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

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

WEEK 42

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 24 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. SARSCoV- 2 is shed from symptomatic and asymptomatic persons in stool but is not transmitted by faecooral route nor in wastewater. This report is based on data collected from June 2020 up until the week ending 22 October 2021 (epidemiological week 41). Generally, levels of SARS-CoV-2 are low and stable or 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 are declining with corresponding decrease in clinical cases
- The areas draining into the following WWTPs need to be monitored as the SARS-CoV-2 levels may be increasing. This increase should be confirmed in subsequent samples:
  - Gauteng Province:
    - City of Tshwane: Sunderland Ridge (Centurion)
    - City of Johannesburg: Northern (Northern Johannesburg)
    - Ekurhuleni: Hartbeesfontein (Tembisa, northern Ekurhuleni)
  - Free State Province
    - Mangaung: Welvaart (Botshabelo)
  - Western Cape Province:
    - City of Cape Town: Zandvleit (Khayelitsha)

NATIONAL INSTITUTE FOR COMMUNICABLE DISEASES
Division of the National Health Laboratory Service
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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, and a high proportion of cases 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.

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
CSIR	Polyethylene Glycol	Omega Bio-tek ENZA total RNA Kit II	2019-nCoV CDC EUA Kit	
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	Allplex™ 2019-nCoV Assay	
SAMRC-TB	Ultra centrifugation	ZymoBIOMICS kit	2019-nCoV CDC EUA Kit	
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

*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 1 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.

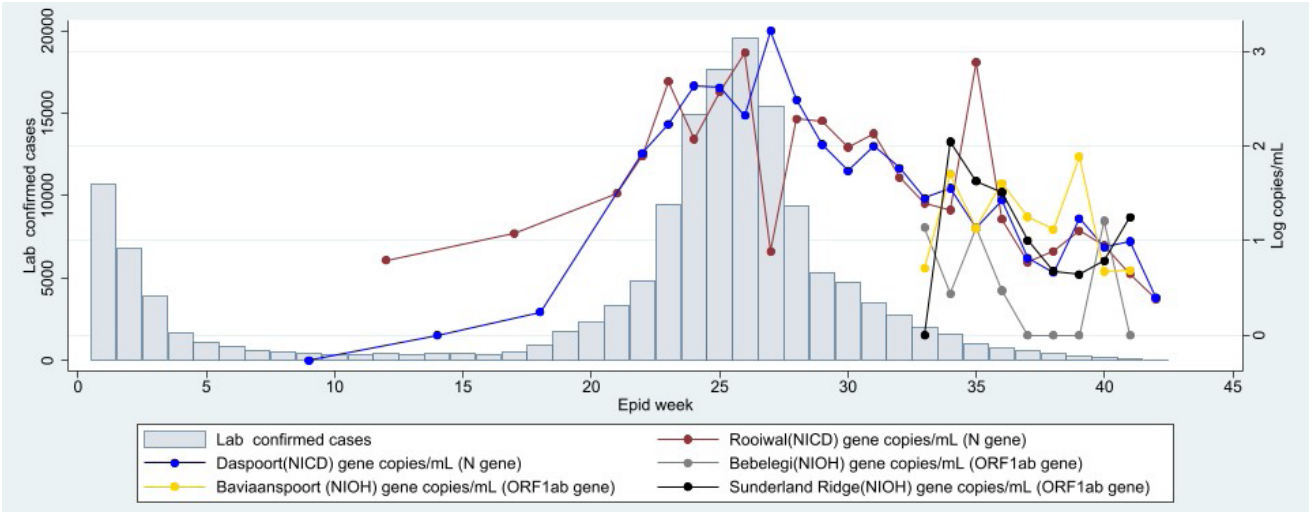
Testing modality	Interpretive principles to support public health responses
Detection of SARS-CoV-2	When a test result changes from • positive to negative, this signifies fewer/no cases in population • negative to positive, this indicates the need for increased population awareness and action • Qualitative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

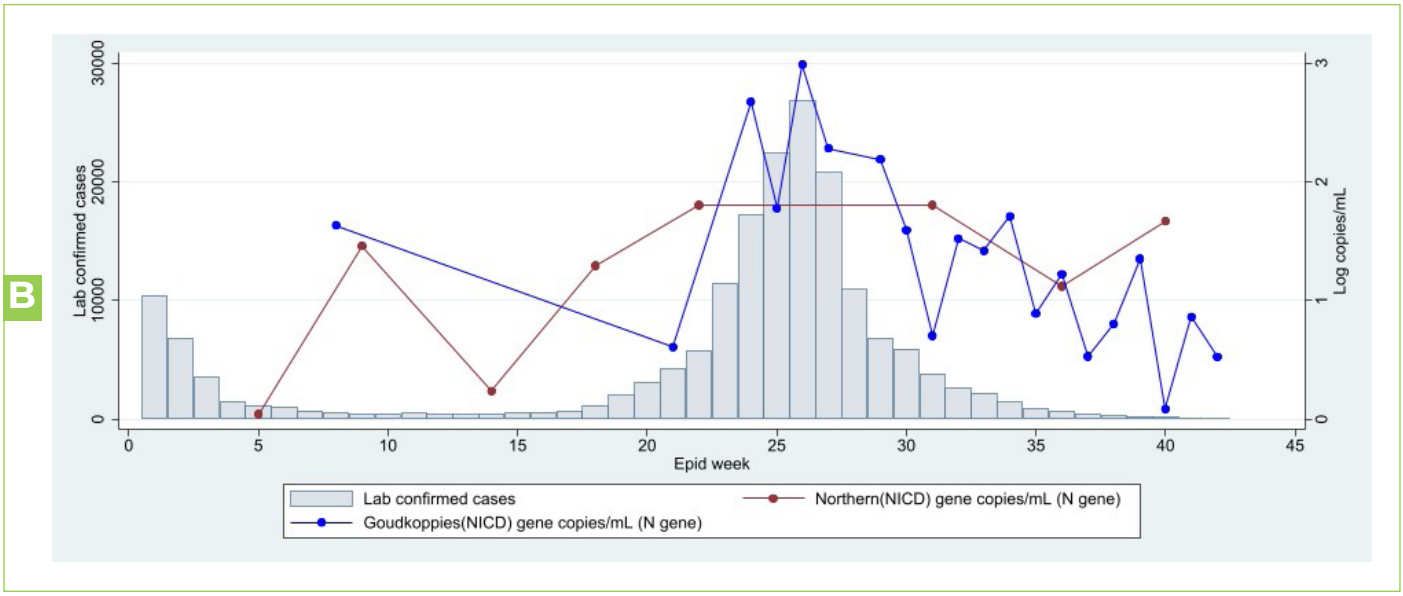
Gauteng Province

A: City of Tshwane Metropolitan Municipality

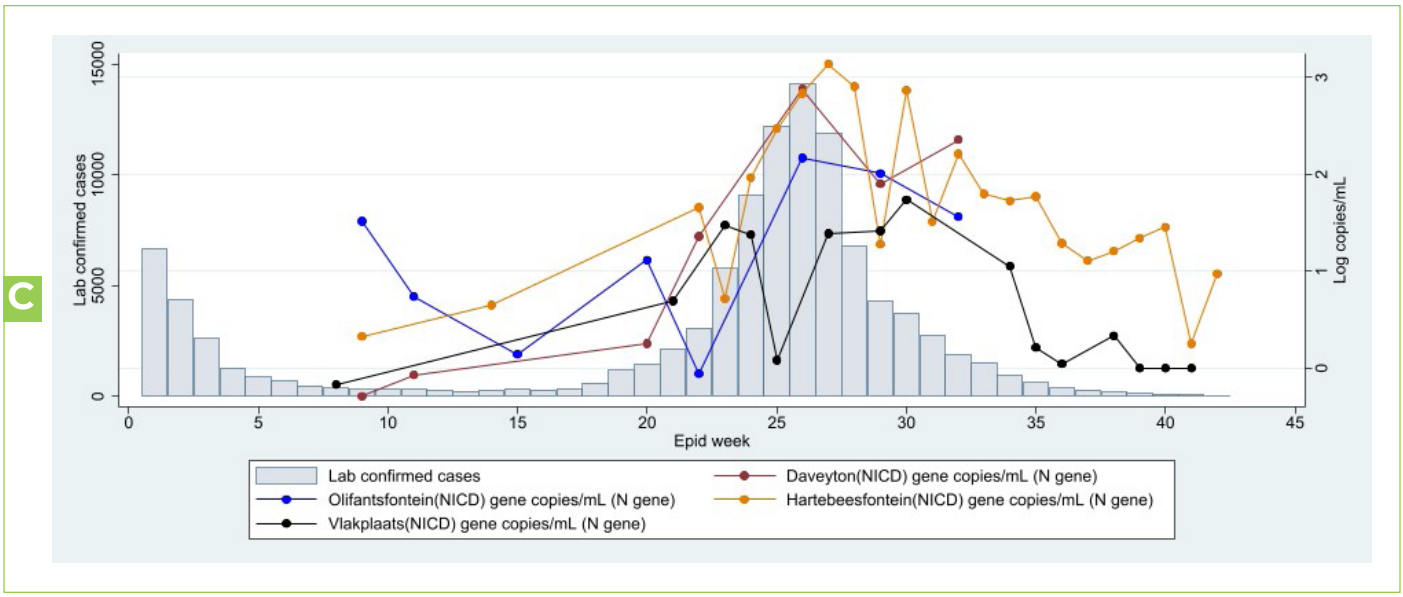
A



B: City of Johannesburg Metropolitan Municipality



C: Ekurhuleni Metropolitan Municipality



Figures 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 Gauteng Province during epidemiological weeks 1-42, 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.

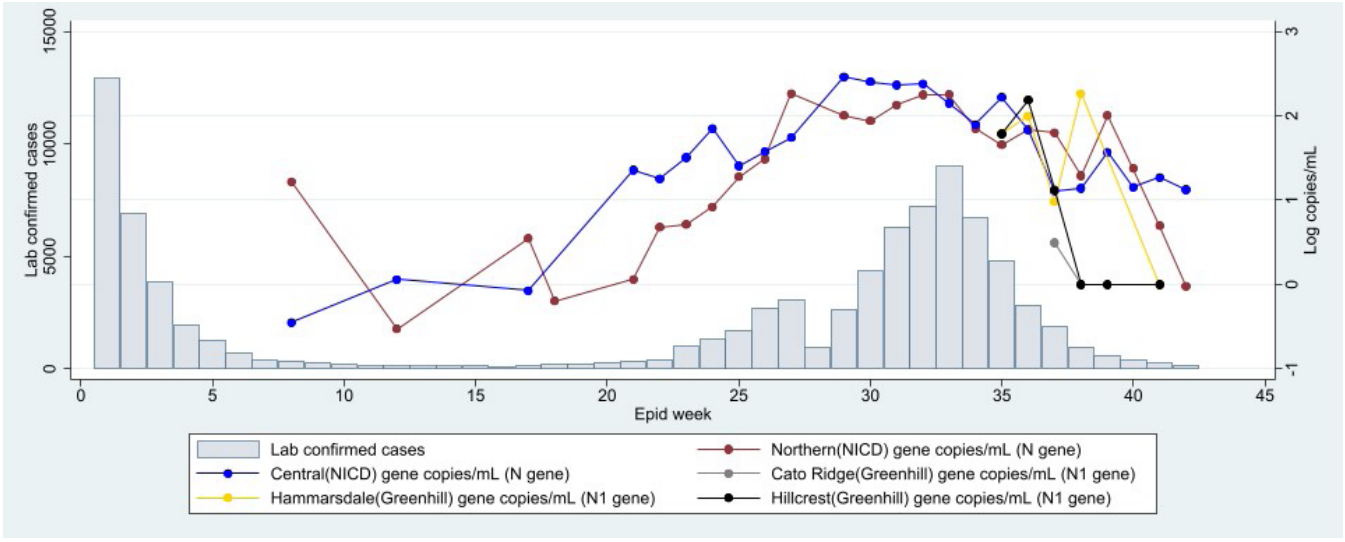
In Gauteng province, wastewater testing for SARS-CoV-2 is currently being conducted in three district municipalities namely, City of Tshwane, City of Johannesburg and Ekurhuleni. Of the five treatment plants where SARS-CoV-2 levels are being monitored in Tshwane (Figure 1A), quantitative testing commenced by the NICD in epidemiological week 9 and 12 at Daspoort and Rooiwal treatment plants respectively. Quantitative testing in the other three treatment plants (Baviaanspoort, Sunderland Ridge and Babelegi commenced in epidemiological week 33 by the National Institute for Occupational Health (NIOH). In the city of Johannesburg (Figure 1B), quantitative testing by the NICD began in epidemiological week 5 in two treatment plants (Goudkoppies and Northern). In Ekurhuleni (Figure 1C), testing began in epidemiological week 8 in one treatment plant (Vlakplaats), and week 9 in three treatments plants (Daveyton, Hartebeesfontein and Olifantsfontein) all at the NICD.

In most treatment plants in Tshwane, City of Johannesburg and Ekurhuleni, the SARS-CoV-2 levels are steadily declining from week 39 till week 42 with a correesponding decrease in clinical cases. SARS-CoV-2 levels may be increasing in Northern WWTP in City of Johannesburg and Hartbeesfontein in Ekurhuleni but these need to be monitored closely. Two consecutive increases in wastewater levels of SARS-CoV-2 have been reported from Sunderland Ridge which services Centurion in City of Tshwane. Authorities should strengthen surveillance in this area.

KwaZulu-Natal Province

A: eThekweni Metropolitan Municipality

A



B: Umgungundlovu District Municipality

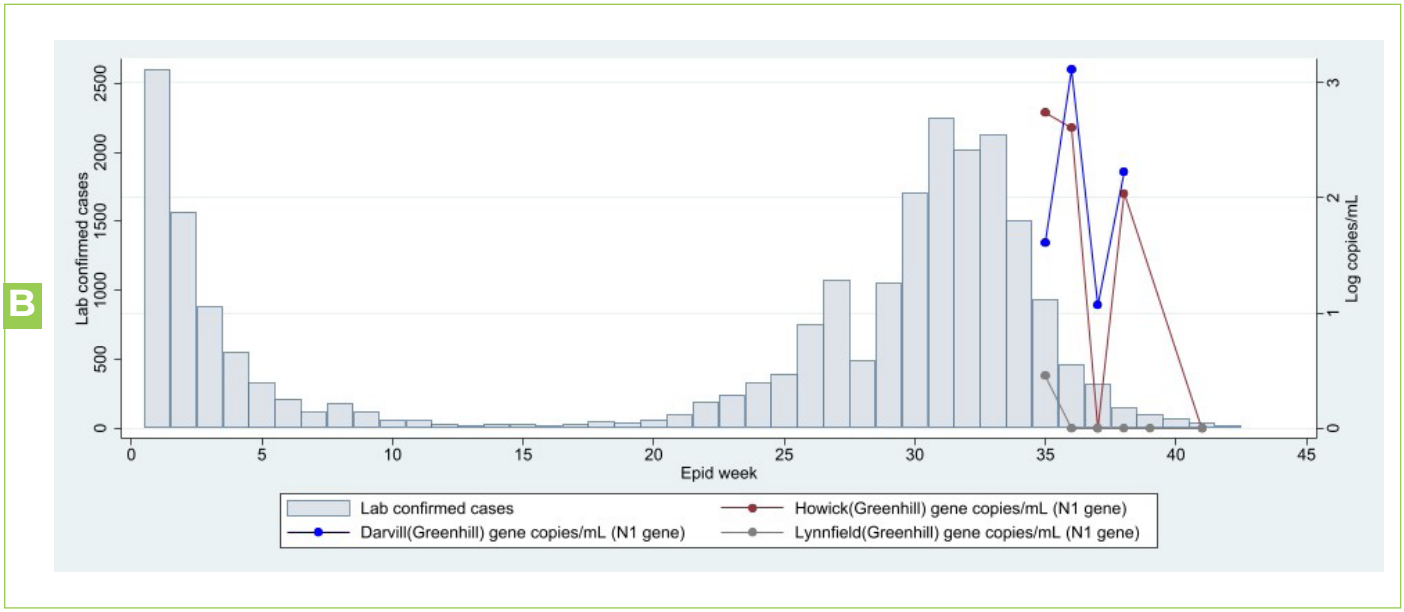


Figure 2A-B. Laboratory confirmed cases of SARS-CoV-2 (bars) and levels of SARS-CoV-2 in log copies/ml of wastewater (coloured lines) from wastewater treatment plants (WWTP) in Ethekwini, (A) and uMgungundlovu Metro (B), KwaZulu Natal Province during epidemiological weeks 1-42, 2021. The testing laboratory 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 by the NICD commenced in epidemiological week 8, 2021 at two WWTPs, Central and Northern. Quantitative testing by GreenHill Laboratories began in week 34, 35, and 37 2021, at the Hillcrest, Hammarsdale and Cato Ridge WWTP respectively. In Umgungundlovu (Figure 2B), quantitative testing by GreenHill Laboratories commenced in epidemiological week 35 in three WWTPs (Darvill, Howick and Lynnfield).

There has been a steady decrease in wastewater levels of SARS-CoV-2 in all the WWTPs tested from week 40, 2021, in both eThekwini and Umgungundlovu , paralleling the decrease in clinical cases.

Free State Province

Mangaung Metropolitan Municipality

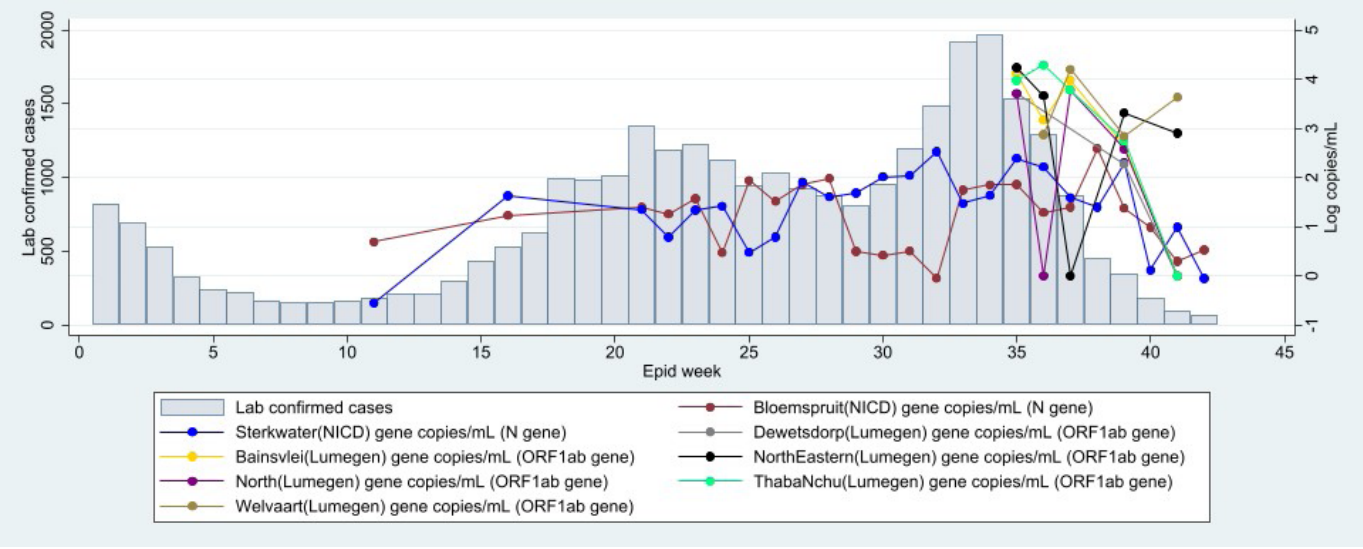


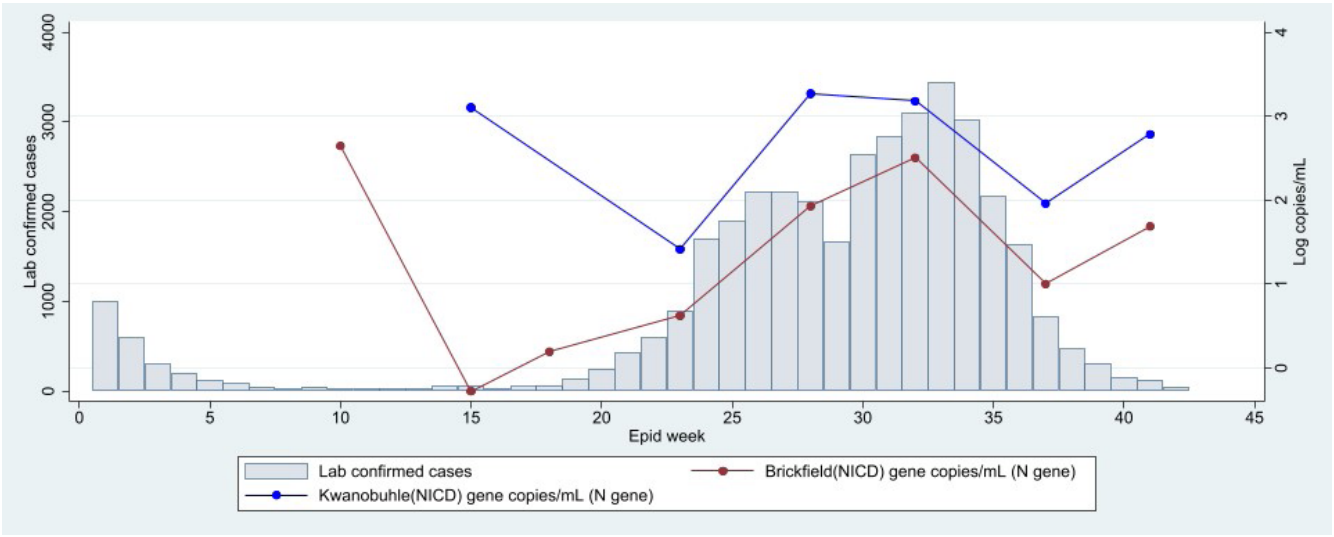
Figure 3. Laboratory confirmed cases of SARS-CoV-2 (bars) and levels of SARS-CoV-2 in log copies/ml of wastewater (coloured lines) from wastewater treatment plants (WWTPs) in Mangaung, Free State Province during epidemiological weeks 1-41, 2021. The testing laboratory and quantified SARS-CoV-2 gene is 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

In Free State, the monitoring of wastewater levels of SARSCov-2 is being conducted at eight WWTPs in Mangaung district municipality, by the NICD and Lumegen Laboratories. The quantitative testing by the NICD 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 six wastewater treatment plants (ThabaNchu, North, Northeastern, Dewetsdorp, Welvaart and Bainsvlei). The SARS-CoV-2 level from all the plants continue to drop in all WWTPs, with the exception of Welvaart This corresponds with the decline in the number of cases. Levels at Welvaart (serving Botshabelo) will be monitored.

Eastern Cape Province

A: Nelson Mandela Metropolitan Municipality

A



B: Buffalo City Metropolitan Municipality

B

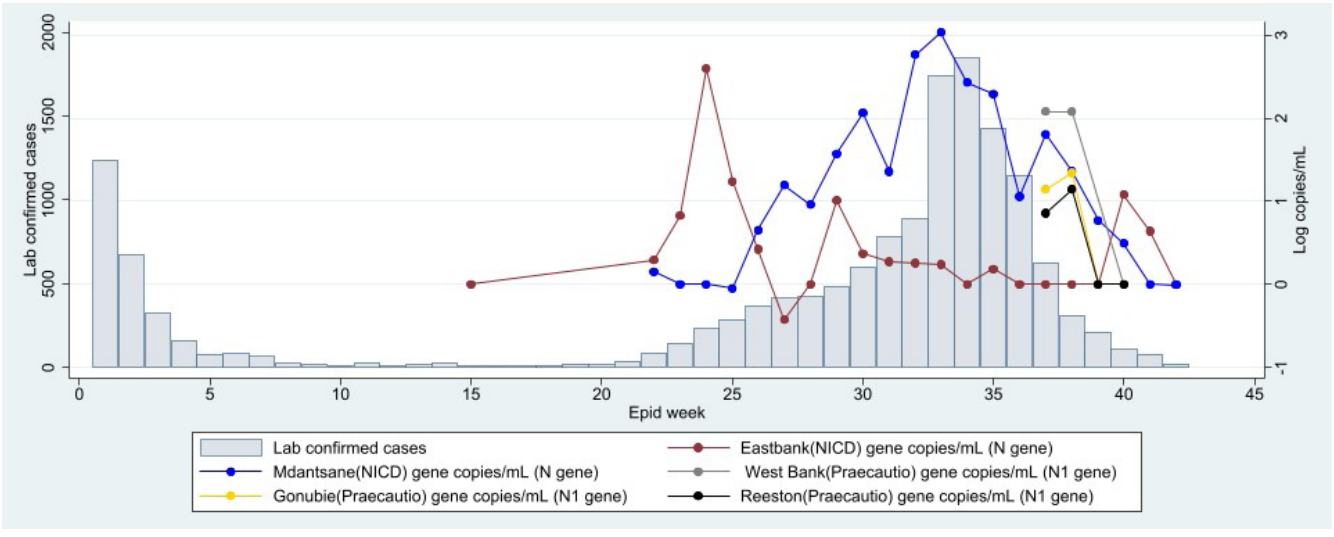


Figure 4A-B. Laboratory confirmed cases of SARS-CoV-2 (bars) and levels of SARS-CoV-2 in log copies/ml of wastewater (coloured lines) from wastewater treatment plants (WWTPs) in Nelson Mandela Metro (A) and Buffalo City Metro (B), Eastern Cape Province during epidemiological weeks 1-40, 2021. The testing 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 NICD commenced quantitative testing in week 10 (Nelson Mandela Metro) and week 15 (Buffalo City Metro) (Figure 4, A-B). Praecautio commenced testing of three WWTPs (West Bank, Gonubie and Reeston) in Buffalo City Metro in epidemiological week 15, 2021.

In Nelson Mandela Metro, SARS-CoV-2 levels in wastewater increased from week 36 to 41 despite decrease in 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/). SARS-CoV-2 levels from wastewater plants in Buffalo City Metro (Mdantsane, Reston, Gonubie and East Bank WWTPs) are stable or showing a downward trajectory from week 36, paralleling a decrease in the burden of clinical cases.

Western Cape Province

City of Cape Town:

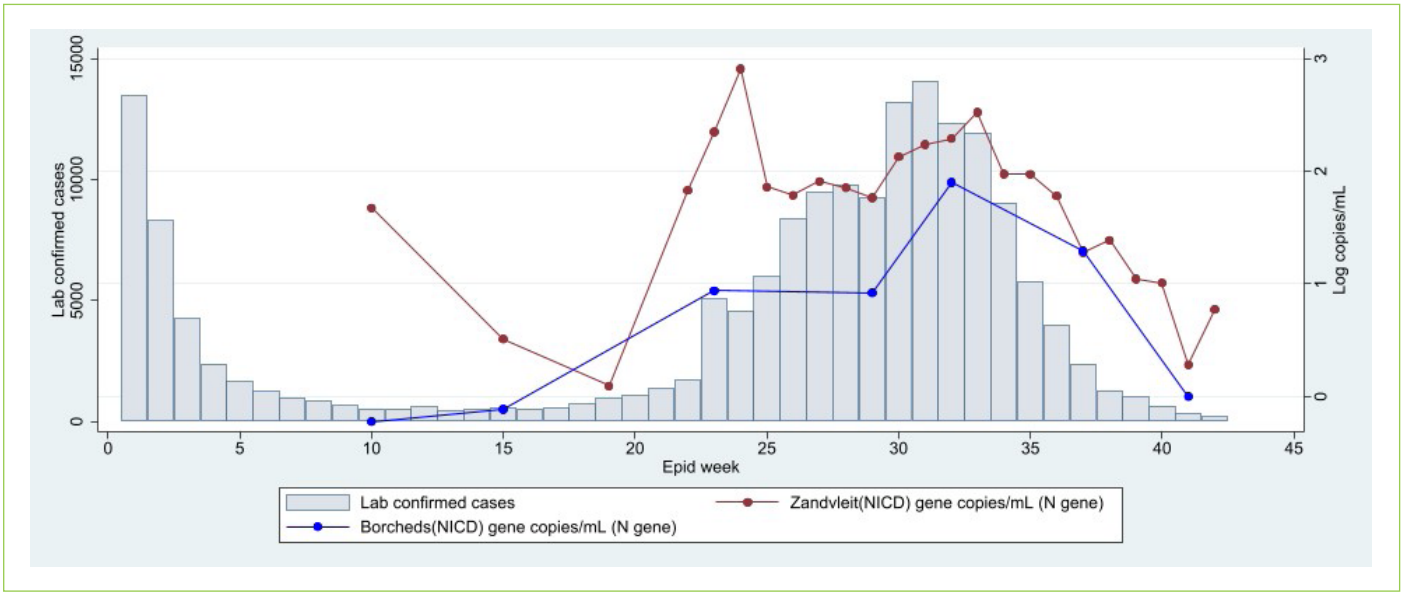
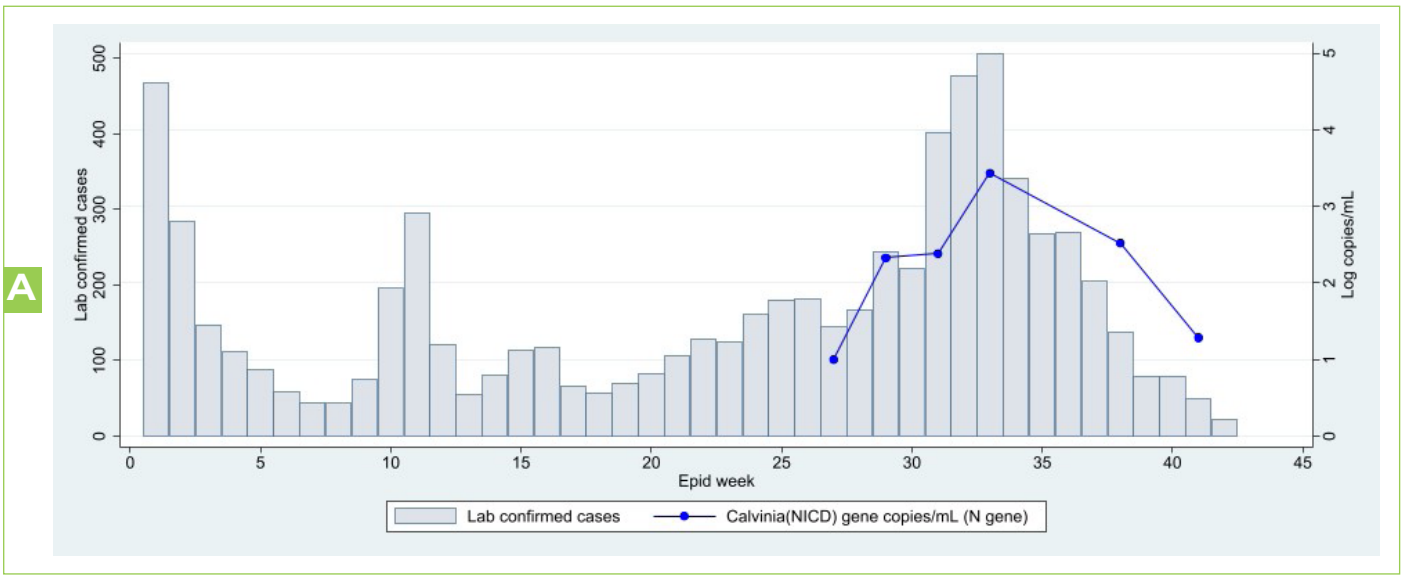


Figure 5. Laboratory confirmed cases of SARS-CoV-2 (bars) and levels of SARS-CoV-2 in log copies/ml of wastewater (coloured lines) from wastewater treatment plants (WWTPs) in City of Cape Town, Western Cape Province during epidemiological weeks 1-41, 2021. The testing laboratory and quantified SARS-CoV-2 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) (Figure 5). From week 36 - 41, there has been a steady decline in SARS-CoV-2 levels in both WWTPs, corresponding to a decrease in clinical cases. The SARS-CoV-2 level in Zandvleit WWTP may be increasing from week 41, but this should be confirmed from subsequent samples. These results should be interpreted with reference to SARS-CoV-2 epidemiology in areas draining into these treatment plants. The MRC website provides data from additional wastewater treatment plants in City of Cape Town and other Western Cape districts (https://www.samrc.ac.za/wbe/).

Northern Cape Province

A: Namakwa District Municipality



B: Frances Baard District Municipality

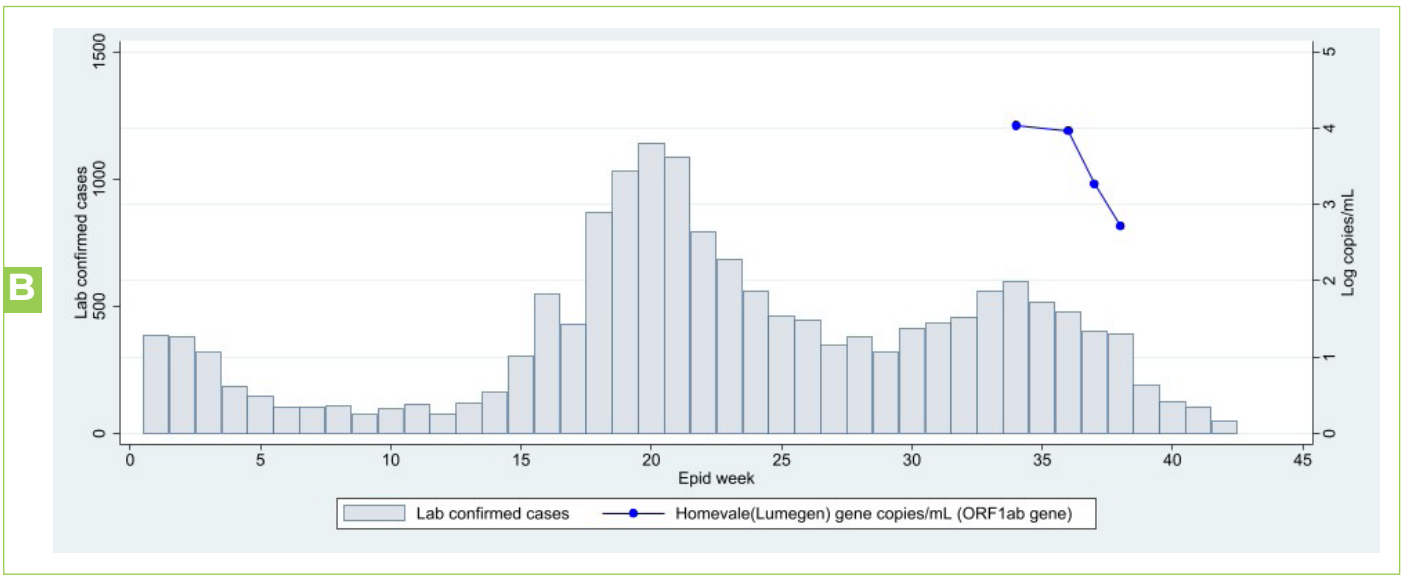


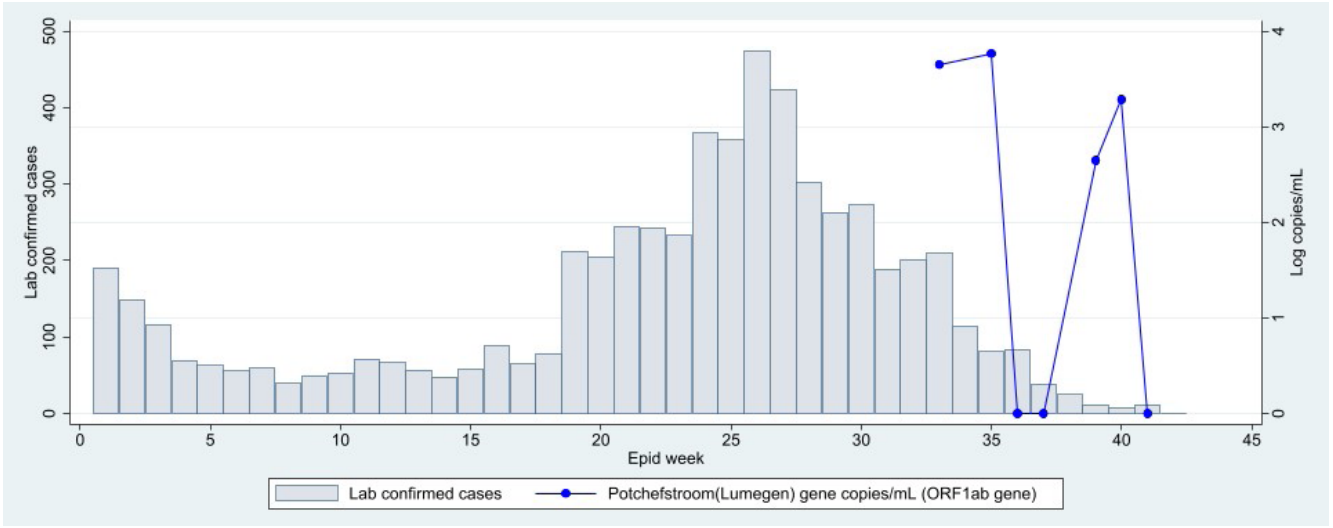
Figure 6A-B. Laboratory confirmed cases of SARS-CoV-2 (bars) and levels of SARS-CoV-2 in log copies/ml of wastewater (coloured lines) in wastewater treatment plants (WWTPs) from Calvinia in Namakwa Metro (A) and Kimberly in Frances Baard District (B), Northern Cape Province during epidemiological weeks 1-40, 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 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 6). In both WWTPs, there has been a decline in SARS-CoV-2 levels corresponding to a decrease in clinical case burden.

North West Province

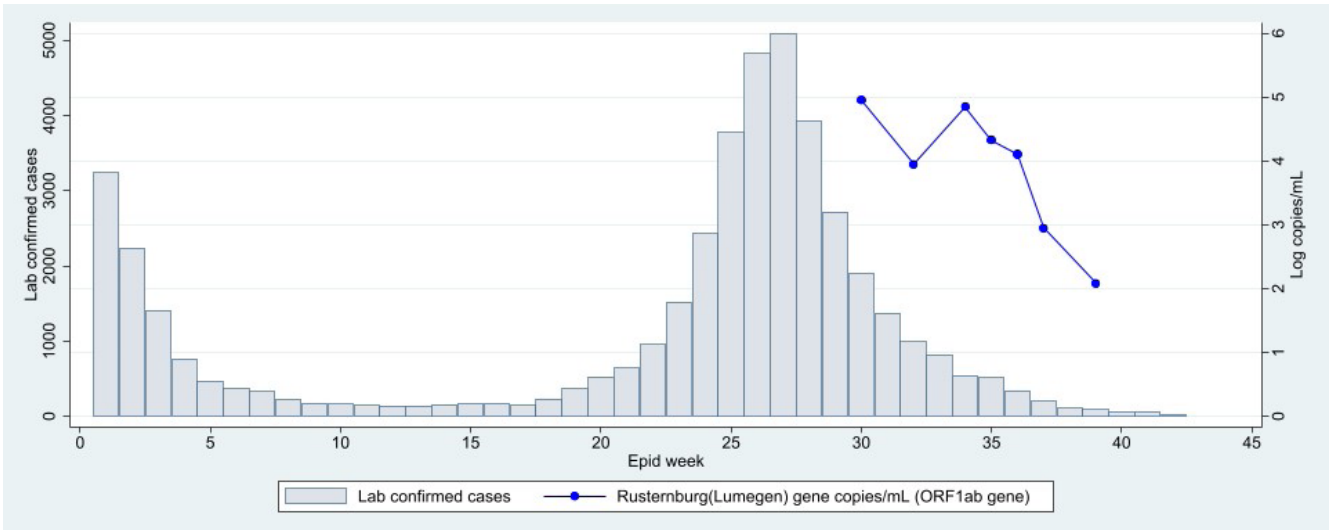
A: JB Marks Local Municipality

A



B: Bojanala District Municipality

B



C: City of Matlosana Municipality

C

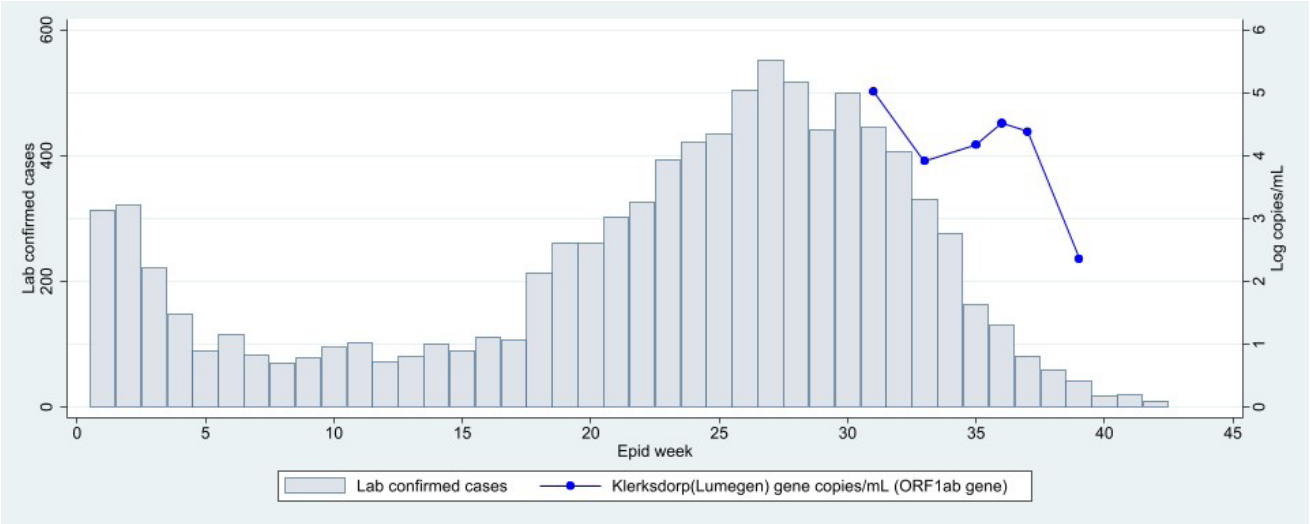


Figure 7A-C. Laboratory confirmed cases of SARS-CoV-2 (bars) and levels of SARS-CoV-2 in log copies/ml of wastewater (coloured lines) in wastewater treatment plants (WWTPs) from Potchefstroom,JB Marks District (A) Rustenberg, Bojanala District (B), and City of Matlosana,. Northwest Province during epidemiological 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 from week 40-41.

Mpumalanga Province

Mbombela Local Municipality

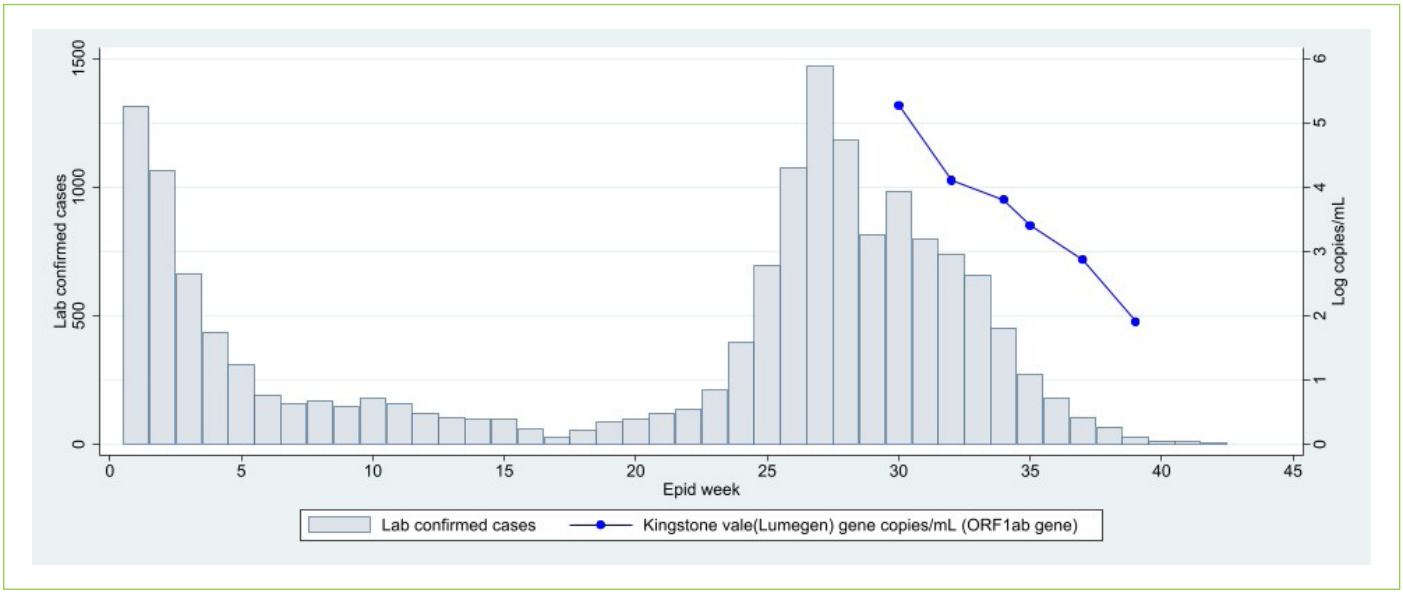


Figure 8. Laboratory confirmed cases of SARS-CoV-2 (bars) and levels of SARS-CoV-2 in log copies/ml of wastewater (coloured lines) in wastewater treatment plants (WWTPs) from Mbombela and Emalahleni Local Municipality, Mpumalanga Province during epidemiological weeks 30-42, 2021. The testing laboratory 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 Mpumalanga, 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 7). From week 37 to week 40, SARS-CoV-2 levels continued to drop in both WWTPs, with a corresponding decrease in the clinical cases.

Limpopo Province

Polokwane Local Municipality

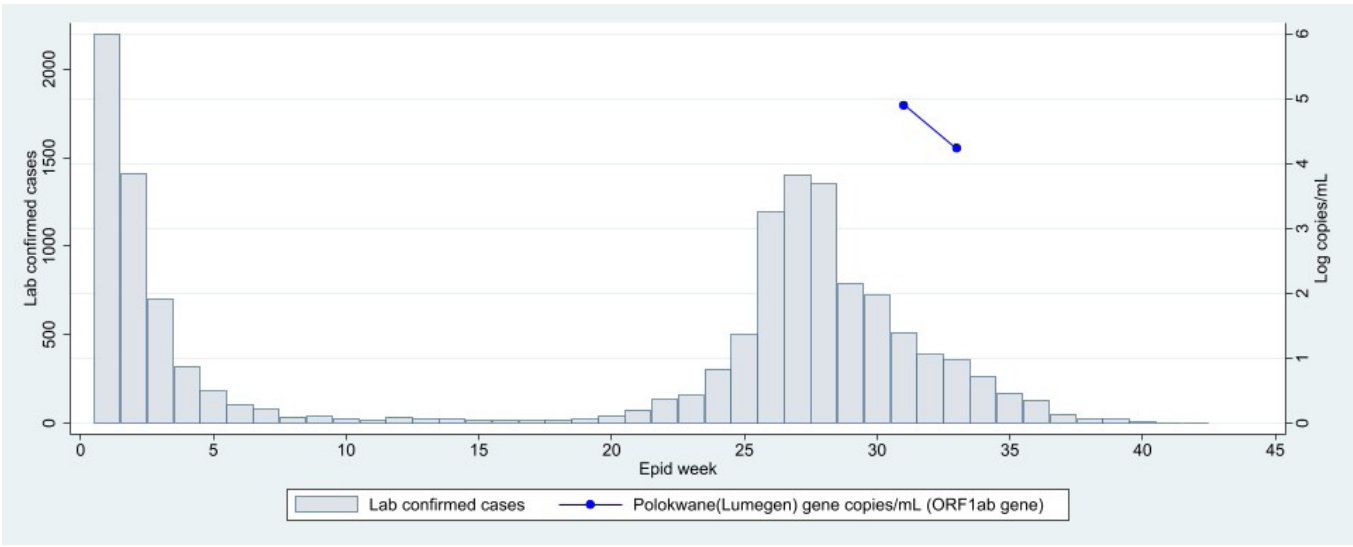


Figure 9. Laboratory confirmed cases of SARS-CoV-2 (bars) and levels of SARS-CoV-2 in log copies/ml of wastewater (coloured lines) in wastewater treatment plants (WWTPs) from Polokwane Local Municipality, Limpopo Province during epidemiological weeks 31-33, 2021.

Quantitative testing commenced by Lumegen laboratories in epidemiologic week 31, 2021, in Polokwane (Figure 8). A downward trajectory in SARS-CoV-2 levels in wastewater was seen between week 31 and 33, with a corresponding decrease in clinical cases.

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

WEEK 42

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.

ACKNOWLEDGEMENTS

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