WASTEWATER-BASED EPIDEMIOLOGY FOR SARS-COV-2 SURVEILLANCE IN SOUTH AFRICA

WEEK 45

NATIONAL INSTITUTE FOR COMMUNICABLE DISEASES
Division of the National Health Laboratory Service

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 18 wastewater treatment plants (WWTP) across five provinces tested by the NICD, and 77 additional plants tested by SACCESS partners including the National Institute for Occupational Health, Lumegen Laboratories, GreenHill Laboratories 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 2020 up until the week ending 12 November 2021 (epidemiological week 45). Generally, levels of SARS-CoV-2 are low and stable for decreasing across the country. Detailed analyses are described in figures and text below.

HIGHLIGHTS

- In most of the WWTPS in all provinces, wastewater levels of SARS-CoV-2 continue to decline` or remain at constant low levels, corresponding with the low levels of clinical cases reported across South Africa.
- There is, however, a need for increased clinical surveillance in the areas draining into the following WWTPs as the SARS-CoV-2 levels in wastewater are increasing:
  - Gauteng Province
    - ◊ Tshwane District Municipality: Three and two successive increases in SARS-CoV-2 levels have been detected from Daspoort (Pretoria central) and Rooiwal (Pretoria North) WWTPs, respectively. Although levels are low, this increase is concerning.
    - ◊ The City of Johannesburg: Two successive increases have been observed in Goudkoppies. Authorities should strengthen surveillance for clinical cases in these areas
    - ◊ The levels in Ennerdale in the City of Johannesburg and Ekurhuleni North (Hartebeesfontein and Refilwe) may be increasing, requiring close monitoring and more samples for confirmation.

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

CO-FUNDED BY THE WATER RESEARCH COMMISSION AND THE NICD

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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. 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,

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 laboratory-confirmed 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 March 2020, laboratories in the country have conducted over 18 million RT-PCR and antigen tests. Three distinct waves of SARS-CoV-2 infection occurred, peaking in June 2020, December 2020 and July 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 95 WWTPs are being tested for SARS-CoV-2.

SARS-CoV-2 detection and quantitation methodology

At the identified wastewater treatment facilities, one litre grab samples of influent are collected and transported at <5°C to the testing facility. Table 1 summarises the sample collection, concentration, RNA extraction and PCR methods for partner laboratories. A positive PCR test result is defined as detection of any SARS-CoV-2 gene target (amongst the N, E or RdRP genes). A negative PCR test is defined as a positive internal control without a positive N, E or RdRP gene target. An invalid test result is defined as failure to detect the N, E or RdRP genes along with a negative internal control. Quantitative PCR results in genome copies/ml were log-transformed when graphed. All RT-PCR detection methodologies use in-built positive and negative controls to eliminate processing errors or contamination. Quantitative testing (in copies/ml of wastewater) is conducted by the NICD using a fourplex RT-qPCR assay. The Allplex 2019-nCoV assay (Seegene, catalogue number RP10243X) includes proprietary primers and probes that amplify the E, N and RdRP genes. The assay also amplifies an internal control that helps monitor for PCR inhibition. Standard curves, from which SARS-CoV-2 copy numbers are calculated, are constructed using the EDX SARS-CoV-2 Standard (Exact Diagnostic, catalogue number COV019) consisting of synthetic RNA transcripts containing the E, N and RdRP genes.

Table 1. Concentration, extraction and RT-PCR detection methodology used by laboratory partners, South African Collaborative COVID-19 Environmental Surveillance System (SACCESS) network.

Method for virus concentration	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 Laboratories / Praecautio	Ultrafiltration (Amicon® Ultra-15 Centrifugal Filter Unit)	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	QuantiFast® Pathogen RT-PCR + IC kit (Qiagen) with 2019-nCoV-N1 primers and probe	Standard curves using the 2019_nCoV_N positive control plasmid (Integrated DNA Technologies, Inc, Coralville, IA)
SAMRC-BRIP	Centrifugation	RNeasy PowerSoil	2019-nCoV CDC EUA Kit	
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 EZNA total RNA Kit II	2019-nCoV CDC EUA Kit	Relative quantification based on the 2019-nCoV CDC positive control.

*RT-PCR=reverse transcriptase polymerase chain reaction; Ct=cycle threshold

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 and interpretive principles to guide application of test results to support COVID-19 public health responses, South Africa.

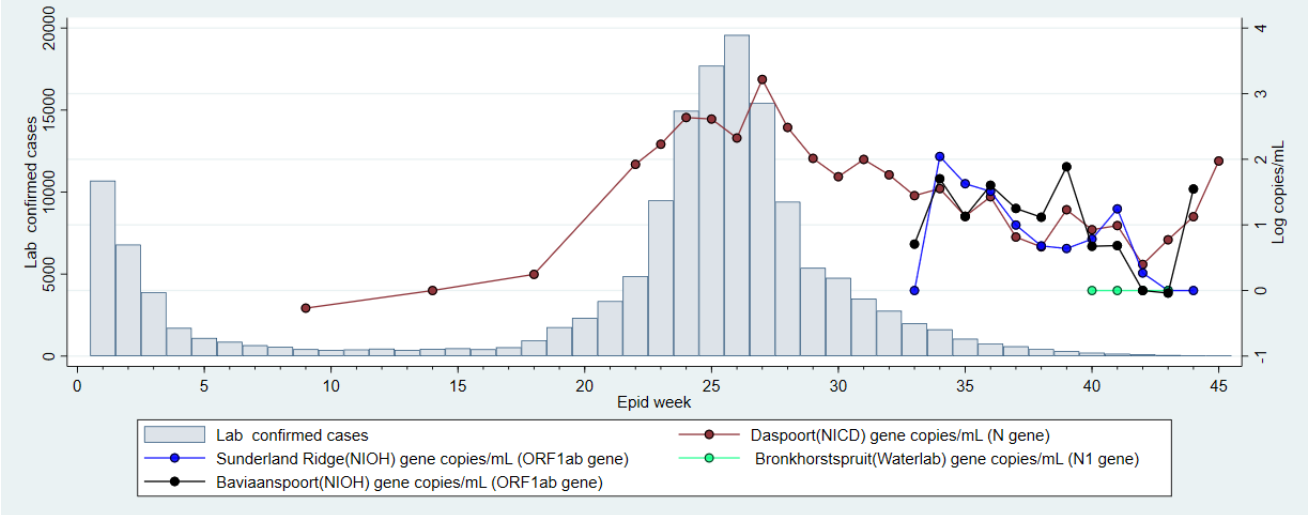
Testing modality	Interpretive principles to support public health responses
Detection of SARS-CoV-2	When a test result changes from • positive to negative, this signifies fewer/no cases in population • negative to positive, this indicates the need for increased population awareness and action • Qualitative results (presence or absence) are comparable between laboratories
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)

A



B: City of Tshwane North (sub-districts 1, 2, 5)

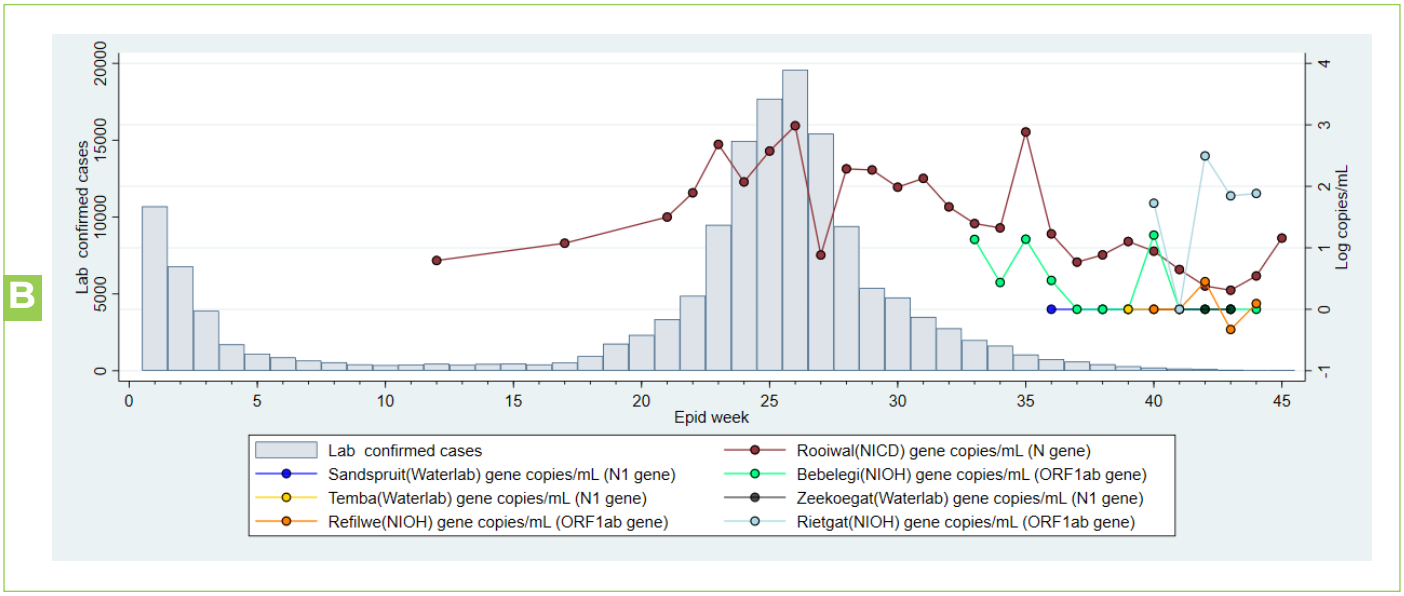


Figure 1 A-B. Laboratory confirmed cases of SARS-CoV-2 (bars) and levels of SARS-CoV-2 in log copies/ml of wastewater (coloured lines) for selected wastewater treatment plants (WWTP) and metropolitan areas in Tshwane District Municipality(Tshwane South and North), Gauteng Province during epidemiological weeks 1-45, 2021. 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.

C: City of Johannesburg Metropolitan Municipality

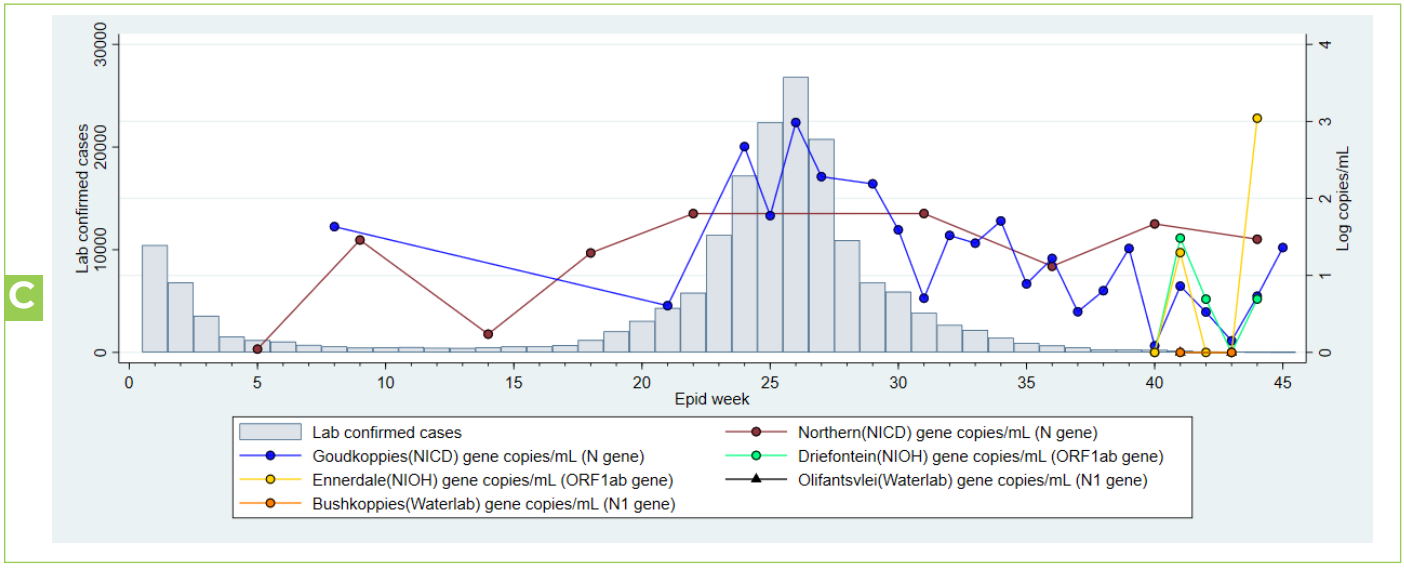
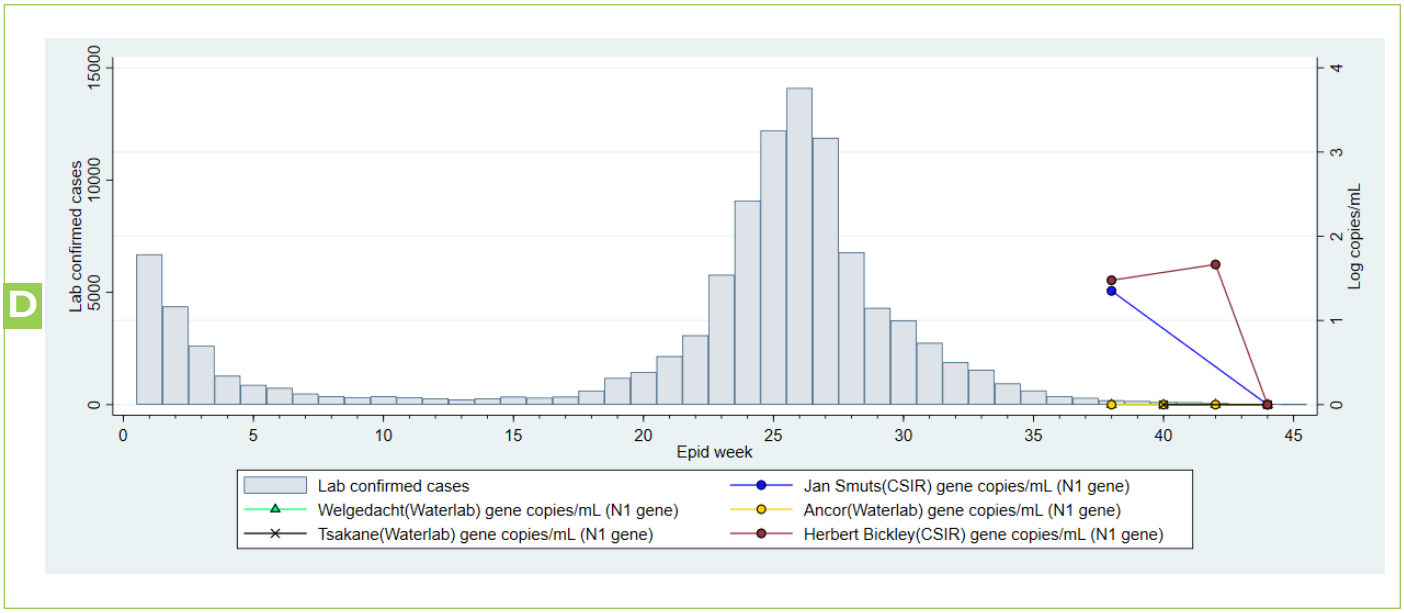
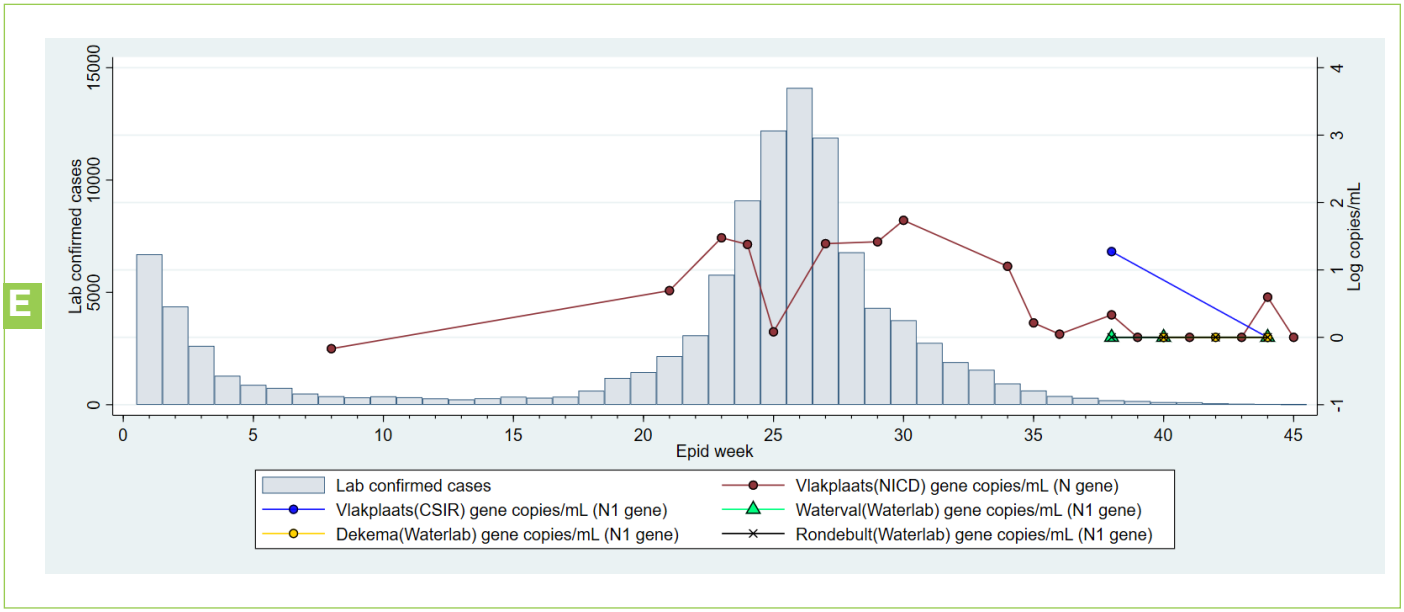


Figure 1 C. Laboratory confirmed cases of SARS-CoV-2 (bars) and levels of SARS-CoV-2 in log copies/ml of wastewater (coloured lines) for selected wastewater treatment plants (WWTP) in City of Johannesburg Metropolitan Municipality, Gauteng Province during epidemiological weeks 1-45, 2021. The testing 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.

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



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



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

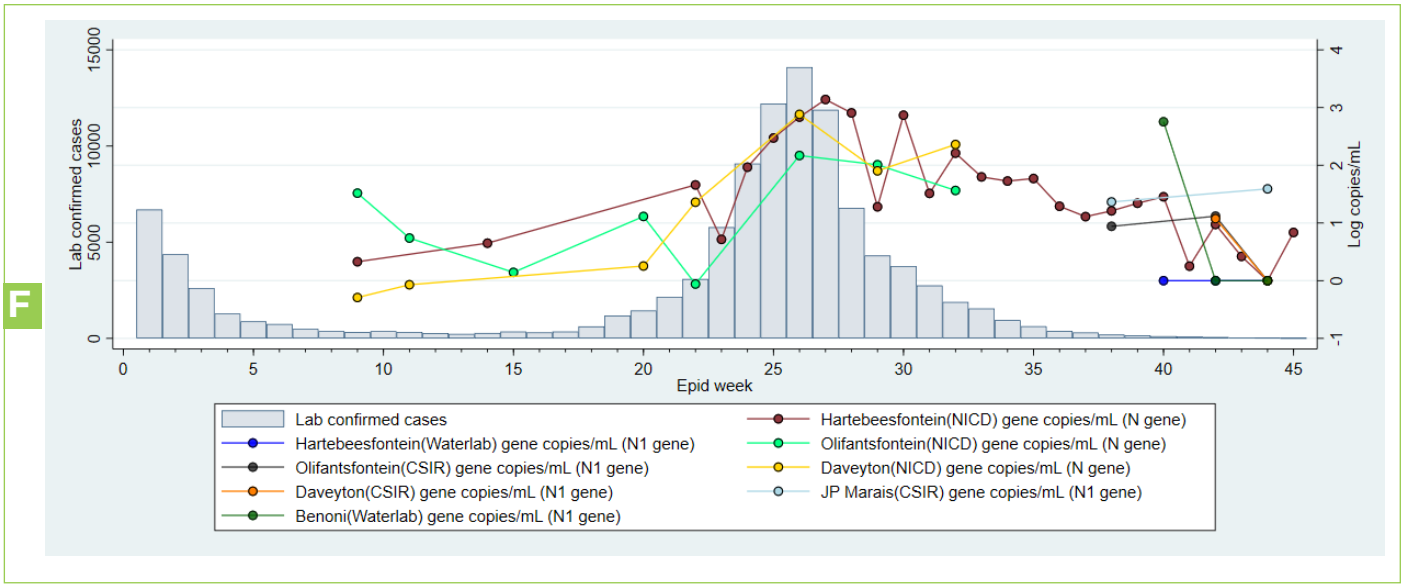


Figure 1 D-F. Laboratory confirmed cases of SARS-CoV-2 (bars) and levels of SARS-CoV-2 in log copies/ml of wastewater (coloured lines) for selected wastewater treatment plants (WWTP) in Ekurhuleni Metropolitan Municipality, Gauteng Province during epidemiological weeks 1-45, 2021. The testing laboratory 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.

G: West Rand District Municipality

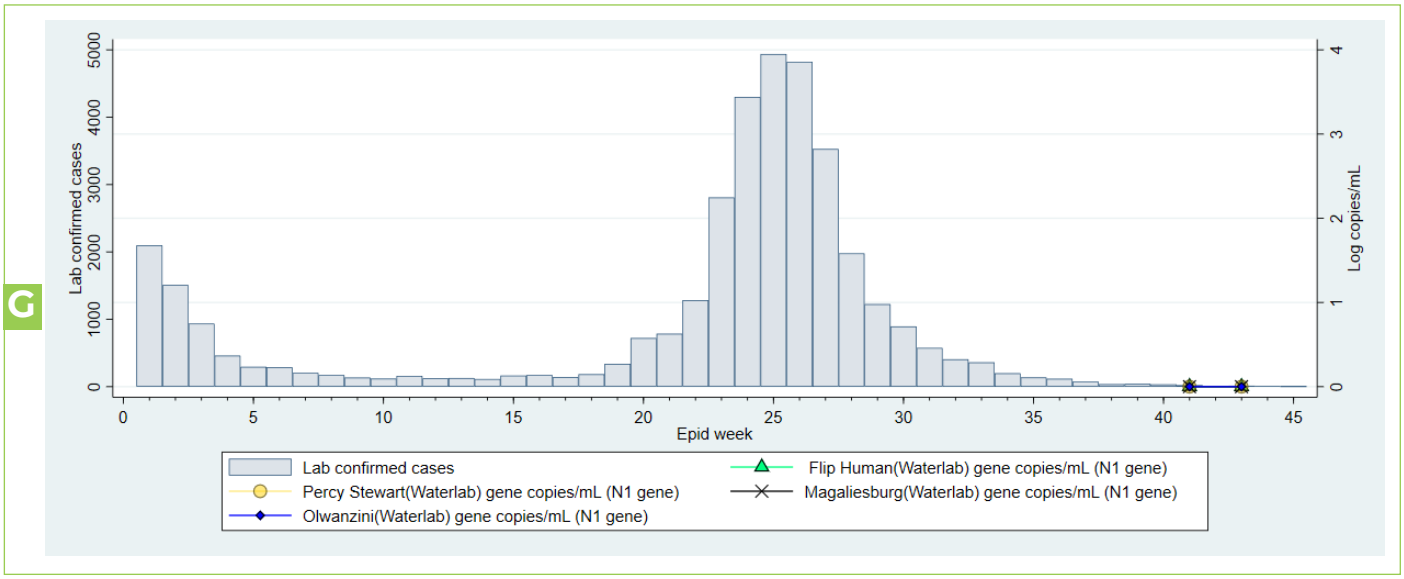


Figure 1 G. Laboratory confirmed cases of SARS-CoV-2 (bars) and levels of SARS-CoV-2 in log copies/ml of wastewater (coloured lines) for selected wastewater treatment plants (WWTP) in West Rand District Municipality, Gauteng Province during epidemiological weeks 1-43, 2021. The testing laboratory and 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.

In Gauteng province, wastewater testing for SARS-CoV-2 is currently being conducted in four district municipalities namely, City of Tshwane, City of Johannesburg, Ekurhuleni and West Rand by four different laboratories, namely NICD, NIOH, CSIR and Waterlab. The figures above are arranged so as to reflect the geographical locations of the wastewater treatment plants.

In Tshwane District Municipality, SARS-CoV-2 levels are being monitored in 11 treatment plants in Tshwane South and North (Figures 1A-B). In Tshwane South, the NICD is testing SARS-CoV-2 levels at Daspoort, the Waterlab at Bronkhortspruit while the National Institute for Occupational Health (NIOH) is testing at Sunderland Ridge and Baviaanspoort. In Tshwane North, the NICD is testing at Rooiwal, while the NIOH is testing at four WWTPS namely, Refilwe, Rooiwal, Bebelegi, and Rietgat. Waterlab is testing at three WWTPs namely, Sandspruit, Temba, and Zeekoegat.

Quantitative testing by the NICD commenced in epidemiological weeks 9 and 12 at Daspoort and Rooiwal WWTPs, respectively. Quantitative testing by the NIOH began in epidemiological week 33 in Baviaanspoort, Sunderland Ridge, and Babelegi and week 40 in Refilwe and Rietgat. Testing by the Waterlab started in week 32 in Bronkhortspruit, week 36 in Sandspruit, week 39 in Temba, and week 40 in Zeekoegat (Figures 1A-1B).

In the City of Johannesburg (Figure 1C), quantitative testing by the NICD began in epidemiological week 5 in two WWTPs (Goudkoppies and Northern); week 40 by the NIOH in two WWTPs (Driefontein and Ennerdale); and week 41 by the Waterlab in two WWTPS (Bushkoppies and Olifantsvlei).

In Ekurhuleni (Figures 1D-F), quantitative testing is currently being done in the Eastern, Southern and Northern parts. In the Eastern part, testing is done in Welgedacht, Tsakane and Ancor WWTPs. In the Northern part, testing is being done in Hertesbeesfontein, Benoni, Olifantsfontein, Daveyton and JP Marais. In the Southern part, testing is done in Vlakpaats, Dekema, Waterval and Rondebult WWTPs.

Testing by CSIR (Figure 1D-F) began in epidemiological week 38 in four WWTPs (Herbert Bickley, Jan Smuts and JP Marais. The CSIR is also testing at Daveyton, Olifantsfontein and Vlakplaats, with testing commencing in weeks 42, 38, and 38, respectively (Figure 1E). Quantitiative testing commenced by the NICD in epidemiological week 8 in Vlakplaats, and week 9 in three treatments plants (Daveyton, Hartebeesfontein and Olifantsfontein) (Figure 1E-F). The Waterlab (Fig 1E-F) began testing of two WWTPs in week 38 (Ronderbult and Welgedacht) and week 40 in five WWTPs (Ancor, Dekema, Harteesbonfontein, Tsakane and Waterval, and Benoni).

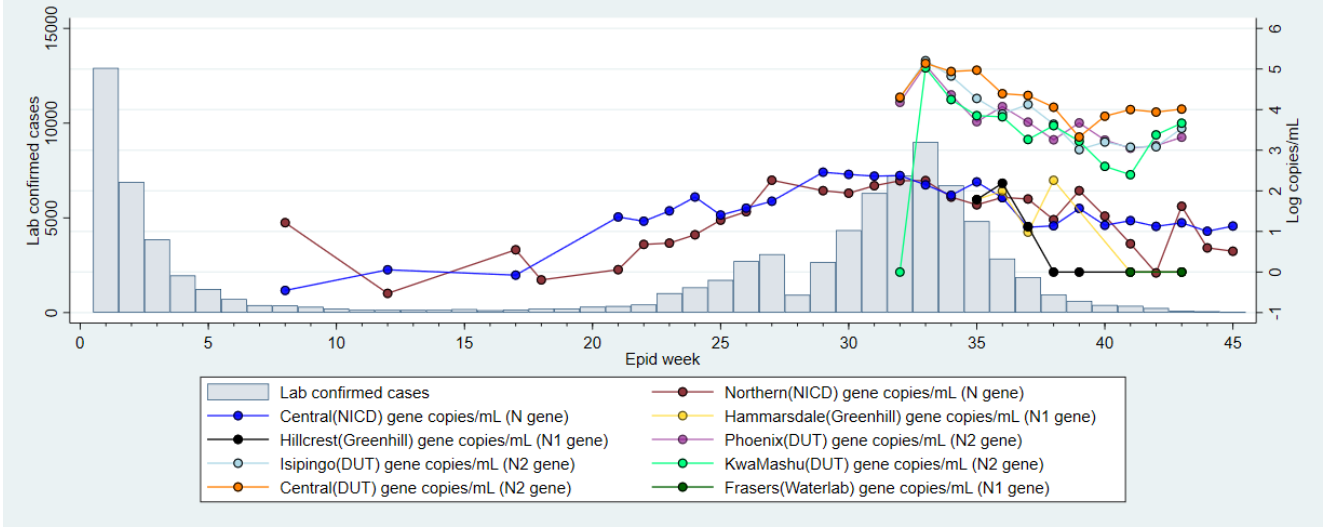
In West Rand (Figure 1G), Waterlab is currently testing three WWTPS (Flip Human, Magaliesburg, and Percy Stewart). Testing in all these WWTPS commenced in week 41.

In most treatment plants in Tshwane, the City of Johannesburg, Ekurhuleni, and West Rand, the SARS-CoV-2 levels continue to decline or remain stable from week 40 till week 45 with a corresponding decrease in clinical cases. All results from Gauteng plants tested by Waterlab have been negative for SARS-CoV-2 this week, while results from adjacent WWTPs have indicated that SARS-CoV-2 is present at low levels. Waterlab has verified internal detection controls and confirmed test validity. These results, therefore, indicate that SARS-CoV-2 levels are below the threshold of detection for this laboratory's methodology. This is in keeping with the decline in clinical case load across Gauteng province. However, three successive increases in levels (between weeks 42-45) have been detected in specimens from Daspoort (which drains Pretoria central), and two successive increases in specimens from Rooiwal WWTPs (between weeks 43-45), both in Tshwane Metropolitan Municipality, signalling a need for increased surveillance around this area. Two successive increases in levels have been observed in Goudkoppies in the City of Johannesburg, also requiring increased surveillance. The levels in specimens from Ennerdale WWTP in the south of the City of Johannesburg and in specimens from Hartebeesfontein and Refilwe in Ekurhuleni North may be increasing, requiring close monitoring and more samples for confirmation.

KwaZulu-Natal Province

2A: eThekwini Metropolitan Municipality

A



B: uMgungundlovu District Municipality

B

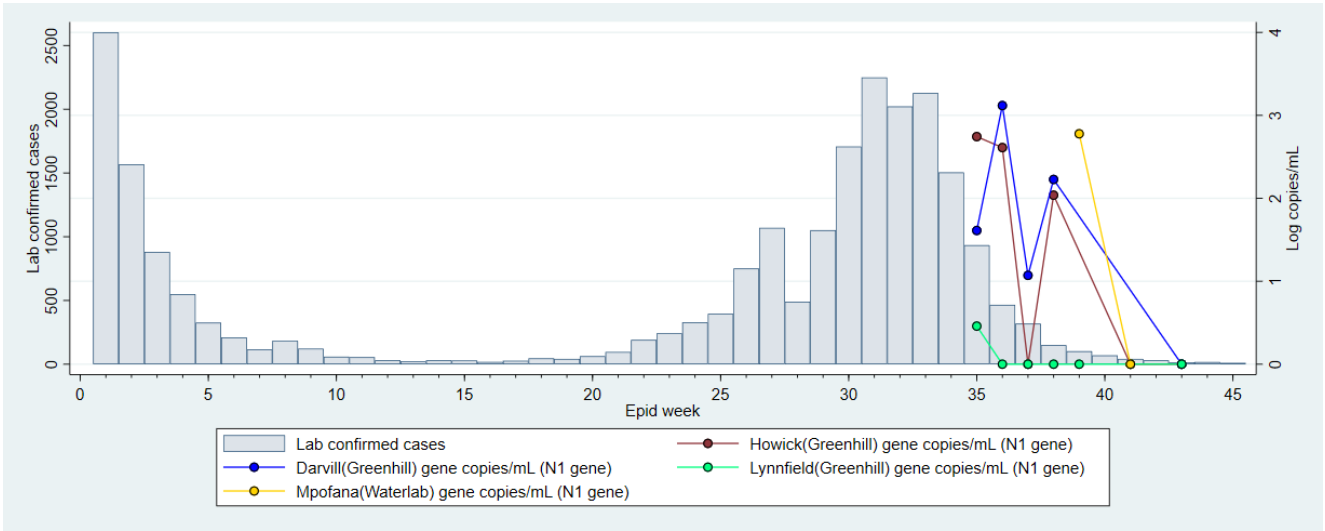


Figure 2A-B. Laboratory confirmed cases of SARS-CoV-2 (bars) and levels of SARS-CoV-2 in log copies/ml of wastewater (coloured lines) from wastewater treatment plants (WWTP) in Ethekwini, (A-B) and uMgungundlovu Metro (C), KwaZulu Natal Province during epidemiological weeks 1-44, 2021. The testing laboratory 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.

In eThekwini (Figure 2A), quantitative testing is conducted by the NICD, GreenHill Laboratory, Durban University of Technology (DUT) and Waterlab. Quantitative testing by the NICD commenced in epidemiological week 8 2021 at two WWTPs, Central and Northern. Quantitative testing by GreenHill Laboratory began in week 34, and 35, 2021, at the Hillcrest and Hammarsdale WWTPs respectively. The DUT began testing of four WWTPs (Isipingo, Phoenix, Central and KwaMashu) in week 32, 2021. Waterlab commenced testing at Frasers WWTP in Ballito in week 41, 2021.

In Umgungundlovu (Figure 2C), quantitative testing is being done by GreenHill Laboratories andWaterlab. Testing by GreenHill Laboratory commenced in epidemiological week 35 in three WWTPs (Darvill, Howick and Lynnfield). Waterlab commenced testing in Mfofana WWTP in week 39, 2021.

SARS-CoV-2 levels tested by DUT are consistently 2-3 log copies/ml higher than those tested by the NICD. This may be accounted for by the fact that DUT is using a more sensitive methodology for PCR detection, namely digital droplet PCR. It also highlights the interpretive principle that comparisons of levels should only be done on specimens tested in the same laboratory using the same test methodology.

SARS-CoV-2 levels continue to remain low or stable in most of the WWTPs tested from week 42, 2021, in both eThekwini and Umgungundlovu, paralleling the decrease in clinical cases. However, two successive increases in samples from KwaMashu and Phoenix WWTPs in eThekwini Metropolitan Municipality were observed in weeks 42-43, as reported last week. KwaMashu and Phoenix are geographically co-located and therefore this is a significant signal. Authorities should strengthen surveillance for cases in these areas, coupled with close monitoring of subsequent samples in these plants. A steady increase in wastewater levels of SARS-CoV-2 occurred between week 39-43 in Central WWTPs whilst two successive increases were detected from Isipingo (southern eThekwini). There is need for increased surveillance for clinical cases and close monitoring of subsequent samples in these plants.

Free State Province

A: Mangaung Metropolitan Municipality

A

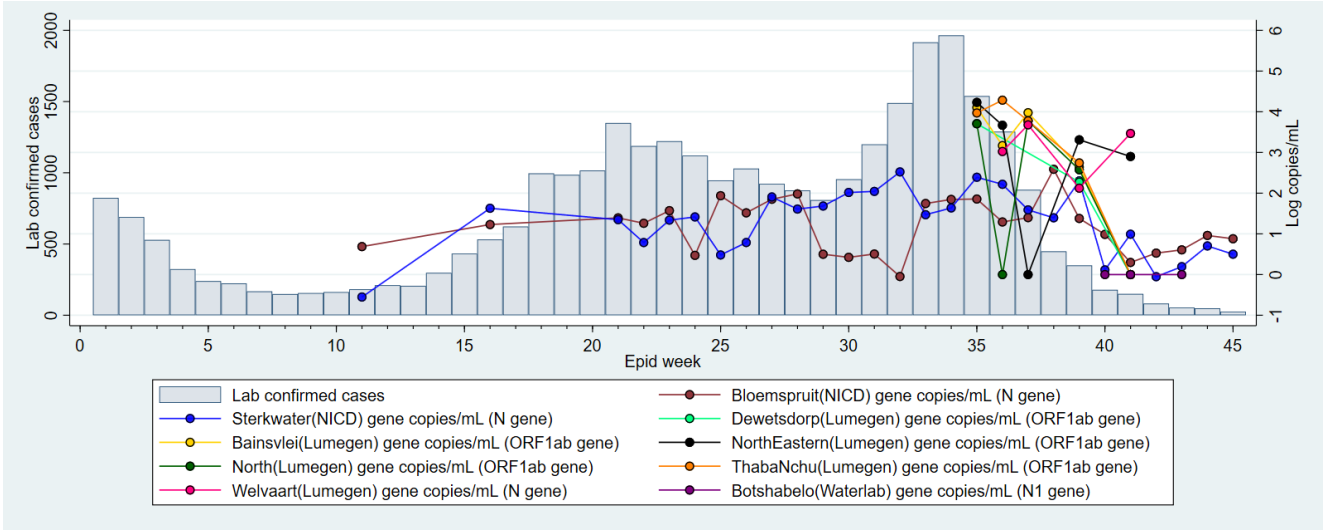


Figure 3A. Laboratory confirmed cases of SARS-CoV-2 (bars) and levels of SARS-CoV-2 in log copies/ml of wastewater (coloured lines) from wastewater treatment plants (WWTPs) in Mangaung, Free State Province during epidemiological weeks 1-45, 2021. The testing laboratory and quantified SARS-CoV-2 gene 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.

In Free State, the monitoring of wastewater levels of SARSCov-2 is being conducted at nine WWTPs in Mangaung district municipality by the NICD, Lumegen, and Waterlab laboratories. Quantitative testing by the NICD (Figure 3A) commenced in epidemiological week 11, 2021 at two WWTPs (Bloemspruit and Sterkwater). Quantitative testing by Lumegen Laboratories commenced in epidemiological week 35 of 2021 in five wastewater treatment plants (ThabaNchu, North, Northeastern, Dewetsdorp, and Bainsvlei) and week 36 in Welvaart WWTP. Waterlab commenced testing in Botshabelo WWTP in epidemiological week 40 of 2021.

Current results suggest that SARS-CoV-2 is circulating at low levels in populations drained by Bloemspruit and Sterkwater, as this weeks samples from these WWTPs showed a decrease.

Eastern Cape Province

A: Nelson Mandela Metropolitan Municipality

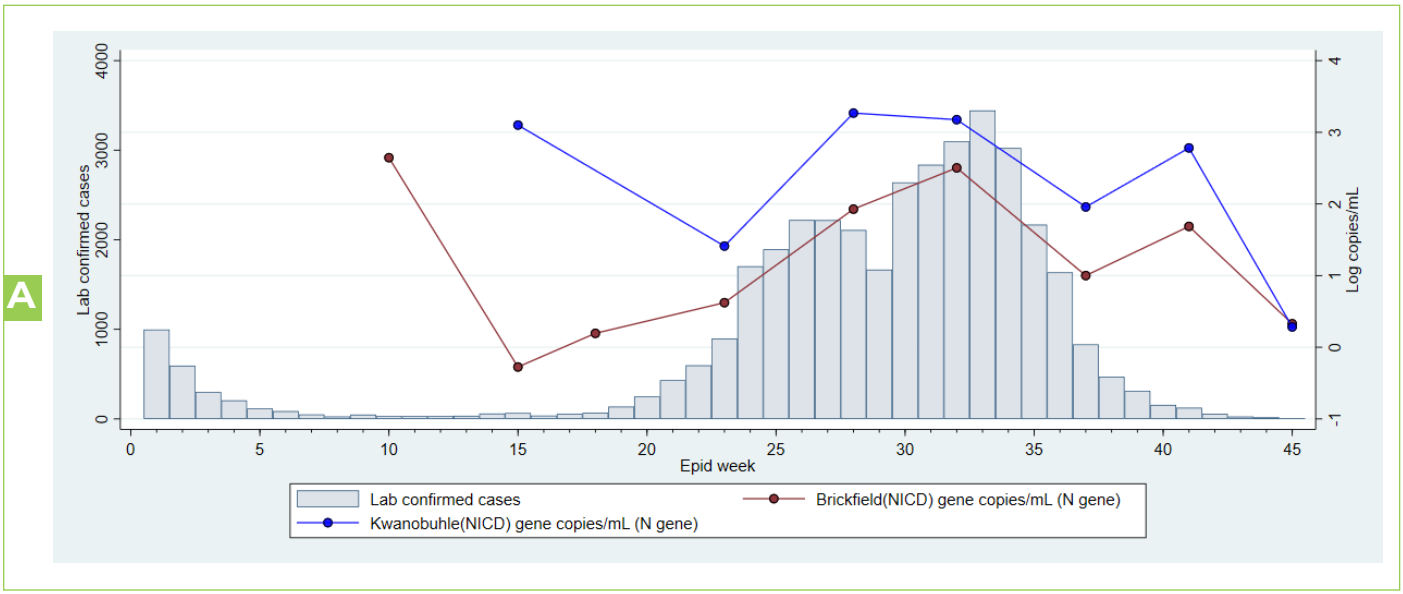


Figure 4A. Laboratory confirmed cases of SARS-CoV-2 (bars) and levels of SARS-CoV-2 in log copies/ml of wastewater (coloured lines) from wastewater treatment plants (WWTPs) in Nelson Mandela Metro, Eastern Cape Province during epidemiological weeks 1-45, 2021. The testing laboratory and quantified SARS-CoV-2 gene 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

B: Buffalo City Metropolitan Municipality

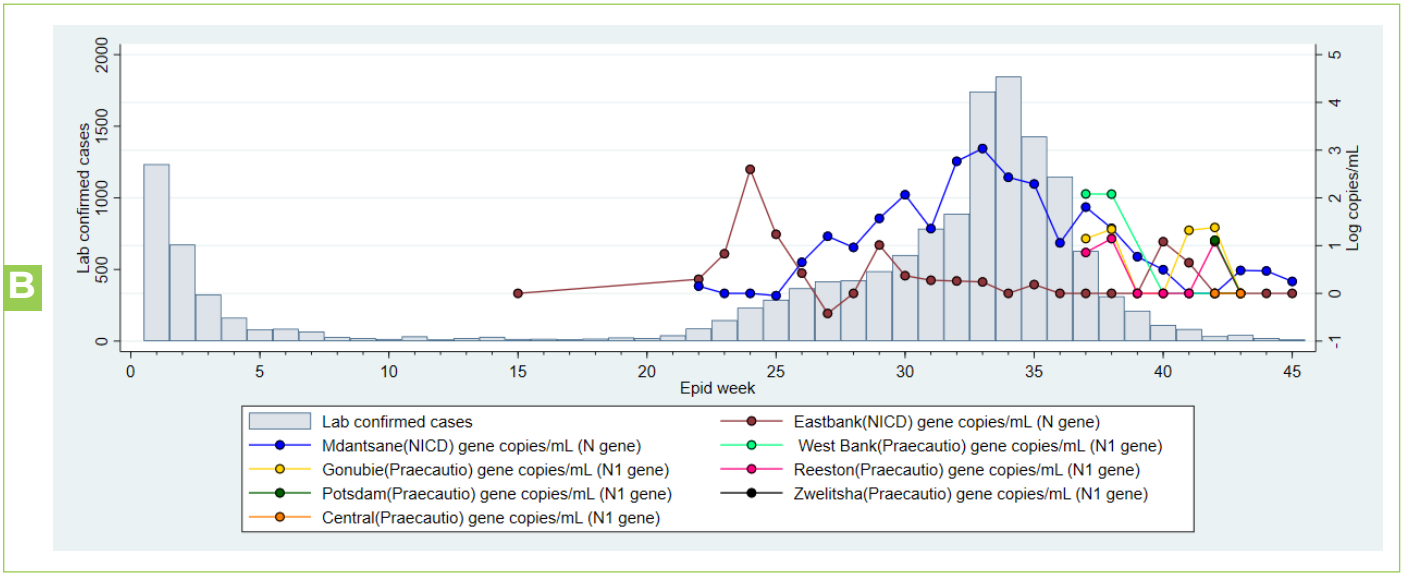


Figure 4B. Laboratory confirmed cases of SARS-CoV-2 (bars) and levels of SARS-CoV-2 in log copies/ml of wastewater (coloured lines) from wastewater treatment plants (WWTPs) in Nelson Mandela Metro, Eastern Cape Province during epidemiological weeks 1-45, 2021. The testing laboratory and quantified SARS-CoV-2 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

In the Eastern Cape Province, the quantitative testing of SARS-CoV-2 levels are currently being done in 10 WWTPs in Nelson Mandela Metro and Buffalo City by the NICD (n=4) and Preacautio laboratories (n=6). The NICD commenced quantitative testing in week 10 at Kwanobuhle WWTP and week 15 at Brickfield`, at Nelson Mandela Metro (Figure 4a). The NICD is also testing two WWTPs in East Bank and Mdantsane in Buffalo City Metro, which commenced in weeks 15 and 22, 2021 respectively (Figure 4B). Praecautio commenced testing of three WWTPs (West Bank, Gonubie and Reeston) in Buffalo City Metro in epidemiological week 37, 2021 and another three WWTPs (Potsdam, Central and Zwelitsha) in week 42, 2021.

In Nelson Mandela Metro and Buffalo City Metro SARS-CoV-2 levels from all WWTPs are low and stable, corresponding with the low numbers of clinical cases. Readers are referred to the SA MRC 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:

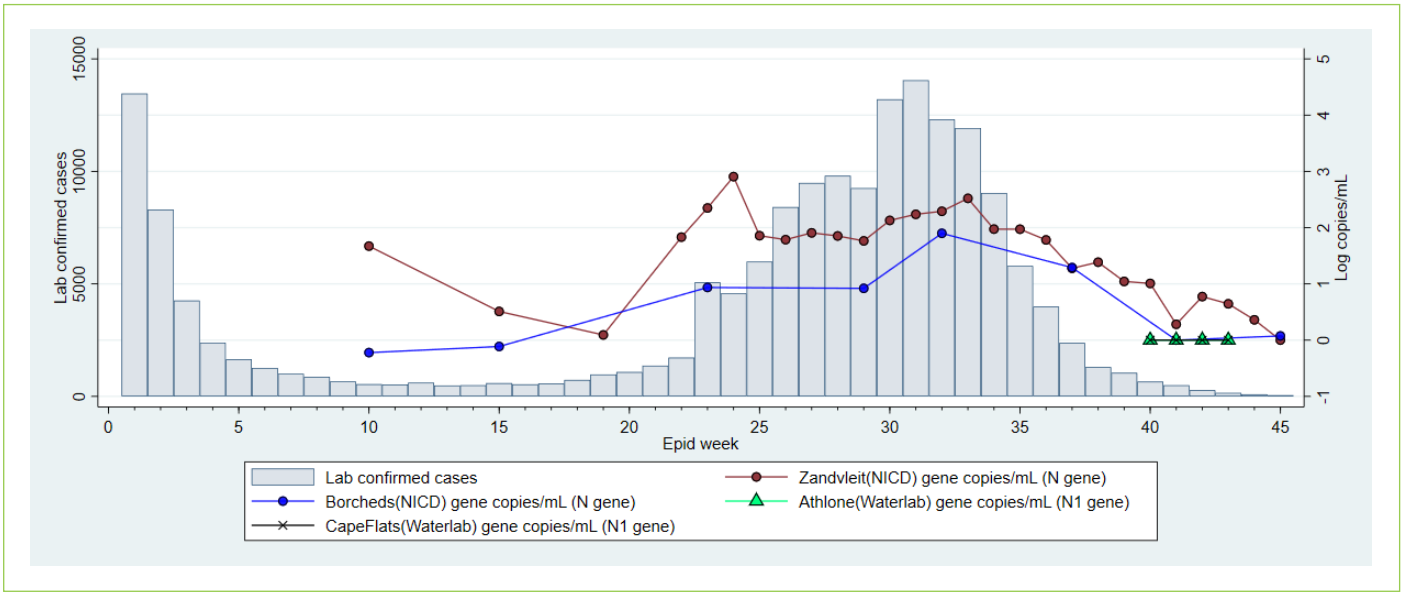


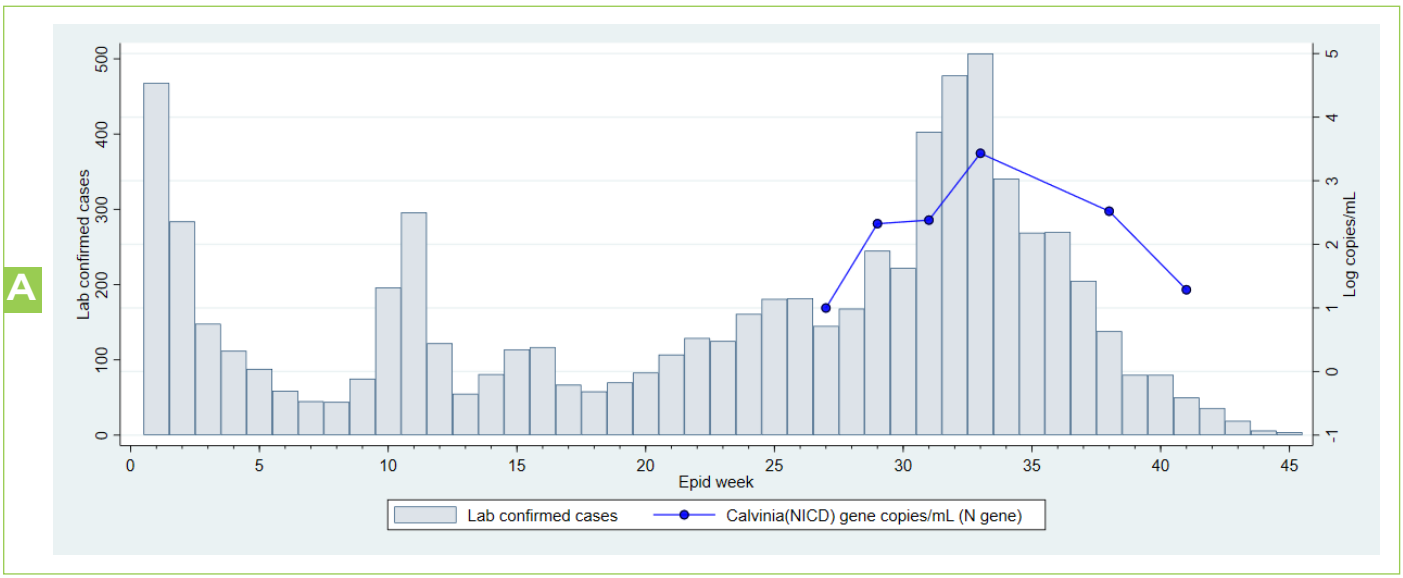
Figure 5. Laboratory confirmed cases of SARS-CoV-2 (bars) and levels of SARS-CoV-2 in log copies/ml of wastewater (coloured lines) from wastewater treatment plants (WWTPs) in City of Cape Town, Western Cape Province during epidemiological weeks 1-45, 2021. The testing laboratory and quantified SARS-CoV-2 gene is named in brackets after the name of the WWTP.

In the Western Cape Province, the NICD commenced quantitative testing in week 10, 2021 in two wastewater treatment plants (Borcherds and Zandvleit) while Waterlab commenced testing in Athlone and CapeFlats in week 40.

SARS-CoV-2 levels have been decreasing steadily or remained stable in all in WWTPs from week 41, corresponding to the decrease in clinical cases. These results should be interpreted with reference to SARS-CoV-2 epidemiology in areas draining into these treatment plants. Readers are referred to the MRC website which provides data from additional wastewater treatment plants in City of Cape Town and other Western Cape districts (https://www.samrc.ac.za/wbe/) to contextualise the results.

Northern Cape Province

A: Namakwa District Municipality



B: Frances Baard District Municipality

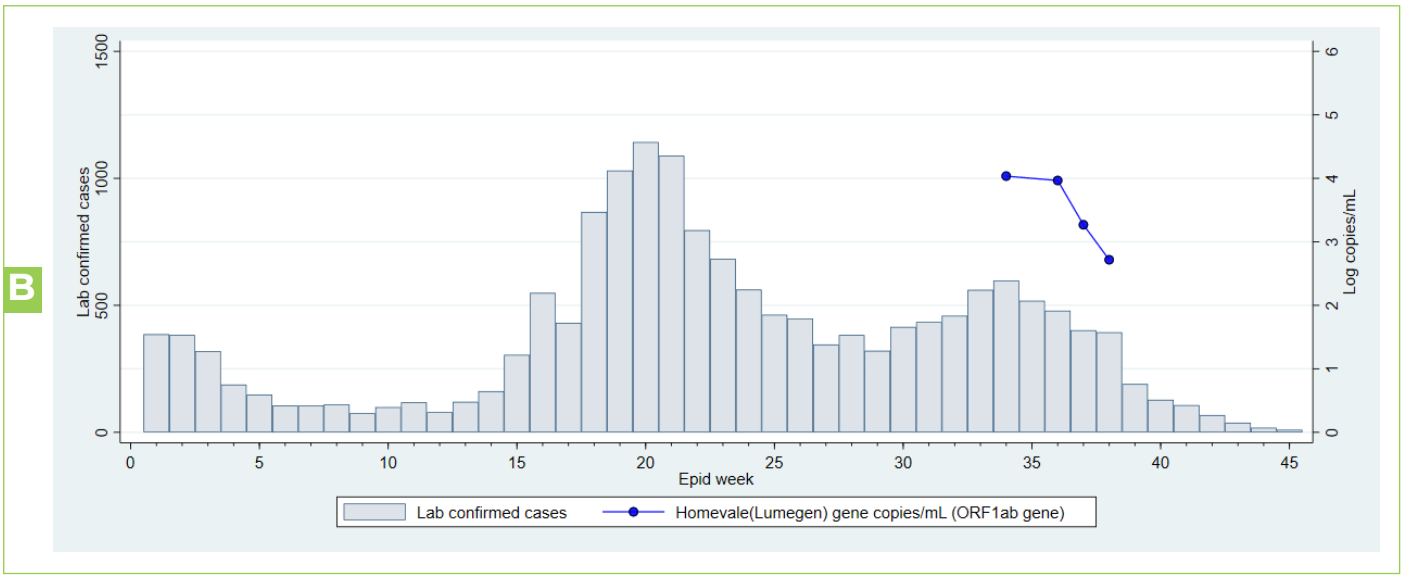


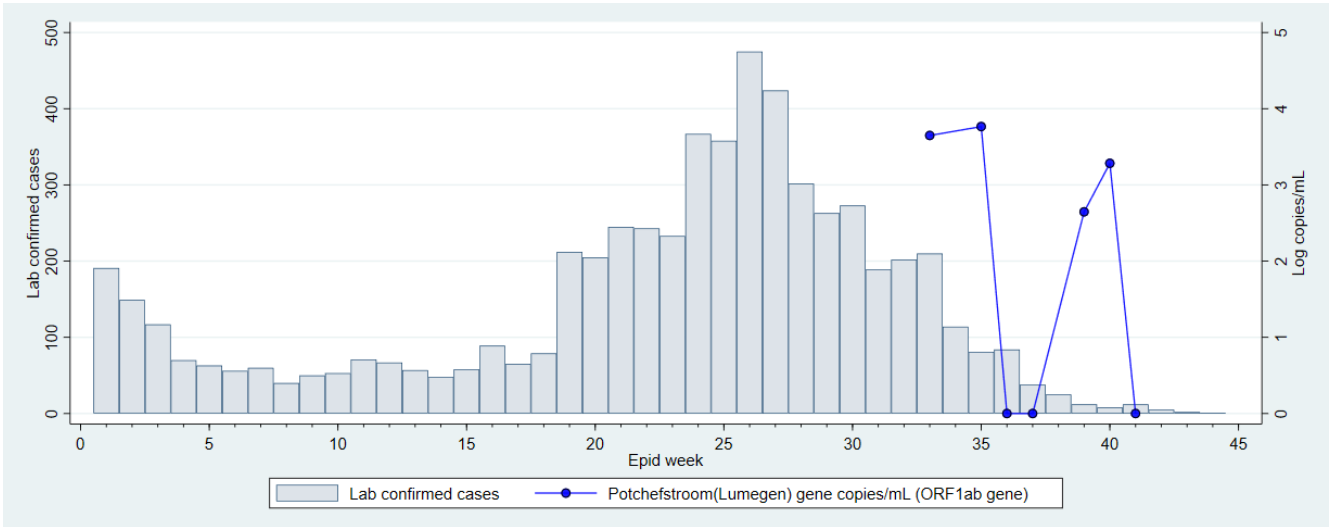
Figure 6A-B. Laboratory confirmed cases of SARS-CoV-2 (bars) and levels of SARS-CoV-2 in log copies/ml of wastewater (coloured lines) in wastewater treatment plants (WWTPs) from Calvinia in Namakwa Metro (a) and Kimberly in Frances Baard District (b), Northern Cape Province during epidemiological weeks 1-41, 2021. The testing laboratory and quantified SARS-CoV-2 gene is named in brackets after the name of the WWTP. Note that comparisons

In Northern Cape province, the NICD and Lumegen laboratories commenced quantitative testing of SARS-CoV-2 levels in wastewater treatment plants in Namakwa (Calvinia) and Frances Baard (Homevale) in epidemiological week 27 and 34 respectively (Figure 6a-b`). In both WWTPs, there has been a decline in SARS-CoV-2 levels corresponding to a decrease in clinical case burden.

North West Province

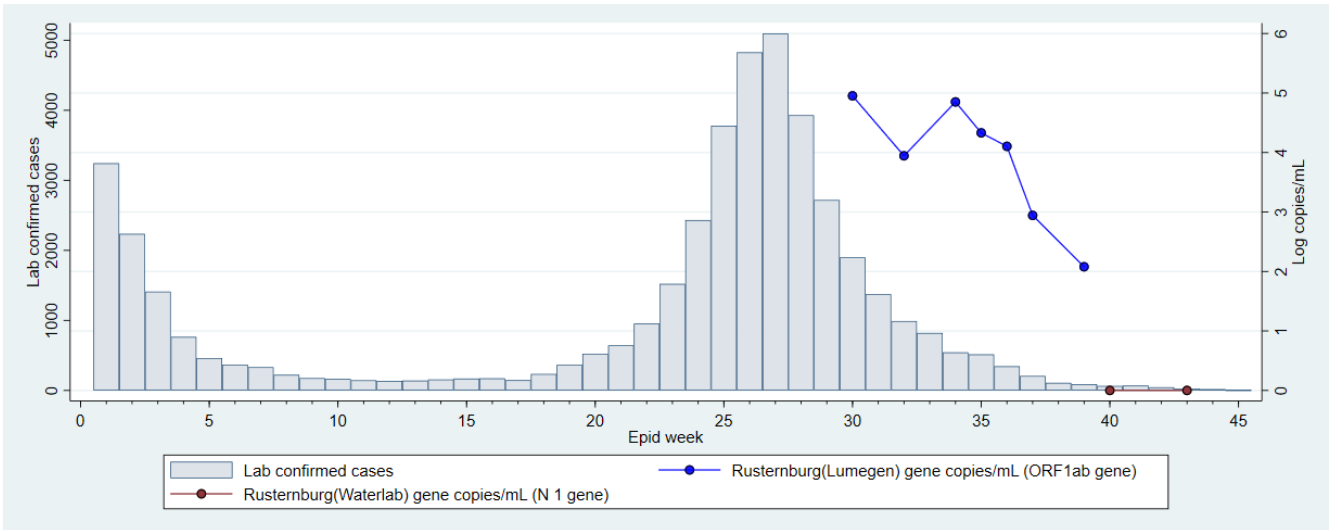
A: JB Marks Local Municipality

A



B: Bojanala District Municipality

B



C: City of Matlosana Municipality

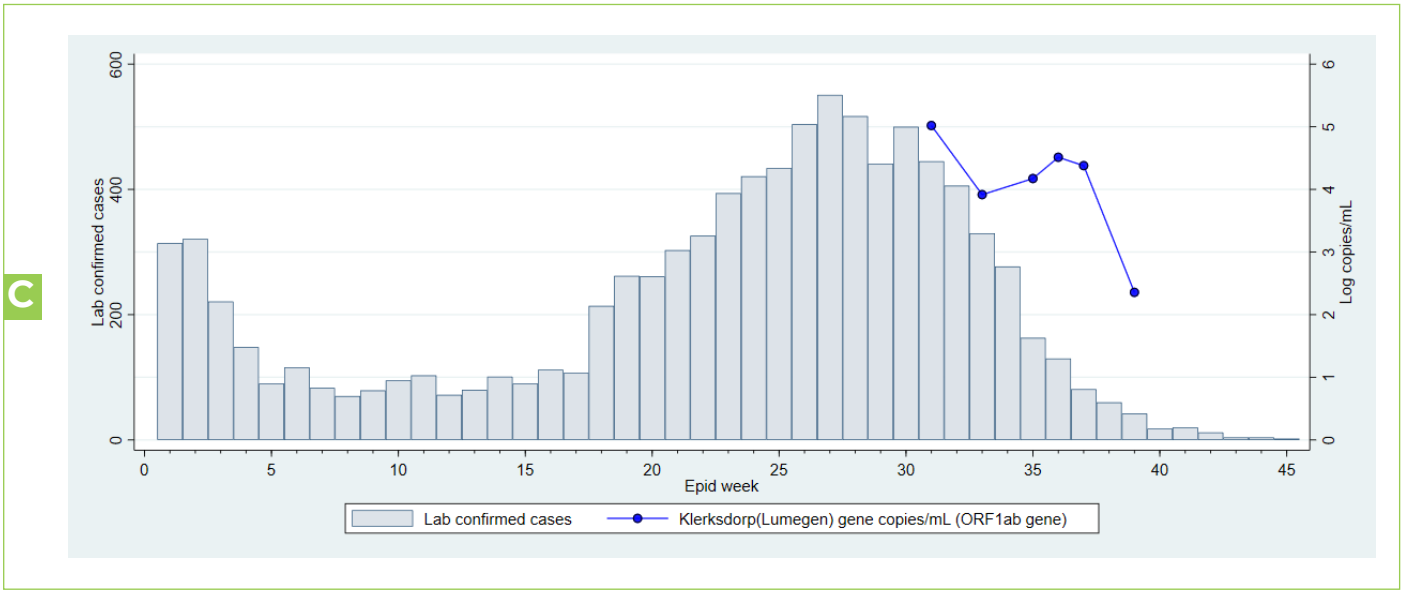


Figure 7A-C. Laboratory confirmed cases of SARS-CoV-2 (bars) and levels of SARS-CoV-2 in log copies/ml of wastewater (coloured lines) in wastewater treatment plants (WWTPs) from Potchefstroom,JB Marks District (A) Rustenberg, Bojanala District (B), and City of Matlosana, Northwest Province during epidemiological weeks 31-41, 2021. 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.

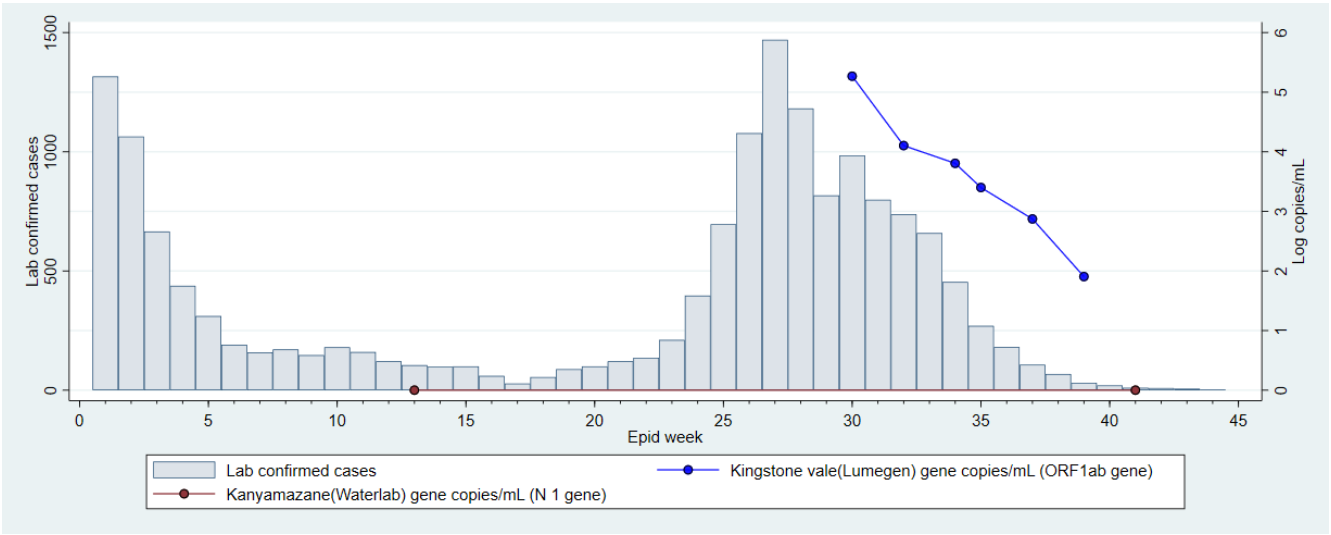
Three WWTPs are currently being tested in the Northwest province by Lumegen Laboratory (Figure 6a-c). Quantitative testing for SARS-CoV-2 levels in wastewater commenced in epidemiologic week 33, in JB Marks Local Municipality (Potchefstroom), week 30 in Bojanala District (Rustenburg) and week 31 in City of Matlosana.

The SARS-CoV-2 levels in WWTPs in Bojanala and Matlosana showed a decline in trend from week 37-39, with a corresponding decrease in the number of clinical cases. In JB Marks, SARS-CoV-2 levels have also been decreasing between week 40-41.

Mpumalanga Province

Mbombela Local Municipality

A



B: Emalahleni Local Municipality

B

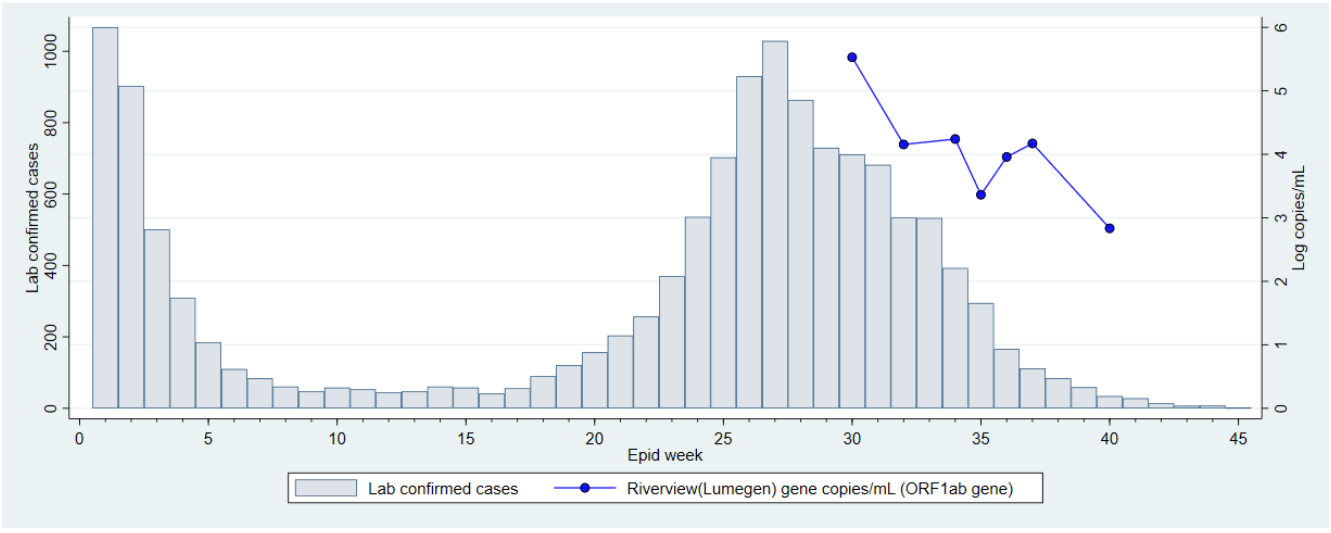


Figure 8A-B. Laboratory confirmed cases of SARS-CoV-2 (bars) and levels of SARS-CoV-2 in log copies/ml of wastewater (coloured lines) in wastewater treatment plants (WWTPs) from Mbombela and Emalahleni Local Municipality, Mpumalanga Province during epidemiological weeks 30-41, 2021. The testing laboratory and quantified SARS-CoV-2 gene is named in brackets after the name of the WWTP. Note that comparisons of levels overtime should only be done for specimens tested in the same laboratory.

In Mpumalanga, three WWTPs are being tested by Lumegen and Waterlab. Lumegen commenced the quantitative testing for SARS-CoV-2 levels in two WWTPs in Mbombela (Kingstone Vale) and Emalahleni (Riverview) Local Municipalities, in epidemiologic week 30 (Figure 8a-8b). Waterlab commenced testing in epidemiologic week 39 in Kanyamazane WWTP in Emalahleni Local Municipality. Between week 37 to week 40, SARS-CoV-2 levels to dropped in Kingstone Vale and Riverview WWTPs, with a corresponding decrease in the clinical cases.

Limpopo Province

Polokwane Local Municipality

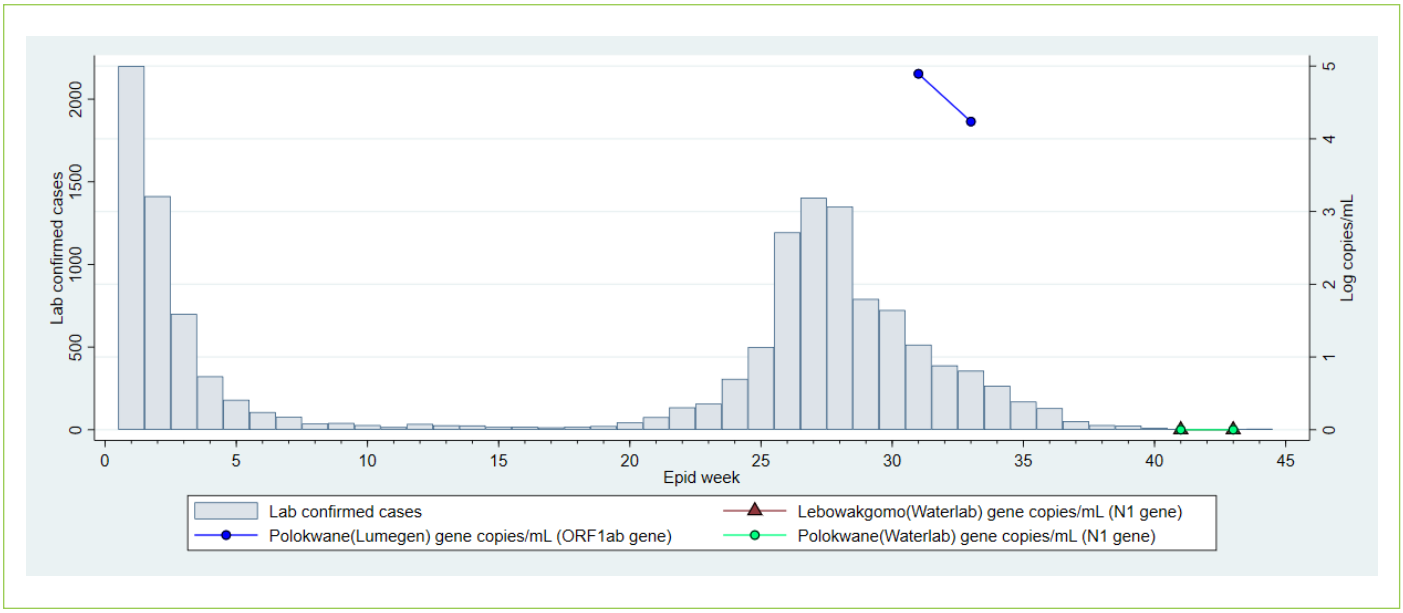


Figure 9. Laboratory confirmed cases of SARS-CoV-2 (bars) and levels of SARS-CoV-2 in log copies/ml of wastewater (coloured lines) in wastewater treatment plants (WWTPs) from Polokwane Local Municipality, Limpopo Province during epidemiological weeks 31-33, 2021.

Quantitative testing commenced by Lumegen laboratories in epidemiologic week 31, 2021, in Polokwane (Figure 9). A downward trajectory in SARS-CoV-2 levels in wastewater was seen between week 31 and 33, with a corresponding decrease in clinical cases.

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 are 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 interpretation of observed fluctuations in RNA levels.

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.

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