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The WRC operates in terms of the Water Research Act (Act 34 of 1971) and its mandate is to support water research and development as well as the building of a sustainable water research capacity in South Africa.



## Understanding and unpacking the third wave of the SARS-CoV-2 infections through wastewater-based epidemiology at sentinel wastewater treatment sites

**Authors:** Kerrigan McCarthy<sup>1,2</sup>, Said Rachida<sup>1</sup>, Mukhlid Yousif<sup>1,3</sup>, Nkosenhle Ndlovu<sup>1</sup>, Wayne Howard<sup>1</sup>, Shelina Moonsamy<sup>1</sup>, Gina Pocock<sup>4</sup>, Leanne Coetzee<sup>4</sup>, Janet Mans<sup>5</sup>, Lisa Schaefer<sup>6</sup>, Wouter J. Le Roux<sup>6</sup>, Annancietar Gomba<sup>7</sup>, Don Jambo<sup>7</sup>, David Moriah de Villiers<sup>8</sup>, Nadine Lee Lepart<sup>8</sup>, Rabia Johnson<sup>9</sup>, Christo Muller<sup>9,10,11</sup>, Natacha Berkowitz<sup>12</sup>, Jay Bhagwan<sup>13</sup>, Melinda Suchard<sup>1,14</sup> for the SACCESS network.

<sup>1</sup>*Centre for Vaccines and Immunology, National Institute for Communicable Diseases, a division of the National Health Laboratory Service;* <sup>2</sup>*School of Public Health, University of the Witwatersrand;* <sup>3</sup>*Department of Virology, School of Pathology, Faculty of Health Sciences, University of the Witwatersrand;* <sup>4</sup>*Waterlab;* <sup>5</sup>*University of Pretoria* <sup>6</sup>*Water Centre, Council for Scientific and Industrial Research;* <sup>7</sup>*National Institute for Occupational Health, a division of the National Health Laboratory Service;* <sup>8</sup>*Lumegen Laboratories;* <sup>9</sup>*Biomedical Research and Innovation Platform (BRIP), South African Medical Research Council;* <sup>10</sup>*Division of Medical Physiology, Faculty of Health Sciences, Stellenbosch University;* <sup>11</sup>*Department of Biochemistry and Microbiology, University of Zululand;* <sup>12</sup>*City of Cape Town Health Department;* <sup>13</sup>*Water Research Commission;* <sup>14</sup>*Department of Chemical Pathology, School of Pathology, University of the Witwatersrand.*

*From 2020 to 2021, 142 and 371 wastewater samples were submitted from 18 and 38 WWTW respectively and tested by or reported to the NICD by partner laboratories. Of these 383 samples, SARS-CoV-2 was identified in 105 samples in 2020 (74%) and 245 in 2021 (66%), with each site submitting an average of 13.5 samples over the two years. In 2020 and 2021 respectively, 24 (17%) and 45 (12%) of samples had invalid results. Of samples tested in 2021, 88 of 245 (36%) positive samples had*

quantitative tests conducted. The SARS-CoV-2 N-gene was found in 85 out of 88 samples.

- *In Gauteng province since week 17 (mid-April) all nine WWTP across Gauteng that submitted samples had tested positive, signifying an increase in population burden of disease across the province. Quantitative results from two plants in the City of Johannesburg, a single plant in City of Tshwane and three plants in Ekurhuleni were first tested in week 16 (end April 2021) and showed consistently increasing concentrations of SARS-CoV-2 in log RNA copies/ml of influent wastewater, consistent with observed increases in reported clinical cases in these districts.*
- *In the Western Cape Province samples from the two WWTP have consistently tested positive. However, quantitative results in wastewater have shown an increase in viral copies per millilitre in June, in parallel with the increasing numbers of clinical cases.*
- *In the Eastern Cape Province, sample results are available from week 38 of 2020 (mid-September 2020), and all tested positive until week 6 and/or week 10 of 2021 (March-April 2021), coinciding with the end of the second wave. Quantitative results from plants in Buffalo City have increased, but a recent sample from week 26 has a lower viral load. In Nelson Mandela Metro, samples from week 24 show high viral load, in correlation with climbing clinical cases. A single positive sample with a high viral load was detected in week 15 and is of uncertain significance as few clinical cases were detected that week.*
- *In Free State province testing commenced in week 31 of 2020. SARS-CoV-2 RNA has been detected at both sites continuously since then, in keeping with the high clinical case load (greater than 200 cases per week). Quantitative results from week 17 have been consistently elevated, but one plant appears to have a decreasing viral load in weeks 26 and 27.*
- *All samples from KwaZulu-Natal Province (from week 36, 2020 to date) have tested positive since commencement of testing in week 36 of 2020. Viral quantification has shown consistent increases from week 19 (mid May 2021) onwards, signifying an increase in cases.*

*The results from detection of SARS-CoV-2 from wastewater at South African sentinel sites, some concordance of qualitative and quantitative results with clinical epidemiologic curves have been demonstrated. Furthermore, the potential of the network to provide descriptive epidemiological data pertaining to geographic variation and burden of disease has been demonstrated as part of the current study.*

*In conclusion, the detection results from sentinel sites across South African urban areas indicating correspondence at some sites with increased numbers of clinical cases during the third wave in Gauteng, KwaZulu-Natal, Free State and Northern Cape provinces support the further exploration of the use of these data to monitor SARS-CoV-2 epidemiology using wastewater based testing.*

## Background and introduction

Globally, detection and monitoring of SARS-CoV-2 epidemiology through wastewater was proposed as early as April 2020<sup>1,2</sup>. First reports describing the feasibility and practical usefulness of this approach emerged simultaneously from a number of countries around August 2020<sup>3,4</sup>. Recent evidence has shown that SARS-CoV-2 can be detected in wastewater prior to the appearance of clinical cases<sup>5</sup>. Longitudinal tracking of SARS-CoV-2 viral load in wastewater is shown to correlate with the burden of clinically diagnosed cases<sup>6</sup>. Sequencing of SARS-CoV-2 RNA fragments in wastewater has identified variants of concern as well as mutations not detected in clinical cases<sup>7</sup>.

Wastewater-based epidemiology (WBE) is emerging as a useful tool to monitor SARS-CoV-2 epidemiology and support public health decision making regarding COVID-19 response activities. Based on the global developments in the field and the opportunity WBE offers in terms of an early warning and hotspot indicator of the viral infections in communities, several South African institutions, including the Water Research Commission (WRC), National Institute for Communicable Diseases (NICD), South African Medical Research Council (MRC), Durban University of Technology (DUT), University of Pretoria, Council for Scientific and Industrial Research (CSIR), National Institute for Occupational Health and private laboratories (including Waterlab, Lumegen, Greenhill and Precautio Laboratories) launched wastewater testing programmes for SARS-CoV-2. The South African Collaborative COVID-19 Environmental Surveillance System (SACCESS) network of laboratories was formed. Since inception in March 2020, seven laboratories tested 513 specimens from 38 wastewater treatment plants across 9 provinces, covering sentinel sites where 760,731 out of 1,973,972 (38,5%) of laboratory-confirmed COVID-19 cases were reported. SARS-CoV-2 was detected in 350 (68%) samples. Detection of SARS-CoV-2 was more frequent during clinical 'waves'. Viral load in wastewater tended to increase during the third wave in parallel with increasing clinical cases. Detection of SARS-CoV-2 with quantitation of viral loads from wastewater may be a helpful additional data source to support population level monitoring of SARS-CoV-2 epidemiology. However, additional geospatial and epidemiological investigations should be done to support the use-cases of wastewater-based epidemiology for COVID-19.

In South Africa, SARS-CoV-2 epidemiology is monitored through laboratory testing of clinical cases by reverse-transcriptase polymerase chain reaction (RT-PCR) tests and rapid antigen tests. Laboratory testing data is relayed by testing laboratories to the National Institute for Communicable Diseases (NICD). Hospital admissions and death data is submitted by the daily hospital surveillance (DATCOV) system to the NICD. 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.

A number of independent initiatives to test for SARS-CoV-2 in wastewater commenced in early 2020. 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 development of common testing methodology, identify and address challenges, and share best practices related to qualitative, quantitative and RNA sequencing of SARS-CoV-2 in wastewater. In South Africa, treatment of wastewater is the responsibility of local government. Approximately 1,050 wastewater treatment works (WWTW) are administered by metropolitan municipalities and local government and treat both industrial and domestic wastewater. The SACCESS partners and the NICD have engaged with local government to support sample collection, interpretation and utilisation of the results for public health purposes.

As at the beginning of July 2021, South Africa has experienced two 'waves' of COVID-19, and is currently experiencing a third 'wave'. The initial rise in case numbers began in the Northern Cape and Free State provinces in March and early April of 2021. As of 6 July 2021, the rise in cases met the criteria for a 'third wave' in all provinces. At the time of writing, the case incidence rate in Gauteng Province was highest at 45 per 100,000 with over 34% of daily tests reportedly positive.

This scientific brief aims to identify epidemiological trends of SARS-CoV-2 in South African urban wastewater treatment plants and compared PCR detection and quantitation results from influent sewage with trends in clinical epidemiology.

## Methods

### *Outbreak context and clinical case epidemiology*

Since the first case of SARS-CoV-2 was detected on 3 March 2020, South African laboratories have conducted over 13 million RT-PCR and antigen tests. Just over 2 million cases and 61,000 deaths have been recorded in South Africa as of 6 July 2021. Three distinct 'waves' of SARS-CoV-2 infection occurred, with the first two peaking in June and December 2020 respectively, whilst the third is still unfolding as of 6 July 2021. The de-duplicated and geospatially allocated national line list of laboratory-confirmed cases of SARS-CoV-2 (identified by RT-PCR or antigen test) were 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, the Council for Scientific and Industrial Research, the University of Pretoria, the SA-MRC, the Institute for Water and Wastewater Technology at the

Durban University of Technology, the National Institute for Occupational Health and a number of private laboratories (Waterlab, Greenhill and Lumegen Laboratories) commenced with independent projects involving qualitative and quantitative detection of SARS-CoV-2 in local WWTW. A proof of concept was published by the SAMRC in 2021<sup>8</sup>.

In August 2020, laboratories doing this work collectively established the SACCESS network to support communication of challenges and sharing best practice. Through funding and partnership with the WRC, the NICD and partners identified several WWTW for sampling and SARS-CoV-2 testing to maximise coverage across metros and sentinel sites in provinces with smaller populations. A number of webinars were held to share methodologies for concentration, extraction and PCR detection of SARS-CoV-2, and a compendium of methodologies was published<sup>9</sup>. Sampling from selected WWTW and testing was conducted between June 2020 and March 2021 as more funding for partners became available from the WRC. Results from testing funded through the WRC and NICD are reported here.

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

At identified wastewater treatment facilities, one litre grab samples of influent were collected and transported at <5°C to the testing facility. Table 1 summarises testing modalities used in this study. Table 2 provides the concentration, extraction and PCR detection methodology for each network laboratory. 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) was 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 amplifies 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 were calculated and 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. 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**

Testing modality	Test modalities	Interpretive principles to support public health responses
Detection of SARS-CoV-2	Concentration of viruses from influent wastewater samples followed by RT-PCR testing using commercial kits with primers specific for SARS-CoV-2 virus. Interpretive criteria for PCR results are specific to the test kit used for detection. Ct values are recorded for each of the genes detected by the PCR.	When a test result changes from <ul style="list-style-type: none"> <li>• positive to negative this signifies fewer/no cases in population) or from</li> <li>• negative to positive, this indicates the need for increased population awareness and action</li> </ul> Changes in Ct values with time may indicate changing concentrations of virus in the influent (low Ct value equates to high viral load)
Quantification of SARS-CoV-2	Concentration and RT-PCR as above, with comparison to a standard curve drawn from RT-PCR with a known concentration of plasmid containing one/more genes of SARS-CoV-2. The PCR Ct value results are compared to a 'standard curve' to determine quantity of SARS-CoV-2 in the influent sample.	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. Trends in the rate of change of concentration give an indication of whether the burden of disease is increasing or decreasing

### *Interpretation of results*

Table 1 provides interpretive principles to support the application of wastewater detection, quantification of SARS-CoV-2 RNA to public health surveillance and response activities. A positive PCR test result was defined as detection of any SARS-CoV-2 gene target (amongst the N, E or RdRP genes). A negative PCR test was defined as a positive internal control without a positive N, E or RdRP gene target. An invalid test result was 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.

**Table 2. Concentration, extraction and RT-PCR detection methodology used by laboratory partners.**

Name of Laboratory partner	Method for virus concentration	Method for nucleic acid extraction	RT-PCR assay
NICD	Centricon® Plus-70 centrifugal	QIAamp® viral RNA mini kit	Allplex™ 2019-nCoV Assay
CSIR	Polyethylene Glycol	Omega Bio-tek ENZA total RNA Kit II	2019-nCoV CDC EUA Kit
NIOH	Skim milk flocculation	MagMAX Viral and Pathogen Nucleic Acid Isolation Kit	Thermofisher (TaqPath kit)
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	Tangential Flow Filtration	Qiagen extraction Kit	Thermofisher (TaqPath kit)

## Results

### Overview of wastewater and clinical SARS-CoV-2 test results

In 2020, WWTW from 19 sites across five provinces submitted samples for testing, including nine in Gauteng, two in the City of Cape Town, Western Cape Province, two in Mangaung, Free State Province, two in eThekweni (KwaZulu-Natal Province) and four in Eastern Cape Province (two in Buffalo City Metro and two in Nelson Mandela Metro).

In 2021, additional sites in Gauteng (seven sites), Limpopo (two sites), Mpumalanga (three sites), North West (three sites), the Northern Cape (one site) and Western Cape (three sites) provinces were added, bringing the total to 38 sites. Two sites in Gauteng were duplicated, being tested by the NICD and another laboratory.

The de-duplicated and geospatially allocated line list of laboratory-confirmed clinical cases from commencement of the South African pandemic (4 March 2020) until the end of week 25 (26 June 2021) yielded 1,973,972 cases. Amongst these, 760,731 (38,5%) were located in a metropolitan area or local municipality where wastewater-based surveillance for SARS-CoV-2 was being conducted at the time (15,940 in Buffalo City; 36,370 in Nelson Mandela Metro Bay; 30,133 in Mangaung Free State; 135,701 in City of Johannesburg; 110,148 in City of Tshwane; 75,934 in Ekurhuleni; 29,825 in West Rand; 81,931 in eThekweni, 6,496 in Capricorn District Municipality (DM); 7514 in Emalahleni DM, 5,166 in Mbombela DM; 4,762 in Matlosana DM, 2,931 in JBMarks Local Municipality (LM); 10,047 in Rustenburg LM; 7,533 in Sol Plaatje LM, 200,310 in City of Cape Town Metro)

### Provincial trends

From 2020 to 2021, 142 and 371 wastewater samples were submitted from 18 and 38 WWTW respectively and tested by or reported to the NICD by partner laboratories. Of these 383 samples, SARS-CoV-2 was identified in 105 samples in 2020 (74%) and 245 in 2021 (66%), with each site submitting an average of 13.5 samples over the two years. In 2020 and 2021 respectively, 24 (17%) and 45 (12%) of samples had invalid results. Of samples tested in 2021, 88 of 245 (36%) positive

samples had quantitative tests conducted. The SARS-CoV-2 N-gene was found in 85 out of 88 samples. Detection and quantitation results are displayed in Figures 1-6 together with epidemiologic curves for clinical cases detected for the districts or metros where each WWTW is located.

In Gauteng province (Figure 1A-C) all samples were negative up until week 21 of 2020, (commencing 17 May 2020), when samples from two plants (Ekurhuleni and City of Tshwane) tested positive, coinciding with the onset of the first wave. All sites continued to test positive through the inter-wave period (August-December 2020) and through the second wave (ending week 7, February 2021). A number of sites in City of Tshwane, Johannesburg and Ekurhuleni tested negative during weeks 11-16 (mid-March until end April 2021). Since week 17 (mid-April) all nine WWTP across Gauteng that submitted samples had tested positive, signifying an increase in population burden of disease across the province. Quantitative results from two plants in the City of Johannesburg, a single plant in City of Tshwane and three plants in Ekurhuleni were first tested in week 16 (end April 2021) and show consistently increasing concentrations of SARS-CoV-2 in log RNA copies/ml of influent wastewater, consistent with observed increases in reported clinical cases in these districts.

In the Western Cape Province (Figure 2) samples from the two WWTP have consistently tested positive. However, quantitative results in wastewater have shown an increase in viral copies per millilitre in June, in parallel with the increasing numbers of clinical cases.

In the Eastern Cape Province (Figure 3), sample results are available from week 38 of 2020 (mid-September 2020), and all tested positive until week 6 and/or week 10 of 2021 (March-April 2021), coinciding with the end of the second wave. Quantitative results from plants in Buffalo City have increased but a recent sample from week 26 has a lower viral load. In Nelson Mandela Metro, samples from week 24 show high viral load, in correlation with climbing clinical cases. A single positive sample with a high viral load was detected in week 15 and is of uncertain significance as few clinical cases were detected that week.

In Free State province (Figure 4) testing commenced in week 31 of 2020. SARS-CoV-2 RNA has been detected at both sites continuously since then, in keeping with the high clinical case load (greater than 200 cases per week). Quantitative results from week 17 have been consistently elevated, but one plant appears to have a decreasing viral load in weeks 26 and 27.

All samples from KwaZulu-Natal Province (Figure 5) (from week 36, 2020 to date) have tested positive since commencement of testing in week 36 of 2020. Viral quantification has shown consistent increases from week 19 (mid May 2021) onwards, signifying an increase in cases.

## Discussion

In this description of results from detection of SARS-CoV-2 from wastewater at South African sentinel sites, some concordance of qualitative and quantitative results with clinical epidemiologic curves have been demonstrated. Furthermore, the potential of the network to provide descriptive epidemiological data pertaining to geographic variation and burden of disease has been demonstrated as part of the current pilot study.

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 are so mild as not to elicit health seeking and that laboratory-confirmed cases are likely to represent less than 10% of SARS-CoV-2 cases prevalent in a community at any given time point. 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.

Detection results from our network reflect that SARS-CoV-2 is reliably present in wastewater during 'waves', and during the third wave, while quantitation results have reflected the current upward trend observed amongst clinical cases. However, certain anomalies in the results require careful attention. Whilst SARS-CoV-2 cases were present in all metros and areas under surveillance during the inter-wave period, continued positive results even during times of apparent low population burden may signify high sensitivity of detection mechanisms, higher than reported numbers of cases in sewage drainage areas or, less likely, false positive laboratory test results. A single high concentration of SARS-CoV-2 was detected in Nelson Mandela Metro Bay in week 15, when only 60 clinical cases were reported. It is considered that an error in the quantitative PCR result unlikely, therefore this result may reflect a localised transmission event which went undetected or high numbers of asymptomatic cases. In recent weeks (22-27, June-early July 2021) although the general trend of SARS-CoV-2 concentration is upward, fluctuations of quantitation results have been observed. The significance of these fluctuations is presently unclear.

Presently, the results are helpful to retrospectively confirm the presence of SARS-CoV-2 in wastewater and hence in metros of South Africa. However, current data challenge the network partners to exploit and build on the opportunity provided by this network to supply meaningful and timely data to public health decision makers at provincial and national level as has been done elsewhere<sup>6</sup>. These data include temporal and geographical changes in qualitative and quantitative detection patterns within metros by determining population sizes and case loads within sewage drainage areas, mapping of sewage reticulation networks by metropolitan officials with nodal sampling and testing of wastewater to identify localised areas of high SARS-CoV-2 prevalence. These data may guide the implementation of public health measures to curb transmission and contain the economic and social impact of SARS-CoV-2. Some work has been done globally attempting to calculate population burdens of disease from quantitative PCR results<sup>10</sup>. However, this requires knowledge of duration and quantity of SARS-CoV-2 shedding from infected persons as well as the rate of decay of RNA in wastewater. With frequent sampling, it is possible to determine the effective reproductive rate of SARS-CoV-2 using wastewater based epidemiology<sup>11</sup>. Genomic sequencing of SARS-CoV-2 viral fragments in wastewater also has potential to monitor population trends in circulation of variants<sup>7</sup>. Trends in quantitation of viral RNA may also allow inferences to be made regarding the success of vaccine roll-out. Lastly, the network may prove useful to provide surveillance data for other waterborne or excreted communicable diseases including agents of gastroenteritis, hepatitis and other respiratory illness such as influenza<sup>12</sup> and for monitoring of antimicrobial resistance<sup>13</sup>.

Limitations of wastewater-based epidemiology include restriction of the surveillance methodology to urban populations with functional sewage reticulation networks, inability to measure disease amongst non-sewered populations (e.g. in informal settlements or rural areas) and reliance on repeated measurements from the same plant tested with identical methodology to demonstrate temporal trends. 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. The use-case scenarios SARS-CoV-2 wastewater based epidemiology to support public health interventions at local, provincial and national level in South Africa need to be established.

## Conclusion

In conclusion, the detection results from sentinel sites across South African urban areas indicating correspondence at some sites with increased numbers of clinical cases during the third wave in Gauteng, KwaZulu-Natal, Free State and Northern Cape provinces support the further exploration of the use of these data to monitor SARS-CoV-2 epidemiology using wastewater-based testing.

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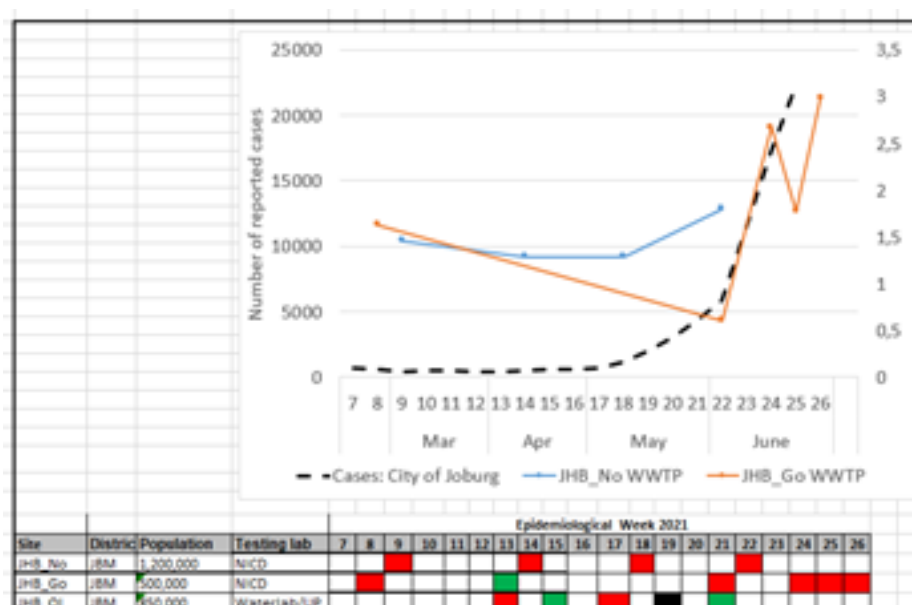
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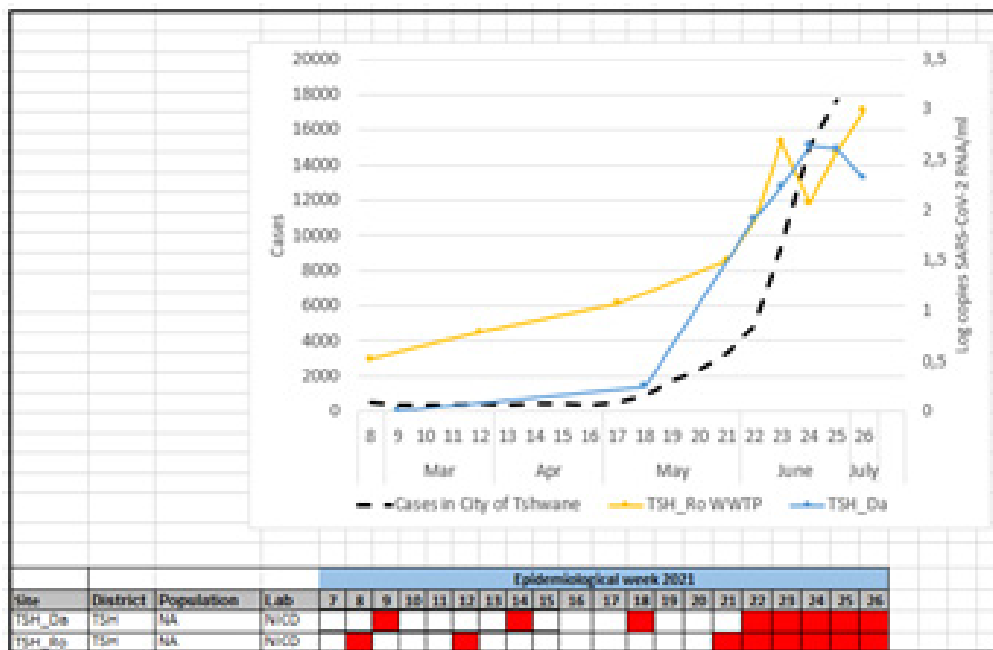
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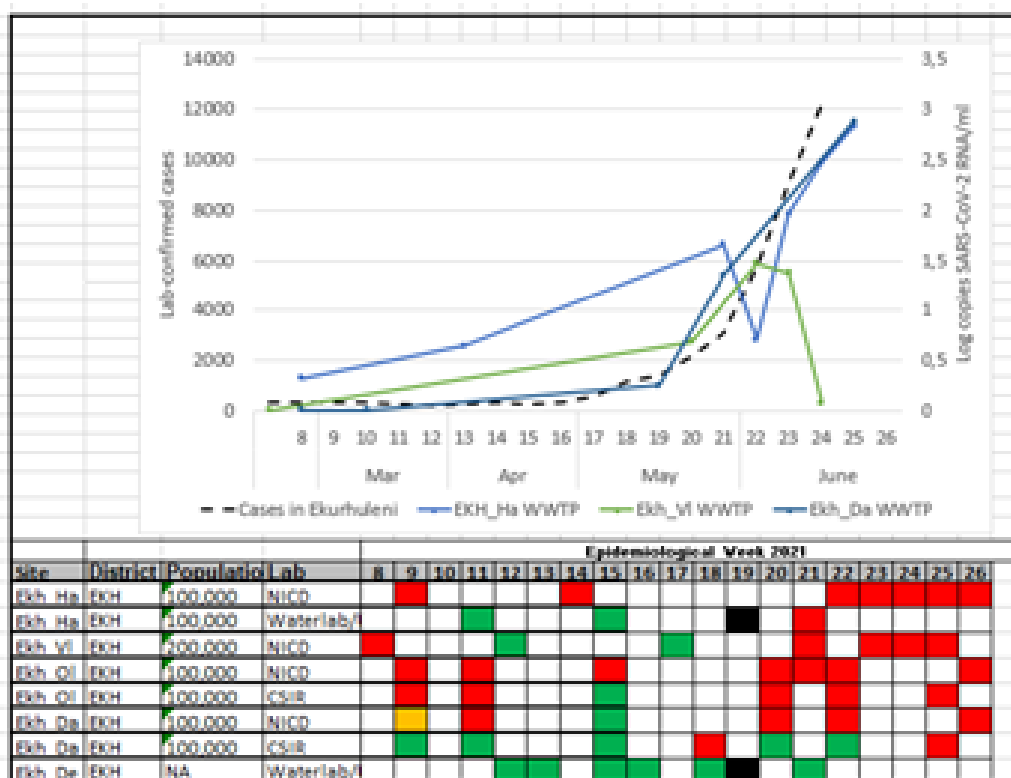
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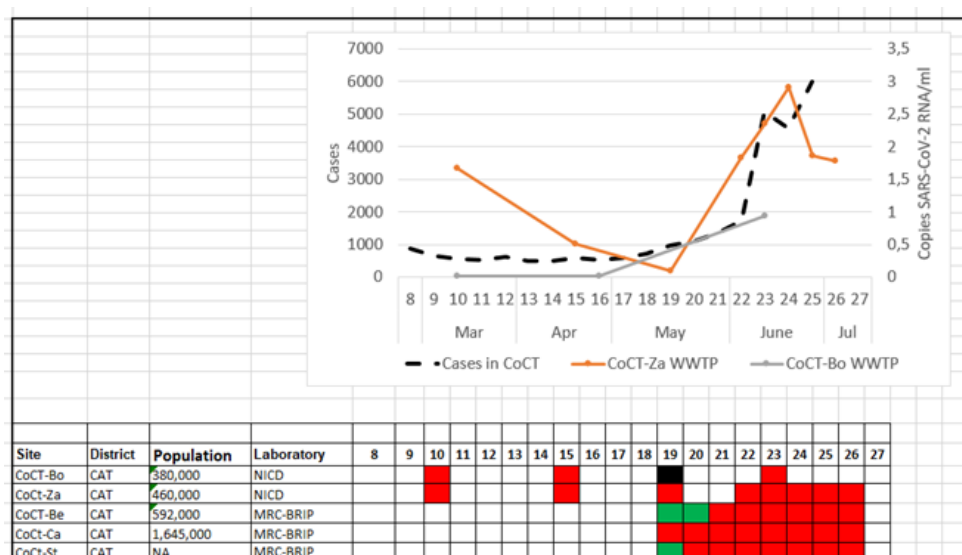


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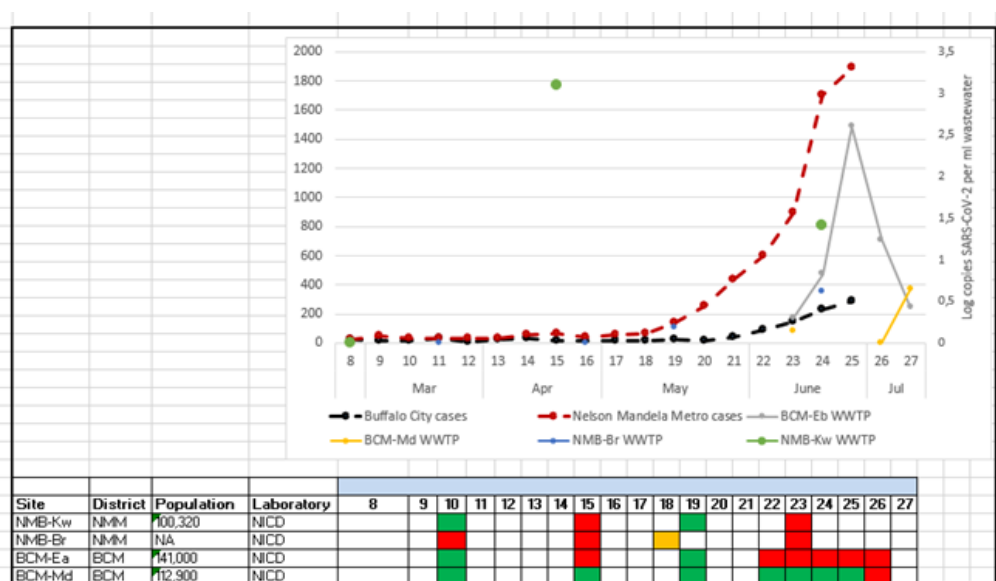
**Figure 1. Epidemiological curves of laboratory-confirmed SARS-CoV-2 cases, SARS-CoV-2 viral loads in wastewater and SARS-CoV-2 PCR detection results in wastewater by district, Gauteng Province, 2020-2021.**

Districts shown include City of Johannesburg (A), City of Tshwane (B) and Ekurhuleni Metropolitan area (C). Black dashed line represents laboratory-confirmed COVID-19 cases (left axis) by epidemiological weeks 8-26, 2021 (horizontal axis). Coloured lines represent quantitative viral loads in copies per millilitre (right axis) in specimens collected in given epidemiological week from wastewater treatment facilities (horizontal axis). Green squares represent negative PCR results, red squares represent positive results and black squares represent invalid results. JHB = City of Johannesburg. TSH = City of Tshwane. EKH = Ekurhuleni. WWTP = wastewater treatment plant. \*Population is the approximate size of the population served by the wastewater treatment plant. Lab is the name of the testing laboratory



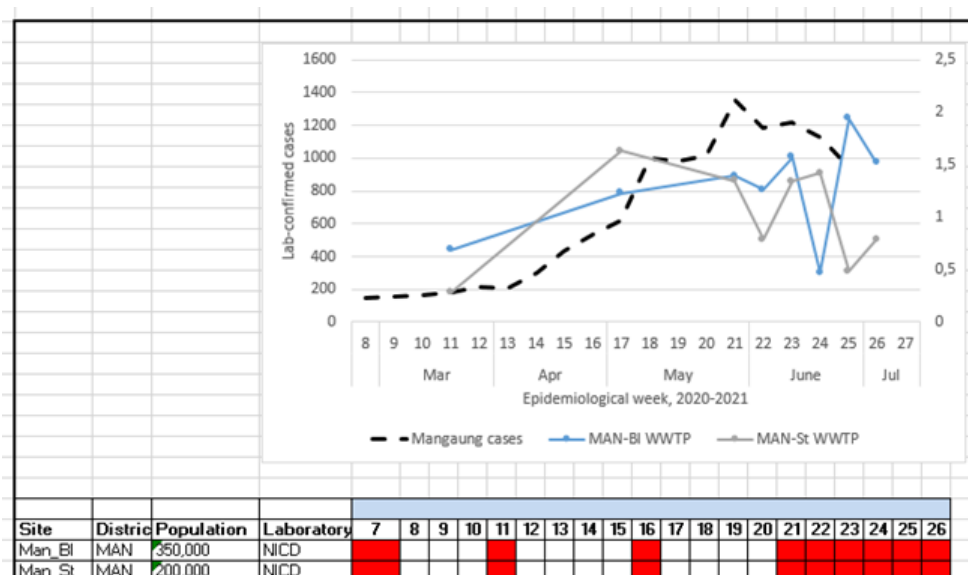
**Figure 2. Epidemiological curves of laboratory-confirmed SARS-CoV-2 cases, SARS-CoV-2 viral loads in wastewater and SARS-CoV-2 PCR detection results in wastewater for the City of Cape Town, Western Cape province, 2020-2021.**

Black dashed line represents laboratory-confirmed COVID-19 cases (left axis) by epidemiological weeks 8-26, 2021 (horizontal axis). Coloured lines represent quantitative viral loads in copies per millilitre (right axis) in specimens collected in given epidemiological week from wastewater treatment facilities (horizontal axis). Green squares represent negative PCR results, red squares represent positive results and black squares represent invalid results. CoCT = City of Cape Town. WWTP = wastewater treatment plant.\*Population is the approximate size of the population served by the wastewater treatment plant. \*Lab is the name of the testing laboratory



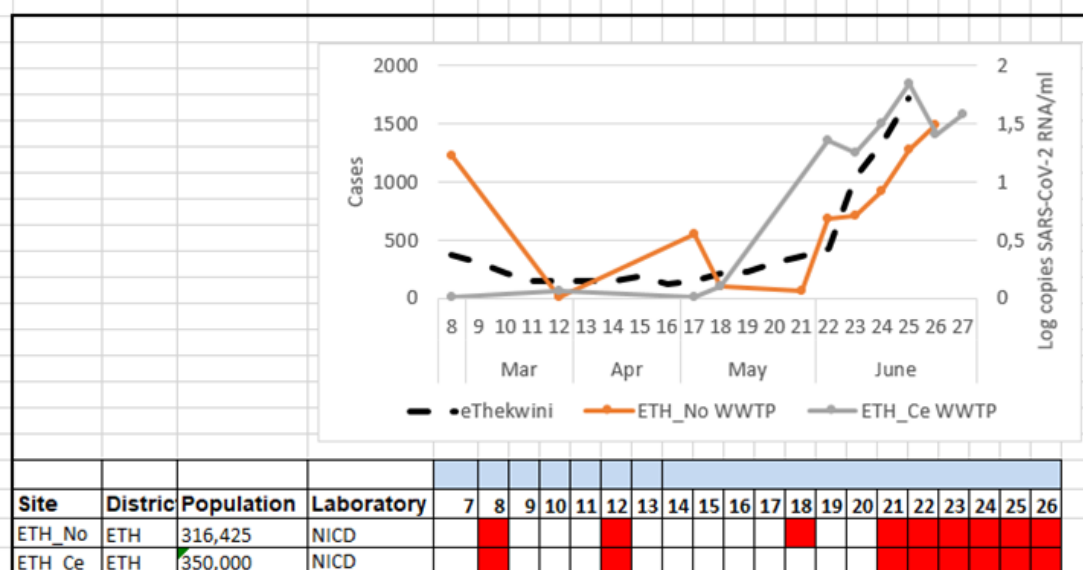
**Figure 3. Epidemiological curves of laboratory-confirmed SARS-CoV-2 cases, SARS-CoV-2 viral loads in wastewater and SARS-CoV-2 PCR detection results in wastewater for Nelson Mandela Metropolitan area (NMMB), and Buffalo City Metropolitan area (BCM) in the Eastern Cape Province, 2020-2021.**

Black dashed line represents laboratory-confirmed COVID-19 cases in BCM, red dashed line represents laboratory-confirmed COVID-19 cases in NMM (left axis) by epidemiological weeks 8-26, 2021 (horizontal axis). Coloured lines represent quantitative viral loads in copies per millilitre (right axis) in specimens collected in given epidemiological week from wastewater treatment facilities (horizontal axis). Green squares represent negative PCR results, red squares represent positive results and black squares represent invalid results. NMB = Nelson Mandela Bay. BCM = Buffalo City Metro. WWTP = wastewater treatment plant.\*Population is the approximate size of the population served by the wastewater treatment plant. \*Lab is the name of the testing laboratory



**Figure 4. Epidemiological curves of laboratory-confirmed SARS-CoV-2 cases, SARS-CoV-2 viral loads in wastewater and SARS-CoV-2 PCR detection results in wastewater for Mangaung Metropolitan area in Free State Province, 2020-2021.**

Black dashed line represents laboratory-confirmed COVID-19 cases (left axis) by epidemiological weeks 8-26, 2021 (horizontal axis). Coloured lines represent quantitative viral loads in copies per millilitre (right axis) in specimens collected in given epidemiological week from wastewater treatment facilities (horizontal axis). Green squares represent negative PCR results, red squares represent positive results and black squares represent invalid results. MAN = Mangaung Metro. WWTP = wastewater treatment plant. \*Population is the approximate size of the population served by the wastewater treatment plant. \*Lab is the name of the testing laboratory



**Figure 5. Epidemiological curves of laboratory-confirmed SARS-CoV-2 cases, SARS-CoV-2 viral loads in wastewater and SARS-CoV-2 PCR detection results in wastewater for eThekweni Metropolitan area in KwaZulu-Natal Province, 2020-2021.**

Black dashed line represents laboratory-confirmed COVID-19 cases (left axis) by epidemiological weeks 8-26, 2021 (horizontal axis). Coloured lines represent quantitative viral loads in copies per millilitre (right axis) in specimens collected in given epidemiological week from wastewater treatment facilities (horizontal axis). Green squares represent negative PCR results, red squares represent positive results and black squares represent invalid results. ETH = eThekweni Metro. WWTP = wastewater treatment plant. \*Population is the approximate size of the population served by the wastewater treatment plant. \*Lab is the name of the testing laboratory