Khayelitsha, WSU Potsdam, Campus, Mbekweni		20-10-2021
6		Eastern Cape			No subdistrict	Buffalo City Local Municipality			15-09-2021
7		Eastern Cape			No subdistrict	Buffalo City Local Municipality	Duncan Village, Leach Bay, Nahoon		15-09-2021
8		Eastern Cape			No subdistrict	Buffalo City Local Municipality			20-10-2021
9		Eastern Cape	Nelson Mandela Metropolitan Municipality		No subdistrict	Nelson Mandela Metropolitan Municipality	KwaNobuhle, Uitenhage, Van Riebeekhoogte		
10		Eastern Cape	Nelson Mandela Metropolitan Municipality		No subdistrict	Nelson Mandela Metropolitan Municipality	KwaNobuhle, Uitenhage		

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S/ No	Wastewater plant name	Province	Metro or District	Official subdistrict SD or Local municipality	Subdistrict as represented on the graphs	Water service authority	Suburbs in drainage reticulation	Testing laboratory	Date quantitative testing started
11			Mangaung			Mangaung		Lumegen	01-09-2021
12			Mangaung			Mangaung	Langenhoven Park, Bloemfontein		16-03-2021
13			Mangaung			Mangaung		Lumegen	01-09-2021
14			Mangaung			Mangaung	Fonteintjie, Rooidam		16-03-2021
15	Botshabelo		Mangaung	Botshabelo SD	Botshabelo	Mangaung	Bonolo, Botshabelo, Poklenberg, Dankbaar, Roodekop	Lumegen	04-10-2021
16			Mangaung	Botshabelo SD	Botshabelo	Mangaung	Kagisanong, Fichardtpark, Bochebela, Phahameng, Generaal deWet, Willows, Batho, Rocklands, Universitas	Lumegen	09-09-2021
17			Mangaung			Mangaung	Midway, Bloemspruit, Grasslands	Lumegen	01-09-2021
18	Dewetsdorp		Mangaung		Naledi and Thabanchu	Mangaung	Dewetsdorp, Frankfort, Glengary	Lumegen	01-09-2021
19	Thaba Nchu		Mangaung	Thaba N'chu SD	Naledi and Thabanchu	Mangaung	Thaba Nchu, Mokwena, Selosesha, Abramskraal, Roodekop, Strydom College, Bultfontein Number Three, Ratlau, Serwalo, Bultfontein Number One, Bultfontein Number Two, Motlala, Lusaka		01-09-2021
20			City of Johannesburg Metropolitan Municipality		No subdistrict	City of Johannesburg Metropolitan Municipality	Walkerville, Hartsenbergfontein, Althea, Golfview, Blignautrus		04-10-2021

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S/ No	Wastewater plant name	Province	Metro or District	Official subdistrict SD or Local municipality	Subdistrict as represented on the graphs	Water service authority	Suburbs in drainage reticulation	Testing laboratory	Date quantitative testing started
21			City of Johannesburg Metropolitan Municipality		No subdistrict	City of Johannesburg Metropolitan Municipality	Strydompark, Olivedale, Rivonia, Jukskei Park, Douglasdale, Ferndale, Lone Hill, Sandton, North Riding, Fourways, Paulshof		06-04-2021
22	Goudkoppies		City of Johannesburg Metropolitan Municipality		No subdistrict	City of Johannesburg Metropolitan Municipality	Soweto, Rivasdale		24-05-2021
23	Bushkoppies		City of Johannesburg Metropolitan Municipality		No subdistrict	City of Johannesburg Metropolitan Municipality	Baragwanath, Pimville, Johannesburg South, Dube, Willowdene, Nancefield		11-10-2021
24			City of Johannesburg Metropolitan Municipality		No subdistrict	City of Johannesburg Metropolitan Municipality			11-10-2021
25			City of Johannesburg Metropolitan Municipality	Mogale City LM	No subdistrict	City of Johannesburg Metropolitan Municipality	Kelvin, Morningside Manor, Edenburg, Lone Hill, Rivonia, Sandton, Northdene, Fourways, Paulshof		04-10-2021
26			City of Tshwane Metropolitan Municipality			City of Johannesburg Metropolitan Municipality	Wilgerivier, Wonderfontein, Graley Crown Douglas, Bronkhorst		04-10-2021
27	Klipgat		City of Tshwane Metropolitan Municipality			City of Tshwane Metropolitan Municipality	Klipgat, Boekenhoutfontein, Soshanguve, Mabopane, Honeyvale, Boekenhoutfontein, Lebaleng	SAMRC-TB Platform	02-11-2021
28	Sandspruit		City of Tshwane Metropolitan Municipality			City of Tshwane Metropolitan Municipality	Medunsa, Hebron, Rosslyn, Strydfontein, Hornsnek, Kruisfontein		11-09-2021
29			City of Tshwane Metropolitan Municipality			City of Tshwane Metropolitan Municipality	Atteridgeville, Pretoria CBD, Pretoria North, Rosslyn		23-03-2021
30			City of Tshwane Metropolitan Municipality			City of Tshwane Metropolitan Municipality	Majanen, Hammanskraal, Mabopane, Soshanguve, Pyramid, Doornpoort		26-09-2021

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S/ No	Wastewater plant name	Province	Metro or District	Official subdistrict SD or Local municipality	Subdistrict as represented on the graphs	Water service authority	Suburbs in drainage reticulation	Testing laboratory	Date quantitative testing started
31			City of Tshwane Metropolitan Municipality			City of Tshwane Metropolitan Municipality	Groenkloof, Arcadia, Pretoria South, Gezina, Hercules, Rietfontein, Pretoria Central, Sunnyside, Pretoria East, Prinshof, Daspoort, Villieria, Capital Park, Pretoria West, Wonderboom South, Pretoria-Wes, Innesdale		02-03-2021
32	Sunderland Ridge		City of Tshwane Metropolitan Municipality			City of Tshwane Metropolitan Municipality	Centurion, Olivenhoutbosch and some parts of Midrand.		18-08-2021
33	Babelegi		City of Tshwane Metropolitan Municipality			City of Tshwane Metropolitan Municipality			18-08-2021
34			City of Tshwane Metropolitan Municipality			City of Tshwane Metropolitan Municipality	Elandsfontein, Cullinan, Sonderwater		18-08-2021
			City of Tshwane Metropolitan Municipality			City of Tshwane Metropolitan Municipality			05-10-2021
36	Zeekoegat		City of Tshwane Metropolitan Municipality			City of Tshwane Metropolitan Municipality	Zeekoegat, Magalies Water, Buffelsdrif		04-10-2021
37	Godrich		City of Tshwane Metropolitan Municipality			City of Tshwane Metropolitan Municipality	Bronkspruit town Rhema Park Caltura park Venster Park Zithobeni	SAMRC-TB Platform	13-09-2021
38	Summer Place Pack- age Plant		City of Tshwane Metropolitan Municipality			City of Tshwane Metropolitan Municipality		SAMRC-TB Platform	01-09-2021
	Rietgat		City of Tshwane Metropolitan Municipality			City of Tshwane Metropolitan Municipality			05-10-2021
40	Thaba Tsh- wane		City of Tshwane Metropolitan Municipality			City of Tshwane Metropolitan Municipality	Thaba Tshwane, Generaal Kemp Heuwel Radio Uitkyk		05-10-2022

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S/ No	Wastewater plant name	Province	Metro or District	Official subdistrict SD or Local municipality	Subdistrict as represented on the graphs	Water service authority	Suburbs in drainage reticulation	Testing laboratory	Date quantitative testing started
41			Ekurhuleni Metropolitan Municipality			Ekurhuleni Metropolitan Municipality	Welgedag, Persida		NICD:02-03- 2021 CSIR: 21-09- 2021
42			Ekurhuleni Metropolitan Municipality			Ekurhuleni Metropolitan Municipality	New Modder, Lakefield, Benoni, Boksburg, Northmead, Atlasville		21-09-2021
43			Ekurhuleni Metropolitan Municipality			Ekurhuleni Metropolitan Municipality	Welgedag, Payneville, Selcourt, Casseldale, Springs		21-09-2021
44	Carl Grun- dlingh		Ekurhuleni Metropolitan Municipality			Ekurhuleni Metropolitan Municipality	Nigel, Bultfontein, Laversburg		21-09-2021
45			Ekurhuleni Metropolitan Municipality			Ekurhuleni Metropolitan Municipality	Dalpark, Brakpan, Dersley, Dalview, Benoni, New Modder, Schapenrust		21-09-2021
46			Ekurhuleni Metropolitan Municipality			Ekurhuleni Metropolitan Municipality	Benoni, Dersley, Dalpark, Brakpan, Dalview, Schapenrust		05-10-2021
47	Welgedacht		Ekurhuleni Metropolitan Municipality			Ekurhuleni Metropolitan Municipality	KwaThema, Brakpan, Dersley, Schapenrust		21-09-2021
48	Hartebees- fontein WasteWater Treatment Works		Ekurhuleni Metropolitan Municipality		Ekurhuleni North (N1, N2)	Ekurhuleni Metropolitan Municipality	Mid-Ennerdale, Althea, Grasmere, Elandsfontein		NICD:02-03- 2021 Waterlab/UP : 05-10-2021
49			Ekurhuleni Metropolitan Municipality		Ekurhuleni North (N1, N2)	Ekurhuleni Metropolitan Municipality			21-09-2021
50			Ekurhuleni Metropolitan Municipality		Ekurhuleni North (N1, N2)	Ekurhuleni Metropolitan Municipality	Pinedene, Clayville, Tembisa, Midstream Estates, Olifantsfontein		CSIR: 21-09- 2021 NICD: 02-03- 2021
51			Ekurhuleni Metropolitan Municipality	Ekurhuleni N2 SD	Ekurhuleni North (N1, N2)	Ekurhuleni Metropolitan Municipality	Northmead, Dalpark, Dalview, Lakefield, Benoni, New Modder		05-10-2021

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S/ No	Wastewater plant name	Province	Metro or District	Official subdistrict SD or Local municipality	Subdistrict as represented on the graphs	Water service authority	Suburbs in drainage reticulation	Testing laboratory	Date quantitative testing started
52			Ekurhuleni Metropolitan Municipality	Ekurhuleni N2 SD	Ekurhuleni North (N1, N2)	Ekurhuleni Metropolitan Municipality	Northmead, Atlasville, New Modder, Lakefield, Benoni		21-09-2021
53			Ekurhuleni Metropolitan Municipality			Ekurhuleni Metropolitan Municipality	Katlehong, Natalspruit, Randhart, Alrode		05-10-2021
54			Ekurhuleni Metropolitan Municipality			Ekurhuleni Metropolitan Municipality	Bartlett, Atlasville, Boksburg North, Lakefield, Bonaero Park, Ravenswood, Witfield, Boksburg		21-09-2021
55	Vlakplaats WasteWater Treatment Works		Ekurhuleni Metropolitan Municipality			Ekurhuleni Metropolitan Municipality			NICD: 22-02- 2021 CSIR: 21-09- 2021
56			Ekurhuleni Metropolitan Municipality			Ekurhuleni Metropolitan Municipality	Kliprivier, Henley on Klip, Ophir, Glen Donald, Chrissiefontein, Rothdene, Riversdale, Meyerton Farms		21-09-2021
57						Mogale City Local Municipality	Rietvallei, Bhongwem, Brink's Vlakfontein		12-10-2021
58	Magaliesburg			West Rand (Mogale City LM)		Mogale City Local Municipality	Magaliesburg, Mogale City		12-10-2021
59				West Rand (Mogale City LM)		Mogale City Local Municipality	Lewisham, Krugersdorp North		12-10-2021
60	Hammarsdale		eThekwini Metropolitan Municipality			eThekwini Metropolitan Municipality	Hammarsdale, Elangeni, Mpumalanga		02-09-2021
61			eThekwini Metropolitan Municipality			eThekwini Metropolitan Municipality	New Germany, Pinetown, Clermont, Pinelands, KwaDabeka		02-09-2021

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S/ No	Wastewater plant name	Province	Metro or District	Official subdistrict SD or Local municipality	Subdistrict as represented on the graphs	Water service authority	Suburbs in drainage reticulation	Testing laboratory	Date quantitative testing started
62	Isipingo		eThekwini Metropolitan Municipality			eThekwini Metropolitan Municipality	Umbogintwini, Malukaze, Egolokodo, KwaMakhutha, Umlazi		10-08-2021
63			eThekwini Metropolitan Municipality			eThekwini Metropolitan Municipality	Brighton Beach, Grosvenor, King's Rest, Ocean View, Fynnland and Treasure Beach		NICD: 22-02- 2021 DUT: 10-08- 2021 for DUT
64			eThekwini Metropolitan Municipality			eThekwini Metropolitan Municipality	La Lucia, Umhlanga, Prestondale, Phoenix, Duff's Road, Glen Ashley, Mount Edgecombe		10-08-2021
65			eThekwini Metropolitan Municipality			eThekwini Metropolitan Municipality			22-02-2021
66			eThekwini Metropolitan Municipality			eThekwini Metropolitan Municipality	Rietrivier, KwaMashu, Duff's Road, Mount Edgecombe, Phoenix, Richmond, Inanda		10-08-2021
67			eThekwini Metropolitan Municipality			iLembe District municipality			11-10-2021
68			eThekwini Metropolitan Municipality			eThekwini Metropolitan Municipality	Acorn, Albany, Alexander Park		28-10-2021
69			uMgungundlovu District municipality	Msunduzi LM	No subdistrict	The Msunduzi Local Municipality	Pelham, Hayfields, New England, Northdale, Hay Paddock, Scottsville, Cleland, Bishopstowe, Sobantu		02-09-2021
70			uMgungundlovu District municipality	Msunduzi LM	No subdistrict	The Msunduzi Local Municipality	Thornville, Hayfields, Hay Paddock, Lynnfield Park, Cleland		02-09-2021
71			Umgungundlovu District municipality		No subdistrict	Mpofana Local Municipality			28-09-2021

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S/ No	Wastewater plant name	Province	Metro or District	Official subdistrict SD or Local municipality	Subdistrict as represented on the graphs	Water service authority	Suburbs in drainage reticulation	Testing laboratory	Date quantitative testing started
72			Umgungundlovu District municipality	uMngeni LM	No subdistrict	UMgungundlovu District municipality			02-09-2021
73		Limpopo	Capricorn District municipality		No subdistrict	Polokwane Local Municipality	Westenburg, Nirvana, Bendor, Welgelegen, Moregloed, Annadale, Ivydale, Flora Park, Fauna Park, Penina Park, Ivy Park, Ster Park, Dalmada, Broadlands, Woodlands, and Thornhill	Lumegen: /Waterlab/ UP	Lumegen: 02-08-2021 Waterlab/UP: 11-10-2021
74	Lebowakgo- mo	Limpopo	Capricorn District municipality	Lepelle-Nkumpi LM	No subdistrict	Lepelle-Nkumpi LM	Thabamoopo, Vaalboschlaagte, Lekhuswaneng, Moepeng, Makurung, Sekurwaneng, Ga- Matshele, Makurun		12-10-2021
75	Emalahleni (Riverview)	Mpuma- langa	Nkangala District Municipality		No subdistrict		Lynnville, Duvhapark, Paxton, Klipfontein	Lumegen	26-07-2021
76		Mpuma- langa		City of Mbombela LM	No subdistrict	Mbombela/ Umjindi			30-03-2021
77	Mbombela (Kingstonvale)	Mpuma- langa		Mbombela/ Umjindi	No subdistrict	Mbombela/ Umjindi	Gutshwa, eMpumalanga, eNyalungu, Dwaleni, Hlauhlau, Phasha, Ngodini	Lumegen	26-07-2021
78		Northern Cape			No subdistrict	Hantam Local Municipality			
79		Northern Cape		Sol Plaatjie Local Municipality	No subdistrict	Sol Plaatjie Local Municipality		Lumegen	28-08-2021
80					No subdistrict	JB Marks Local Municipality	Harpington, Vyfhoek, Mooibank, Wilgeboom	Lumegen	17-08-2021

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S/ No	Wastewater plant name	Province	Metro or District	Official subdistrict SD or Local municipality	Subdistrict as represented on the graphs	Water service authority	Suburbs in drainage reticulation	Testing laboratory	Date quantitative testing started
81	Klerksdorp main				No subdistrict	Matlosana Local Municipality	Boetrand, Wilkoppies	Lumegen	02-08-2021
82	Rustenburg		Rustenburg Local Municipality	Rustenburg Local Municipality	No subdistrict	Rustenburg Local Municipality		Lumegen and Waterlab/ UP	Lumgen: 26- 07-2021 Waterlab: 11- 10-2021
83		Western Cape	City of Cape Town Metropolitan Municipality		No subdistrict	City of Cape Town Metropolitan Municipality		MRC-BRIP	
84	Cape Flats	Western Cape	City of Cape Town Metropolitan Municipality		No subdistrict	City of Cape Town Metropolitan Municipality			06-10-2022
85		Western Cape	City of Cape Town Metropolitan Municipality	CT Tygerberg	No subdistrict	City of Cape Town Metropolitan Municipality	Crawford, Gleemore, Rondenbosch East		06-10-2021
86	Borcherd's Quarry	Western Cape	City of Cape Town Metropolitan Municipality	CT Tygerberg SD	No subdistrict	City of Cape Town Metropolitan Municipality			09-03-2021
87		Western Cape	City of Cape Town Metropolitan Municipality		No subdistrict	City of Cape Town Metropolitan Municipality			09-03-2021