# **SCIENCE BRIEF**

#### June 2021 - SCIENCE BRIEF NO. 5

NATIONAL INSTITUTE FOR

COMMUNICABLE DISEASES

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.

SAC DESS



# Monitoring for the third wave: detection and sequencing of SARS-CoV-2 at sentinel wastewater treatment sites (National SARS-CoV-2 Wastewater-based Epidemiology -Surveillance Initiative)\*

The results from detection of SARS-CoV-2 trends from wastewater at sentinel sites in South Africa is showing its capabilities as an early warning mechanism as it can predict the increase in infection rates in communities served in the sewer catchments served, way ahead of the reported clinical cases. The surveillance results also demonstrate some concordance of qualitative results with clinical epidemiologic curves, and successful detection of mutations compatible with known circulating SARS-CoV-2 variants.

#### Progress thus far shows that:

- In Gauteng, all samples tested weekly 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). Several 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) 7 sites across all Gauteng metros have tested positive, signifying an increase in population burden of disease across of disease across the province.
- In the Eastern Cape, 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.
- In the Free State, testing commenced in week 31 of 2020. SARS-CoV-2 RNA has been detected at both sites continuously since then.
- In Limpopo, testing commenced in week 7 of 2021 (February 2021) and all samples have tested negative, except for the most recent sample from a treatment works in Capricorn District Municipality, which tested positive in week 15 (mid-April 2021).
- In Mpumalanga, testing commenced in week 6 (February 2021) and all samples have tested negative except for the most recent sample from a works in City of Mbombela, which tested positive in week 15 (mid-April 2021), signifying an increase in local cases.
- All samples from KwaZulu-Natal Province (from week 36, 2020 to date) have tested positive.
- To date, all samples from North West Province (from week 4, 2021 until week 18) have tested negative.
- All samples from a single facility in Kimberley in the Northern Cape Province have tested negative since the onset of testing in week 7 of 2021 (late February 2021) until week 20 (mid May 2021).
- In the Western Cape, all samples reported to NICD from two facilities (commencing week 30, 2020) have tested positive, except for a single sample collected in week 6 (mid-February 2021), which tested negative.

The above progress and trends to date confirm and demonstrate the capabilities of WBES of Covid-19 and has shown that it is a cost-effective means of providing an early warning of the spread and increase in infections.

\* The National Wastewater Based Epidemiology Surveillance for SARS COV-2 Initiative (WBES) is a WRC and SALGA-funded programme, led by the NICD in partnership with SACCESS.

## Background

In response to the pandemic, the WRC, in partnership with SALGA, launched the Water Quality (wastewater and non-sewer) National Wastewater-based Epidemiology Surveillance (WBES) programme aimed to complement national initiatives in dealing with the COVID-19 pandemic. The intent of this programme is to facilitate the implementation of a nationwide initiative for the surveillance of COVID-19 in South African communities using a water and sanitation-focused approach. Through this process, wastewater-based epidemiology (WBE) can contribute to supporting the national COVID-19 responses by identification of areas of intense transmission, as well as serving as an early warning for the resurgence of SARS-CoV-2 and other waterborne disease outbreaks. The network is led and coordinated by the National Institute of Communicable Diseases (NICD) in partnership with SACCESS (South African Collaboration Covid 19 Environmental Surveillance System).

## Progress to date 2020/21

South Africa has experienced two 'waves' of COVID-19 and is currently experiencing a surge in cases heralding a 'third wave'. Gauteng, Free State and the Northern Cape Provinces are presently leading the resurgence, but the proportion testing positive is increasing across all provinces, heralding the emergence of a 'third wave. Many countries that are monitoring resurging cases or new waves are using wastewater-based epidemiology (WBE) to detect and monitor SARS-CoV-2 and support clinical surveillance and response activities.

Globally, detection and monitoring of SARS-CoV-2 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>.

In South Africa, treatment of wastewater is the responsibility of local government. Approximately 1 050 wastewater treatment works (WWTW) are administered by metropolitan councils and local government to treat both industrial and domestic waste. A number of independent initiatives to test for SARS-CoV-2 commenced in early 2020. In November 2020, a network of testing laboratories was established to support development of common methodology, identify and address challenges, and share best practice related to qualitative, quantitative and RNA sequencing of SARS-CoV-2 in wastewater. In this work we report results from detection and sequencing of SARS-CoV-2 from WWTW from sentinel sites in South Africa from 2020/21 and describe the establishment of the network.

# Methods

#### Outbreak context

Since the first case of SARS-CoV-2 was detected on 3 March 2020, over 1.5 million cases and nearly 60 000 deaths have been recorded in South Africa, as at 23 April 2021. Two distinct 'waves' of SARS-CoV-2 infection occurred, peaking in June and December 2020 respectively, with the majority of nine provinces experiencing a bimodal temporal distribution of cases with peaks in June 2020 and January 2021.

Establishment of the laboratory testing network The National Institute for Communicable Diseases (NICD) had been conducting testing of wastewater for polio as part of the National Department of Health's polio surveillance programme. Fortnightly, grab samples from high-volume WWTWs across five provinces (including eight metropolitan areas) were routinely collected by WWTW staff and submitted to the NICD for polio culture in the NICD Centre for Vaccines and Immunology since 2019. In 2020, the NICD, the South African Medical Research Council (SAMRC), the Council for Scientific and Industrial Research (CSIR), the University of Pretoria (UP), the Institute for Water and Wastewater Technology at the Durban University of Technology (DUT), the National Institute for Occupational Health, and several private laboratories (Waterlab, Greenhill and Lumegen) commenced with independent projects involving qualitative and quantitative detection of SARS-CoV-2 in local WWTWs. A proof of concept was published by the SAMRC in 2021<sup>8</sup>.

In August 2020, laboratories doing this work collectively established a forum for communication of challenges and sharing best practice. The group was named the South African Collaborative COVID-19 Environmental Surveillance System (SACCESS) network.

With the partnership and through funding of the Water Research Commission (WRC), the NICD and partners identified WWTWs 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>. Testing of WWTWs commenced between June 2020 and March 2021 as funding for partners became available. Results from testing funded through the WRC and NICD are reported here.

#### Laboratory 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 detection methodologies used by laboratory partners. All RT-PCR detection methodologies use in-built positive and negative controls to eliminate processing errors or contamination.

# Table 1. SARS-CoV-2 testing modalities 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.	<ul> <li>When a test result changes from</li> <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> <li>Changes in Ct values with time may indicate changing concentrations of virus in the influent (low Ct value equates to high viral load).</li> </ul>
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.
Sequencing of SARS-CoV-2	Whole genome characterisation of detected SARS-CoV-2 from influent wastewater samples using 'next generation sequencing' technology. This allows identification of differences (mutations) relative to a 'wild type' strain.	Results of sequencing reactions may be compared with a reference SARS-CoV-2 to identify mutations present in the fragments found in the wastewater sample. Mutations that are unique to certain lineages (e.g. B.1.351) indicate that this lineage is present. Longitudinal (repeat) testing at same facilities will give an indication of how lineages 'move' through populations.

\*Testing methodologies available on request from partners.

#### 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	(to be confirmed)	2019-nCoV CDC EUA Kit
NIOH	Skim milk flocculation	(to be confirmed)	Thermofisher (TaqPath kit)
Waterlab/UP	Skim milk flocculation	(to be confirmed)	Allplex™ 2019-nCoV Assay
SAMRC-TB	Ultra centrifugation	(to be confirmed)	2019-nCoV CDC EUA Kit
SAMRC-BRIP	(to be confirmed)	ZymoBIOMICS kit	2019-nCoV CDC EUA Kit
Lumegen	Tangential Flow Filtration	Qiagen extraction Kit	Thermofisher (TaqPath kit)

#### Interpretation of results

Table 1 provides interpretive principles to support the application of wastewater detection, quantification and sequencing of SARS-CoV-2 RNA to public health surveillance and response activities. Regarding detection PCR, a positive result is defined as at least one SARS-CoV-2 target gene has been amplified with or without the internal control and all PCR controls (positive and negative) are valid.

# Results

#### Testing facilities

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 eThekwini (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) and nine from each of Limpopo (two sites), Mpumalanga (three sites), North West (three sites), the Northern Cape (one site) and Western Cape (three sites) provinces were added, to bring the total to 38 sites. Two sites in Gauteng were duplicated, being tested by the NICD and another laboratory.

#### Provincial trends

Over 2020, and 2021 to date, 136 and 247 wastewater samples were submitted from 18 and 38 WWTW respectively and were tested by or reported to the NICD by partner laboratories. Of these 383 samples, SARS-CoV-2 was identified in 103 samples in 2020 (76%) and 133 in 2021 (54%), with each site submitting an average of 10.7 samples over the two years (range 2-21). Detection results are displayed in Figures 1-6 together with epidemiologic curves for clinical cases detected 2020/21 for the districts or metros where each WWTW is located.

In Gauteng province (Figure 1) 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 sites across all Gauteng which have been tested metros have tested positive, signifying an increase in population burden of disease across the province.

In the Eastern Cape province (Figure 2), 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. In Buffalo City Metro, all sites tested negative in week 20, but one site tested positive in week 22, coinciding with an increase in cases this week.

In Free State province (Figure 3) testing commenced in week 31 of 2020. SARS-CoV-2 RNA has been detected at both

sites continuously since then, in keeping with case load of clinical disease that has infrequently been lower than 200 cases per week. In Limpopo (Figure 4), testing commenced in week 7 of 2021 (February 2021) and all samples have tested negative until week 15 (mid-April) at a single plant, and week 19 in a second plant, in keeping with an increase in case load.

In Mpumalanga (Figure 5), testing commenced in week 6 (February 2021) and all samples have tested negative with the exception a single sample from a works in City of Mbombela which tested positive result in week 15 (mid-April 2021), and a sample each from two plants in Emalahleni in week 20, signifying an increase in local cases. All samples from KwaZulu-Natal Province (Figure 6) (from week 36, 2020 to date) have tested positive.

All samples from North West Province (Figure 7) (from week 4, 2021 until week 18) have tested negative until week 19 (end May 2021) when samples from plants in Matlosana and JB marks tested positive. All samples from a single facility in Kimberley in the Northern Cape Province have tested negative since the onset of testing in week 7 of 2021 (late February 2021, Figure 8) until week 20 (mid May 2021). In the Western Cape Province all samples reported to NICD from two facilities (commencing week 30, 2020) have tested positive, with the exception of a single sample collected in week 6 (mid-February 2021), which tested negative. Three additional sites in the City of Cape Town have commenced testing through WRC funding (week 19, 2021) and two of three sites have had positive results to date.

#### RNA sequencing

SARS-CoV-2 has been amplified and sequenced from 11 samples obtained from 10 WWTW from September 2020 until April 2021. Sample collection dates and consensus sequence lineages are displayed in Table 2. The majority of mutations that were identified (not shown) had been previously reported in clinical samples and were common to the lineage 20A or 20B (Nextclade, or B.1, B.1.177, B1.1.174 Pangolin). Signature mutations for strain 501Y.V2 (Nextclade) (B1.351 Pangolin) were present in a single sample from Olifantsfontein WWTW, in week five, 2021 (early February 2021).

Site	Province	District	Collection	Epi Week	Consensus sequences	
			date		Nextclade	Pangolin
Man-St	Free State	Mangaung	22/09/2020	39	20B	B.1.1.448
Man-Bl	Free State	Mangaung	20/10/2020	43	20A	B.1
NMB-Br	Eastern Cape	Nelson	2020/11/09	46	20H/501Y.V2	B.1.351
		Mandela Bay				
Ekh-Ol	Gauteng	Ekurhuleni	2021/02/02	5	20H/501Y.V2	B.1
Ekh-Ol	Gauteng	Ekurhuleni	2021/02/16	7	20B	B.1.1.174
CoCT-Za	Western Cape	City of Cape	2021/02/09	6	20A	B.1.177
		Town				

# Table 2. Results of SARS-CoV-2 sequencing tests performed on samples collected from wastewater treatment facilities across South Africa, September 2020-April 2021, along with Nextclade and Pangolin lineages of consensus sequences.

Site	Province	District	Collection date	Epi Week	Consensus sequences	
					Nextclade	Pangolin
CoT-Ro	Gauteng	City of Tshwane	2021/02/22	8	20H/501Y.V2	Not assigned
JBM-Go	Gauteng	City of Johannesburg	2021/02/22	8	20A	B.1
CoCt-Za	Western Cape	City of Cape Town	2021/03/09	10	20A	B.1
NMB-Br	Eastern Cape	Nelson Mandela Bay	2021/03/10	10	20A	B.1
NAM-Ca	Northern Cape	Namakwa	2021/04/07	14	20H/501Y.V2	B.1.351

## Discussion

In this description of results from detection of SARS-CoV-2 from wastewater at sentinel sites in South Africa, we demonstrate some concordance of qualitative results with clinical epidemiologic curves, and successful detection of mutations compatible with known circulating SARS-CoV-2 variants. We demonstrate potential of the network to provide descriptive epidemiological data pertaining to geographic variation, burden of disease and variant tracking.

Clinical epidemiology based on reporting of laboratoryconfirmed cases of SARS-CoV-2 has limitations. Firstly, laboratory-confirmed cases likely represent less than 10% of SARS-CoV-2 cases prevalent in a community at any given time point, as demonstrated by household transmission studies (personal communication, Cheryl Cohen, NICD), as a large proportion of cases are asymptomatic, and a lesser proportion of cases so mild as not to elicit health seeking. 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, laboratorydiagnosis is increasingly less representative of the burden of disease.

Whilst results from our network reflect detection of SARS-CoV-2 during peak waves, in many WWTW results were less frequently positive during the inter-wave periods. 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 unlikely false positive laboratory test results. Determination of the quantity of RNA in wastewater will add to the usefulness of these data for public health application.

Our preliminary sequencing results are concordant with known temporal distribution of SARS-CoV-2 strains across time and space in South Africa<sup>10</sup>. The variant 501Y.V2 was first detected in December 2020 and became the dominant South African circulating strain<sup>11</sup>. We detected mutations compatible with this strain in Gauteng province (Ekurhuleni and City of Tshwane) in February 2021 and in Northern Cape

Province (Calvinia) in April 2021. The absence of mutations compatible with this variant in our samples obtained prior to December 2020 supports the potential of this methodology to identify and monitor for emerging mutations.

Presently, our results are helpful to retrospectively confirm the presence of SARS-CoV-2 in wastewater and hence in metros of South Africa. However, our data challenge the decision-makers and 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 gualitative and guantitative detection patterns, determination of lineages and early detection of new variants<sup>7</sup>, 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 (https://www.samrc.ac.za/wbe/). 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>12</sup>. 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>13</sup>. 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>14</sup> and for monitoring of antimicrobial resistance<sup>15</sup>.

Limitations of wastewater-based epidemiology include the limitation of findings to populations corresponding with 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.

Our detection results from sentinel sites across South African urban areas indicating correspondence at some sites with

increased numbers of clinical cases 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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# **About SACCESS**

The South African Collaboration Covid 19 Environmental Surveillance System (SACCESS) was initially established by a collective of researchers in the Western Cape. Through a spontaneous and organic process, a network was established which rapidly expanded to involve a wide range of actors including university researchers, municipal sanitation and public health officials, provincial health departments, the NICD, private laboratories and research councils. Driven by a common goal of harnessing science to generate public health tools for better control of the epidemic, participants developed locally appropriate methods, shared experiences, refined tools and built common approaches.

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Figure 1. Epidemiological curves of laboratory-confirmed SARS-CoV-2 cases by district of Gauteng (graph) 2020-2021, with results of SARS-CoV-2 detection test results by epidemiologic week (table). EKH=Ekurhuleni, JBM=City of Johannesburg Metropolitan area, TSH=City of Tshwane Metropolitan Area, WR=West Rand.



Figure 2. Epidemiological curves of laboratory-confirmed SARS-CoV-2 cases for Nelson Mandela Metropolitan area (NMMB), and Buffalo City Metropolitan area (BCM) in the Eastern Cape Province (graph) 2020-2021, with results of SARS-CoV-2 detection test results by epidemiologic week for wastewater treatment facilities in these metros (table).



Figure 3. Epidemiological curves of laboratory-confirmed SARS-CoV-2 cases for Mangaung Metropolitan area in Free State province (graph) 2020-2021, with results of SARS-CoV-2 detection test results by epidemiologic week for wastewater treatment facilities in this metro(table).



Figure 4. Epidemiological curves of laboratory-confirmed SARS-CoV-2 cases for Capricorn Local Municipality in Limpopo province (graph) 2021, with results of SARS-CoV-2 detection test results by epidemiologic week for wastewater treatment facilities in this area (table).



Figure 5. Epidemiological curves of laboratory-confirmed SARS-CoV-2 cases for Mbombela and Emalahleni District Municipalities area (BCM) (graph) in Mpumalanga province 2021, with results of SARS-CoV-2 detection test results by epidemiologic week for wastewater treatment facilities in these districts(table).



Figure 6. Epidemiological curves of laboratory-confirmed SARS-CoV-2 cases for eThekwini Metro (graph) in KwaZulu-Natal Province 2020-2021, with results of SARS-CoV-2 detection test results by epidemiologic week for two wastewater treatment facilities in this metro (table).



Figure 7. Epidemiological curves of laboratory-confirmed SARS-CoV-2 cases for Matlosana, JB Marks and Bojanala Local Municipalities (graph) in North West Province 2021, with results of SARS-CoV-2 detection test results by epidemiologic week for wastewater treatment facilities in these areas (table).



Figure 8. Epidemiological curves of laboratory-confirmed SARS-CoV-2 cases for Sol Plaatjie Local Municipalities (graph) in Northern Cape Province 2021, with results of SARS-CoV-2 detection test results by epidemiologic week for wastewater treatment facility in this area (table).