Wastewater-based genomic epidemiology for SARS-CoV-2 surveillance in South Africa

15 March 2024

Sample collection dates up to 8 March 2024 (Epidemiological week 10)

C. Sankar*, P. Ntuli*, F. Els, N.S Msomi, N.L Ndlovu, V. Mabasa, E. Phalane, M.E Macheke, S. Gwala, N. Singh, T. Mangena, M Motloung, L. Monametsi, L.P Rabotapi, S. Maposa, M. Yousif[#], K. McCarthy[#]

*Joint first authors #Joint last authors

Centre for Vaccines and Immunology, National Institute for Communicable Diseases, A division of the National Health Laboratory Service



Division of the National Health Laboratory Service

Summary: SARS-CoV-2 transmission and genomics based on evaluation of wastewater at sentinel sites across RSA

Wastewater <u>levels</u> Epidemiological weeks 48 (2023) –10 (2024)

- From weeks 48-10 the cumulative SARS-CoV-2 levels measured at wastewater treatment works (WWTW) has remained around one log genome copies/ml of wastewater. This has followed on from the increases observed in weeks 31-41, when the cumulative SARS-CoV-2 levels in wastewater at sentinel sites in South Africa showed increases to levels above 2 log (100) genome copies/ml of wastewater, up from below one log copy/ml in epidemiological week 22 (first week in June 2023). From week 45, levels measured below 2 log genome copies/ml of wastewater.
- In weeks 48-52 increases and/or higher levels have been seen in Gauteng (Goudkoppies WWTW). In weeks 1-6 lower levels were observed nationally, with an increase seen in week 7 and a subsequent decrease in week 8. Levels remain low in Epi week 10.
- Correlation with syndromic surveillance for influenza-like illness (ILI) and severe acute respiratory infection findings (SARI) is required to determine the clinical and public health significance of ongoing transmission. <u>https://www.nicd.ac.za/diseases-a-z-index/disease-index-covid-19/surveillancereports/weekly-respiratory-pathogens-surveillance-report-week/</u>

Wastewater genomics

Epidemiological weeks 48 (2023) – 10 (2024)

- Omicron lineage BA.2.86, JB.2*, JN.1* and XBB sub-lineages were the dominant lineages circulating in wastewater samples between November 2023 and January 2024
- In clinical samples, **BA.2.86** was also the dominant lineage circulating throughout November 2023 and January 2024, followed by **XBB.1.5***, **XBB.1.19*** and **JN.1***.
- The **Omicron lineage BA.2.86 and JN.1*** is circulating in KwaZulu-Natal in eThekwini (in the catchments of Northern and Central WWTWs), and in Gauteng, in the City of Johannesburg (in catchments of Northern and Goudkoppies WWTWs), in Ekurhuleni (in the catchments of Olifantsfontein, Vlakplaats, and Hartebeesfontein WWTWs), and the City of Tshwane (in the catchment of Daspoort WWTP). It is also circulating in Eastern Cape in Buffalo City (in the catchment of Mdantsane WWTW), in Western Cape, in the City of Cape Town (in the catchment of Borches Quarry WWTW), and Free State, in Mangaung (catchments Bloemspruit and Sterkwater WWTWs).

Interpretation: Ongoing transmission of SARS-CoV-2 due to Omicron lineages including the new BA.2.86 and JN.1* lineages.

Wastewater-based Epidemiology for COVID-19 How is wastewater tested for SARS-CoV-2?

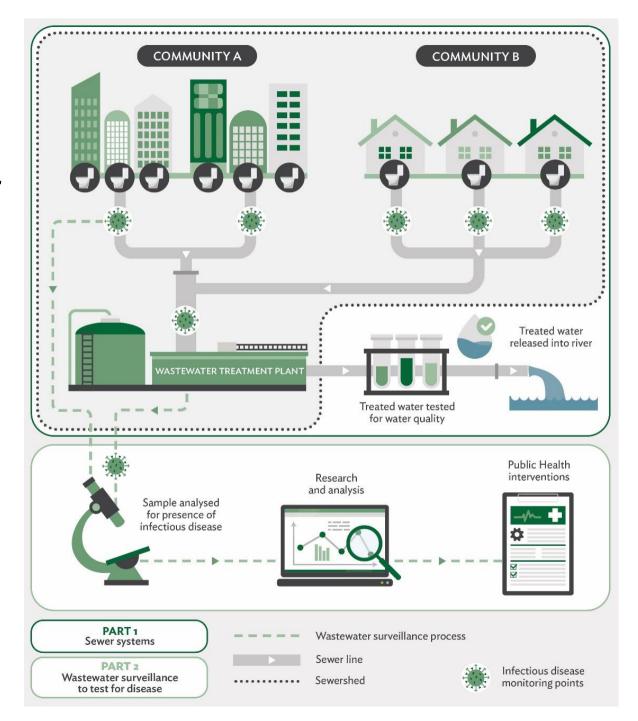
For a full description of this process, see the photo essay developed in collaboration with the Gauteng

City Region Observatory https://www.gcro.ac.za/outputs/photo-essay-sewersheds-what-can-wastewater-tell-us-about-community-health/

For a technical description and analysis of wastewater levels and results see

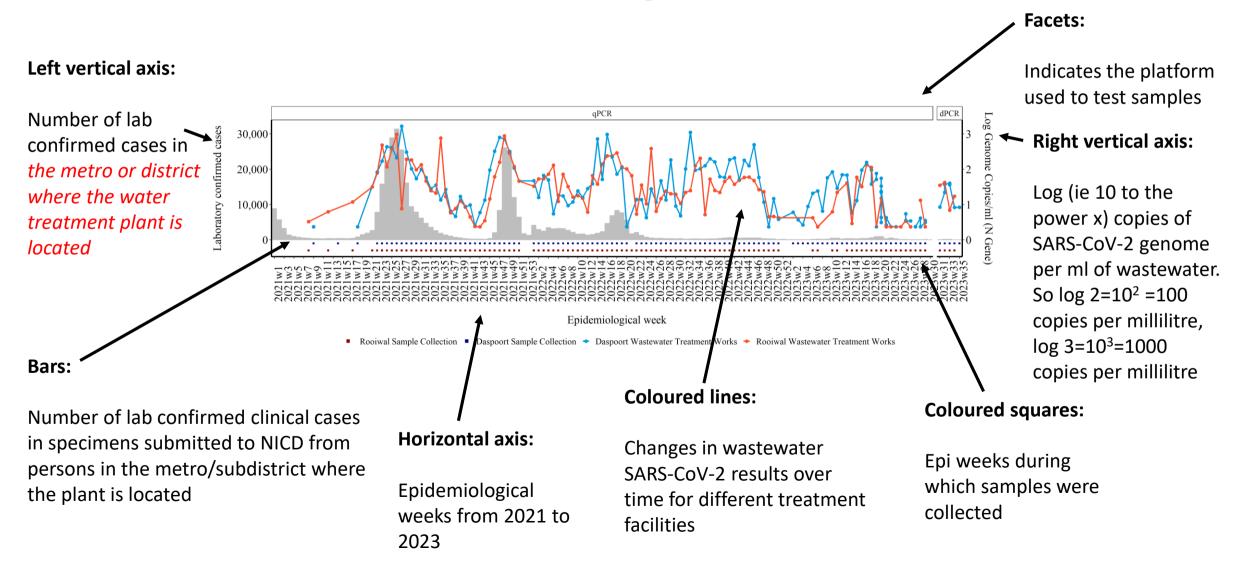
https://pubmed.ncbi.nlm.nih.gov/37506905/ https://www.medrxiv.org/content/10.1101/2022.12.15.22283506v1 (accepted by Nature Communications, publication pending)

SARS-CoV-2 is not transmitted by faeco-oral route. Wastewater with SARS-CoV-2 is not infectious



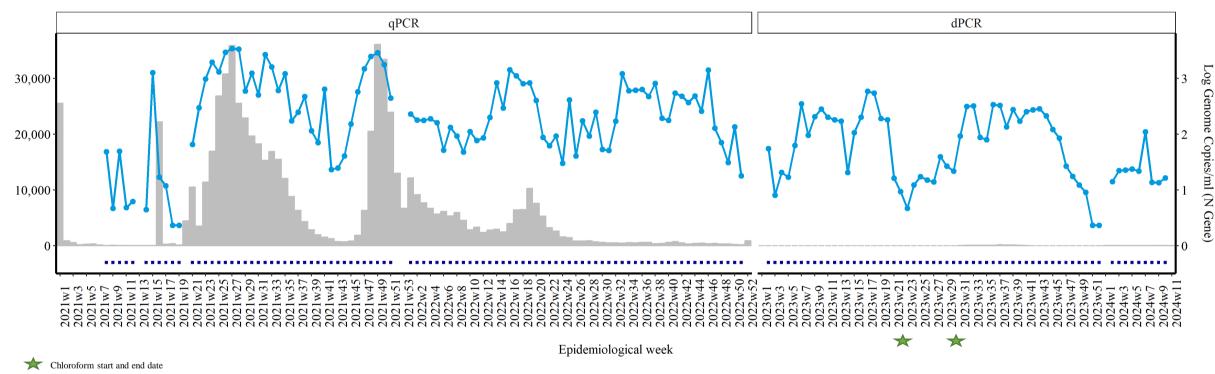
Wastewater-based Epidemiology for COVID-19

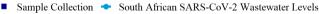
What does wastewater testing for SARS-CoV-2 mean?



South Africa at a glance:

Summed total of clinical and genome copies





Changes in levels of SARS-Cov-2 in log-transformed genome copies/ml (right vertical axis, line graph) in in-flowing untreated wastewater from plants tested by NICD, compared with <u>laboratory-confirmed cases from Tshwane</u>, <u>Johannesburg</u>, <u>Ekurhuleni</u>, <u>eThekwini</u>, <u>Mangaung</u>, <u>Nelson</u> <u>Mandela</u>, <u>Buffalo City</u>, <u>City of Cape Town</u>, <u>Vhembe</u>, <u>Ehlanzeni and Bonjanala Platinum (left vertical axis, grey bars)</u>, by epidemiological week, 2021-2023. Coloured dots below the horizontal axis signify if a sample was collected. SARS-CoV-2 wastewater level data points are only joined if samples on sequential weeks register positive values. The top bar indicates when the change from qPCR to dPCR occurred for SARS-CoV-2 testing.



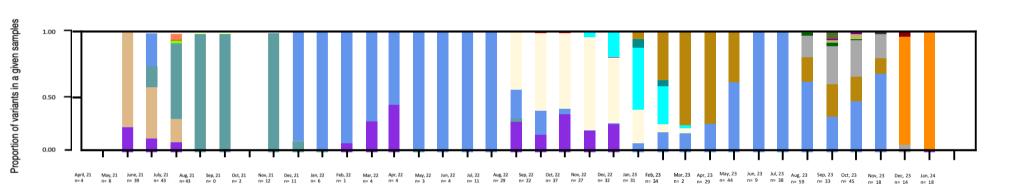
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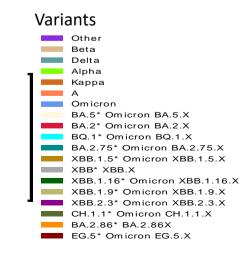


South Africa at a glance: Circulating variants as determined by Freyja deconvolution of sequence data

Inferred variants in wastewater samples from South African wastewater treatment plants by month, between April 2021- November 2023

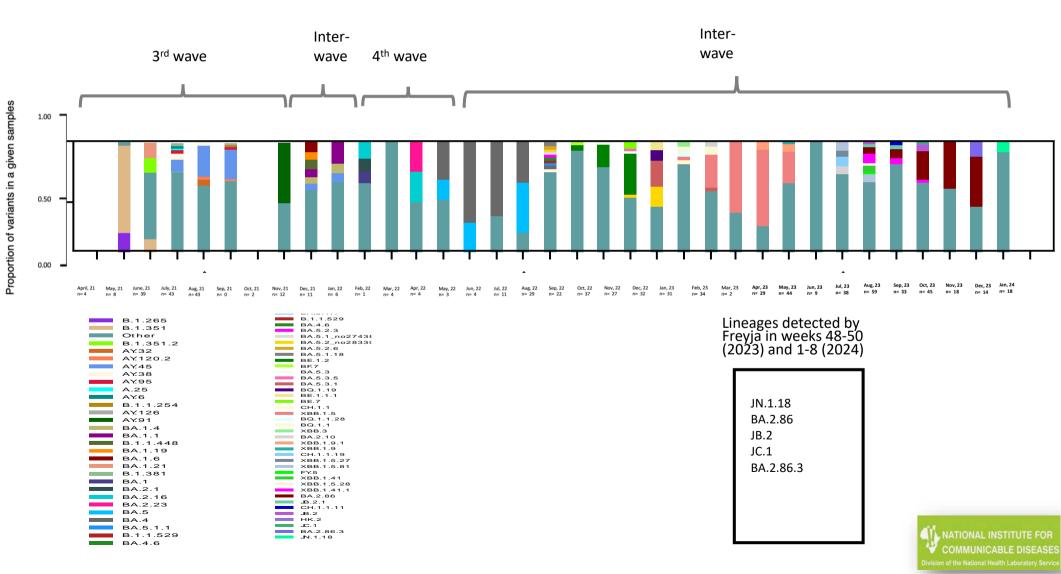
- SARS-CoV-2 variants in wastewater as determined by the 'Freyja' tool (Scripps Institute)
 - Allows determination of variants in each wastewater sample
- Results from sequencing data ending in epi week 8 (23 January 2024)
 - Omicron lineages XBB* and BA.2.86* were circulating from December to January, with BA.2.86* dominating in January.



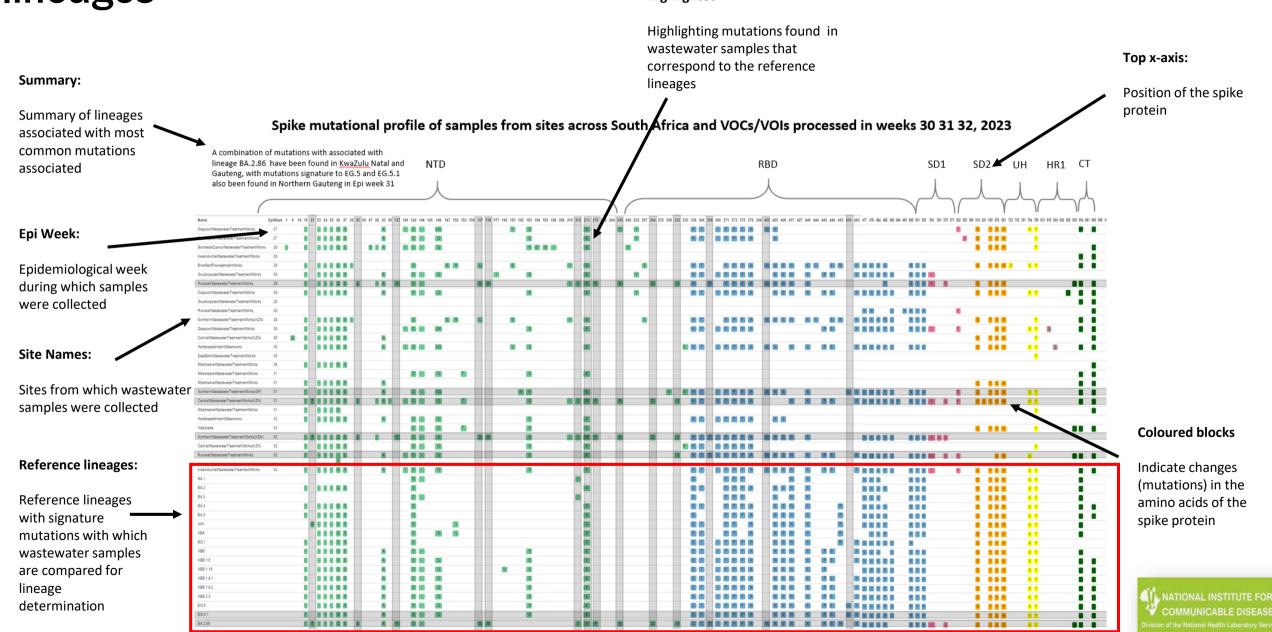


South Africa at a glance: Circulating <u>lineages</u> as determined by Freyja deconvolution of sequence data

- Results from sequencing data ending in epi week 46 (17 November 2023)
 - Omicron lineages XBB.1.41.1 and BA.2.86 were circulating from October to November with BA.2.86 dominance in November.
 - Omicron XBB sublineages were in circulation throughout October.
 - The predominant lineage circulating in clinical samples in the recent week is BA.2.86.



What mutations in the spike protein tell us about the circulating lineages



A. combination of mutations associated with lineage BA.2.86 and XBB sublineages have been found in sites across all South African provinces during Eni weeks 12-15

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Spike mutational profile of samples from sites across South Africa and VOCs/VOIs processed in weeks 42 - 46, 2023

A combination of mutations associated with lineage BA.2.86 and XBB sublineages have been found in sites across all South African provinces auring Epi weeks 42-46.

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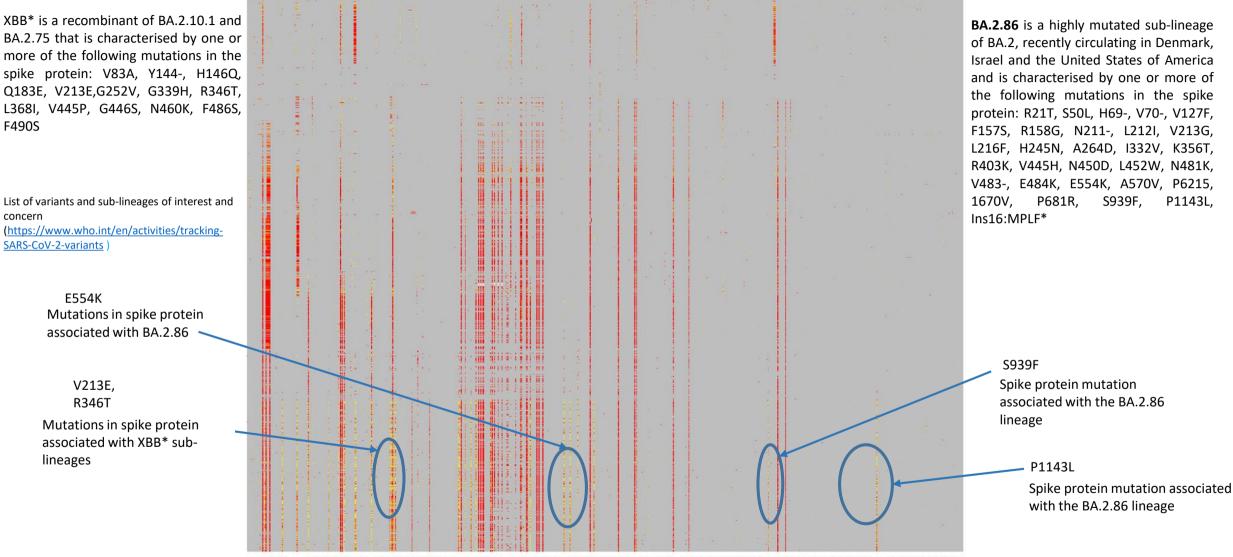
Spike mutational profile of samples from sites across South Africa and VOCs/VOIs processed in weeks 42 - 46, 2023

A combination of mutations associated with lineage BA.2.86 and XBB sublineages have been found in sites across all South African provinces during Epi weeks 42-46.

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Amino acid mutations and frequency – Spike protein

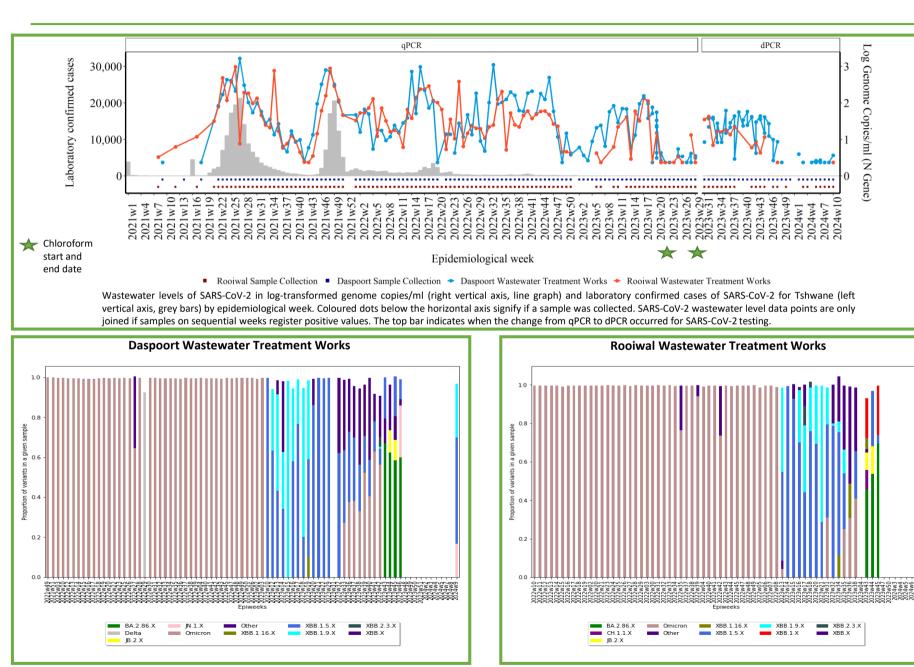


Heatmap showing patterns of emerging mutations in the spike region of SARS-CoV-2, collected from April, 2021 - November, 2023. Mutations appearing in yellow have a low read frequency, those appearing in orange have a medium read frequency and those appearing in red have a high read frequency. Mutations are included and updated weekly.

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Gauteng - Tshwane



SARS-CoV-2 levels and Genomic Results in Epi week 10:

- SARS-CoV-2 levels in Daspoort WWTW decreased to low in Epi week 4 and remain low in Epi week 10.
- SARS-CoV-2 levels in Rooiwal WWTW have decreased in Epi week 47. Levels remain low in Epi week 10.

* Sequencing data ending in Epi week 9 in Daspoort and 8 in Rooiwal.

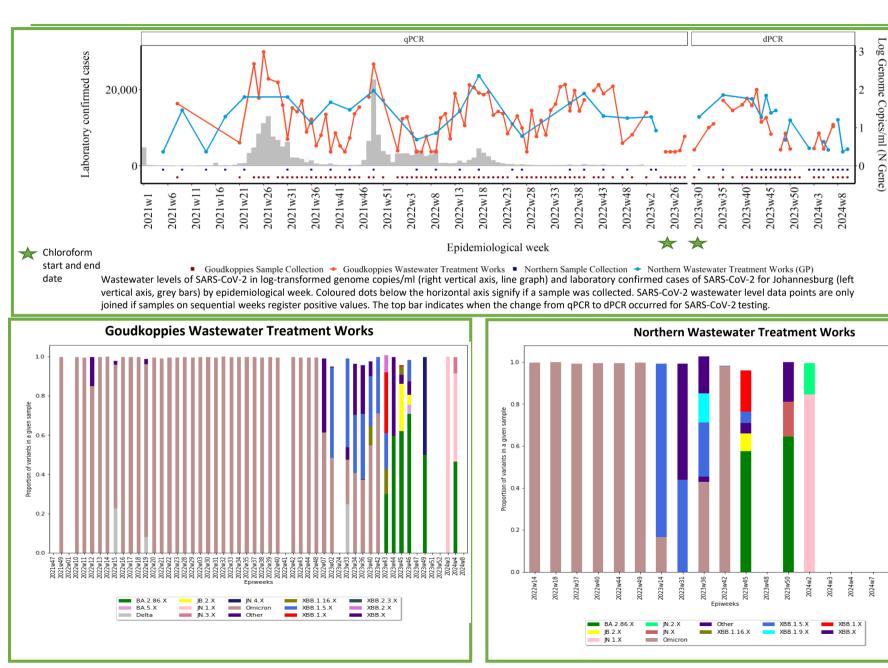
- Omicron lineages XBB.1.5.X, XBB.1.19.X and JN.1.X were circulating in Daspoort during Epi week 9.
- BA.2.86 was also the dominant lineage detected in Rooiwal during Epi week 45. Other lineages including XBB.1.41, XBB.1.5.28 and XBB.1.42.1 were also in circulation.

SNP Analysis:

 A combination of mutations (V127F, L212I, V213G, L216F, H245N, A264D, I332V, K356T) associated with lineage BA.2.86 were found in both Daspoort and Rooiwal.



Gauteng - Johannesburg



SARS-CoV-2 levels and Genomic Results in Epi week 10:

- As of Epi week 5, SARS-CoV-2 levels in Goudkoppies WWTW have decreased from levels in week 4. Levels increased from Epi week 5 to Epi week 7, but levels remain low. No new results for Epi week 10 are available.
- In Northern WWTW. SARS-CoV-2 levels increased slightly in week 5 from levels seen in week 2, and decreased in week 6. Levels increased in Epi week 8 to moderate. Levels decreased back to low in Epi week 9 and remain low in Epi week 10.

* Sequencing data ending in Epi week 8 in Goudkoppies and 9 in Northern Wastewater Treatment Works.

- JN.1.X lineage was dominating during epiweek 3 and 4. In Epiweek 4 BA.2.86 and JN.3.X were also circulating in Goudkoppies.
- In Epiweek 50 BA.2.86, JN and XBB lineages were circulating in Northern (GP). In Epiweek 2 JN.1.X and JN.2.X were circulating.

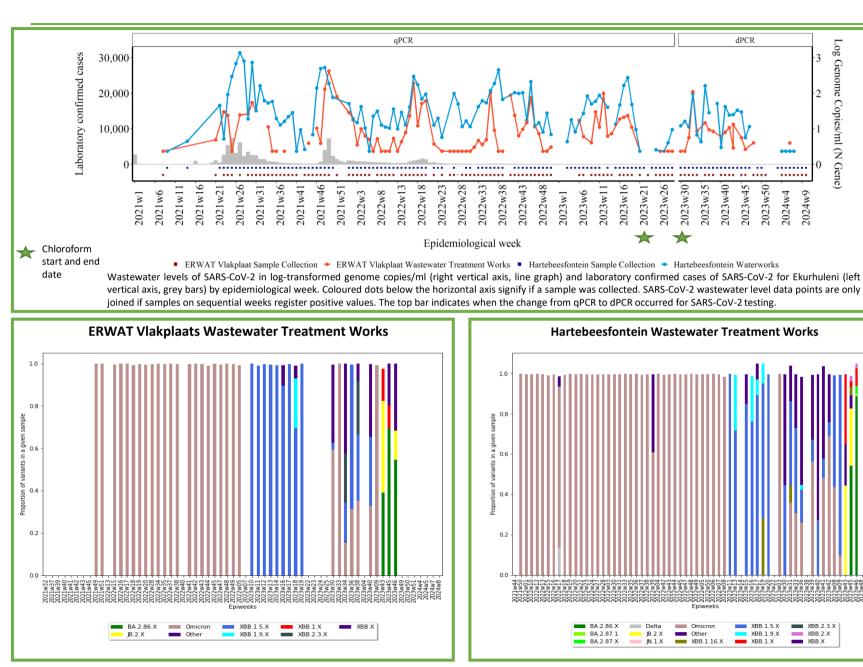
SNP Analysis:

Gene

- A combination of mutations (V127F, L212I, V213G, L216F, H245N, A264D, I332V, K356T) associated with lineage BA.2.86 were found in Goudkoppies.
- A combination of mutations (V127F, L212I, V213G, L216F, H245N, A264D, I332V, K356T) associated with lineage BA.2.86 were found in Northern Johannesburg.



Gauteng - Ekurhuleni



SARS-CoV-2 levels and Genomic Results in Epi week 10:

- The SARS-CoV-2 levels in Hartebeesfontein WWTW decreased in Epi weeks 4 and remain low in Epi week 7. No new results for Epi week 10 are available.
- As of Epi week 43, there was a slight increase in SARS-CoV-2 levels in Vlakplaats WWTW, after a decrease from Epi week 35. Levels remain low in Epi week 6. No new results for Epi week 10 are available.

* Sequencing data ending in Epi week 8 in Vlakplaats and 9 in Hartebeesfontein.

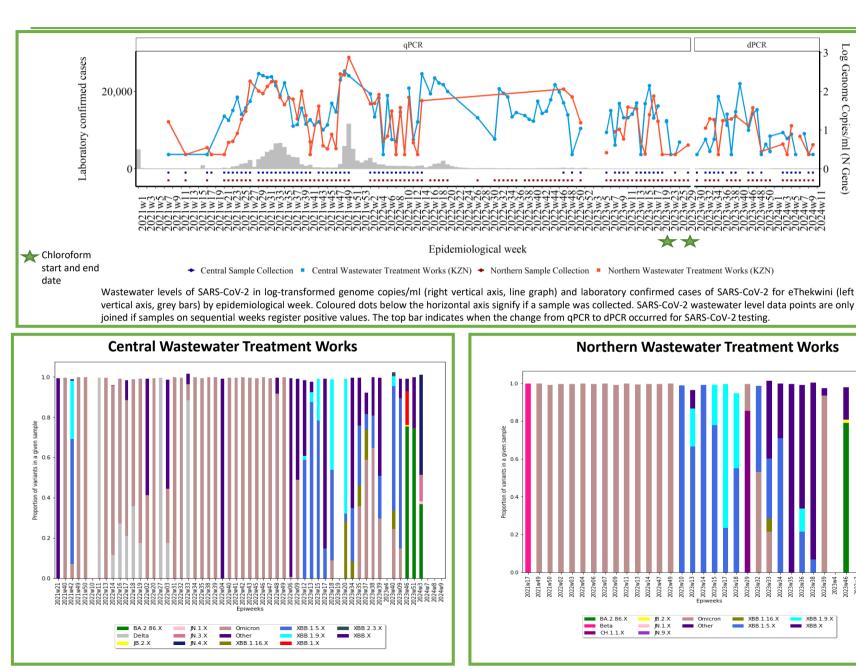
- Omicron lineages BA.2.86.X, JB.2.X and other Omicron lineages were circulating in Vlakplaats during Epi week 46.
- Lineages BA.2.86 and JN.1.X were predominantly circulating during Epi week 4 at the Hartebeesfontein water treatment plant.

SNP Analysis:

A combination of mutations (V127F, L212I, V213G, L216F, H245N, A264D, I332V, K356T) associated with lineage BA.2.86 were found in both Ekurhuleni treatment plants.



KwaZulu-Natal - eThekwini



SARS-CoV-2 levels and Genomic Results in Epi week 10:

- SARS-CoV-2 levels in Central WWTW in Epi week 6 decrease from Epi week 5, as levels remain low. Levels increase in Epi week 8 but remain low. Levels decrease further in Epi week 9 and remain low in Epi week 10.
- SARS-CoV-2 levels increased in Epi week 5 in Northern WWTW. There was a slight decrease in levels in Epi week 7, as levels remain low. Levels remain low in Epi week 10.

* Sequencing data ending in Epi week 9 in Central eThekwini and in eThekwini North.

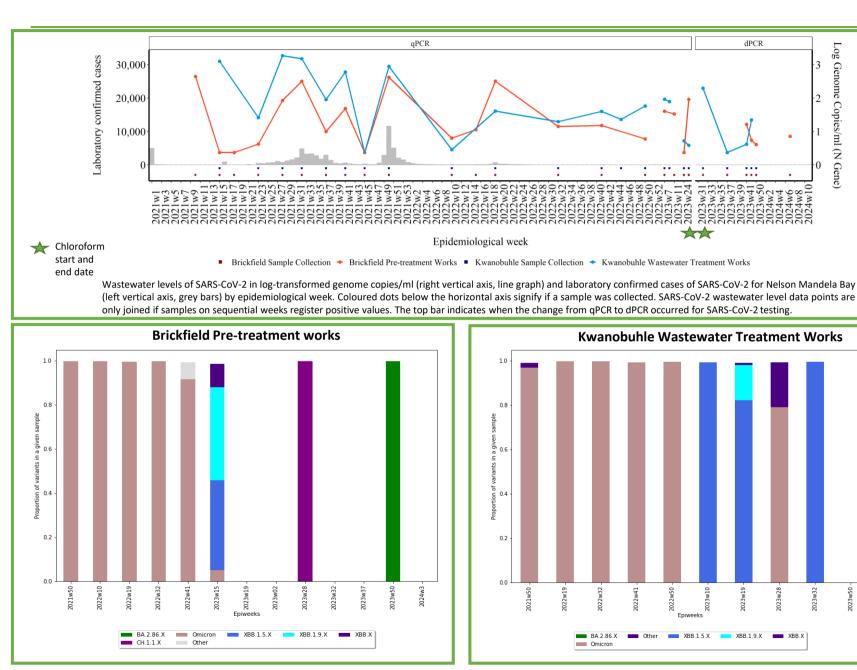
- During Epiweek 51 Lineage BA.2.86 was dominating and other Omicron lineages in circulating in eThekwini Central. Subsequently, JN.4.X, JN.3.X and JN.1.X were circulating in Epiweek 3.
- In eThekwini North, JN.1.X lineages were dominating. In circulation there were also lineages BA.2.86, JN.9.X and other Omicron lineages during Epi week 46.

SNP Analysis:

 A combination of mutations (V127F, L212I, V213G, L216F, H245N, A264D, I332V, K356T) associated with lineage BA.2.86 were found in both eThekwini wastewater treatment plants.



Eastern Cape – Nelson Mandela



SARS-CoV-2 levels and Genomic Results in Epi week 10:

- As of Epi week 46, SARS-CoV-2 levels are moderate after an increase was observed after Epi week 36 in the Kwanobuhle WWTW. No new results for Epi week 10 are available.
- SARS-CoV-2 levels decreased from moderate to low from Epi week 43 to Epi week 46 in Brickfield Pre-treatment works. There was a slight increase in level in Epi week 7, but levels remain low. No new results for Epi week 10 are available.

* Sequencing data ending in Epi week 3 in Brickfield and 50 in Kwanobuhle.

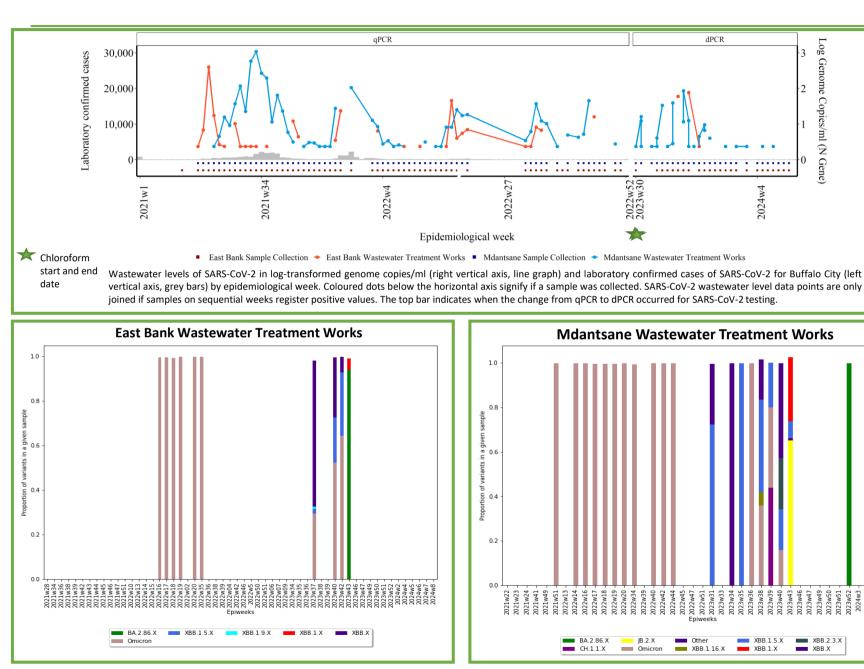
- BA.2.86 lineage was dominating during Epiweek 50 in Mdantsane
- Omicron lineages XBB.1.5.81, XBB.1.5.32 and XBB.1.5.27 were circulating in Kwanobuhle during Epi week 32.

SNP Analysis:

- SNP analysis could not be performed as the SARS-CoV-2 sequencing coverage in the Brickfield samples collected during Epi weeks 30-39 were too low for meaningful interpretation.
- A combination of mutations (V127F, L212I, V213G, L216F, H245N, A264D, I332V, K356T) associated with lineage BA.2.86 were found in the Kwanobuhle wastewater treatment plants.



Eastern Cape – Buffalo City



SARS-CoV-2 levels and Genomic Results in Epi week 10:

- In Epi week 46, SARS-CoV-2 levels in Mdantsane WWTW decreased and levels are low. Levels in Epi week 7 remain low. No new results for Epi week 10 are available.
- SARS-CoV-2 levels in East Bank WWTW in Epi week 44 remain low after decrease was observed from Epi week 38. No new results for Epi week 10 are available.

* Sequencing data ending in Epi week 8 in Eastbank and in Mdantsane.

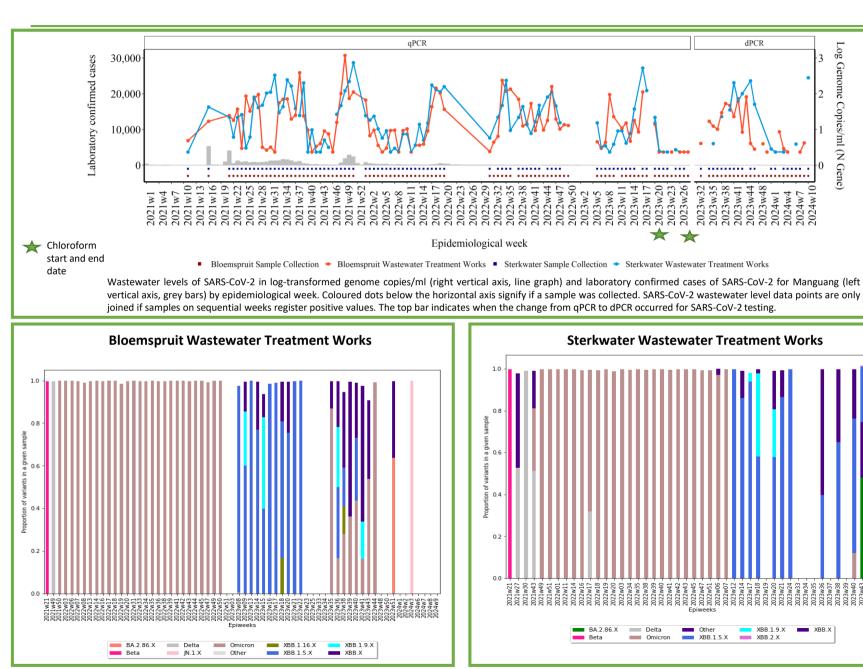
- Omicron lineages BA.2.86 and XBB.1.X JB.2were circulating in Eastbank during Epi week 43.
- Lineage BA.2.86 was dominating in Mdantsane during Epi week 52.

SNP Analysis:

 A combination of mutations (V127F, L212I, V213G, L216F, H245N, A264D, I332V, K356T) associated with lineage BA.2.86 were found in both Eastbank and Mdantsane.



Free State – Mangaung



SARS-CoV-2 levels and Genomic Results in Epi week 10:

- In Bloemspruit WWTW, a 2-fold increase in SARS-CoV-2 levels were seen in Epi week 44. In Epi week 4 levels decreased and remain low in Epi week 9. No new results for Epi week 10 are available.
- A decrease in SARS-CoV-2 levels was seen in Sterkwater WWTW in Epi week 52 and levels remain low in Epi week 4. There is a slight increase in levels in Epi week 7 but levels remain low. Levels in Epi week 10 increase to high (above 2 genome copies/ml).

* Sequencing data ending in Epi week 9 in Bloemspruit and in Epi week 8 in Sterkwater.

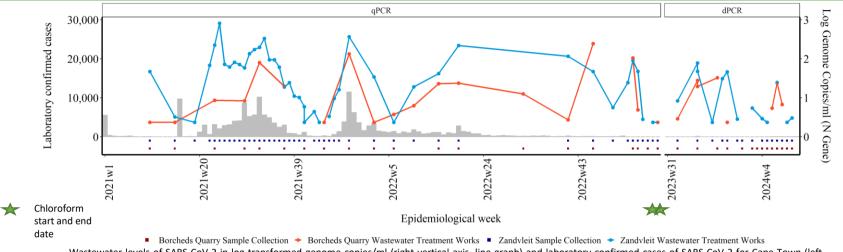
- BA.2.86.X (BA.2.86 and BA.2.86.1) were the dominant lineage circulating in Bloemspruit during Epi week 51.
- JN.1.X lineages were predominantly circulating in epiweek 3 in Bloemspruit
- BA.2.86.X and XBB lineages were circulating in Sterkwater in epiweek 1

SNP Analysis:

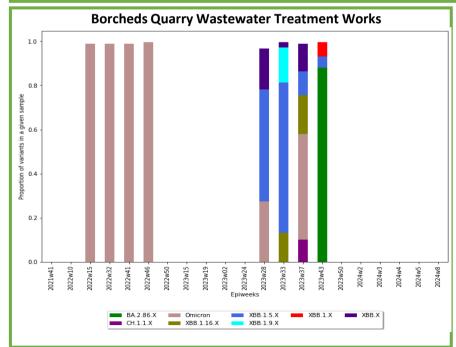
 A combination of mutations (V127F, L212I, V213G, L216F, H245N, A264D, I332V, K356T) associated with lineage BA.2.86 were found in both Bloemspruit and Sterkwater.

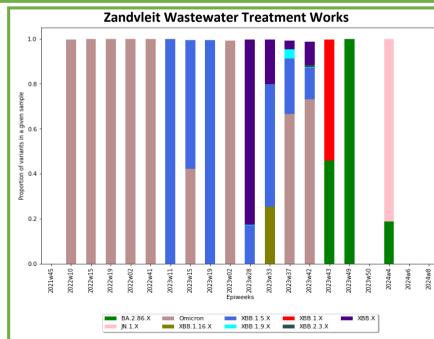
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Western Cape – City of Cape Town



Borcheds Quarry Sample Collection Sourceds Quarry Wastewater Treatment Works Zandvleit Sample Collection Zandvleit Wastewater Treatment Works Wastewater levels of SARS-CoV-2 in log-transformed genome copies/ml (right vertical axis, line graph) and laboratory confirmed cases of SARS-CoV-2 for Cape Town (left vertical axis, grey bars) by epidemiological week. Coloured dots below the horizontal axis signify if a sample was collected. SARS-CoV-2 wastewater level data points are only joined if samples on sequential weeks register positive values. The top bar indicates when the change from qPCR to dPCR occurred for SARS-CoV-2 testing.





SARS-CoV-2 levels and Genomic Results in Epi week 10:

- After a sharp increase in SARS-CoV-2 levels was seen in Epi week 37, a subsequent decrease in SARS-CoV-2 levels in Borcheds Quarry WWTW was seen. Levels remain low in Epi week 49. Levels are low in Epi week 6 and increase to moderate in Epi week 7. Levels decrease in Epi week 8 to low. No new results for Epi week 10 are available.
- In Epi week 4, SARS-CoV-2 levels were low at Zandvleit WWTW. Levels decrease slightly in Epi week 5. In Epi week 7 levels increase to moderate. Levels drop to low in Epi week 9 and remain low in Epi week 10.

* Sequencing data ending in Epi week 8 in Borcheds Quarry and in Zandvleit.

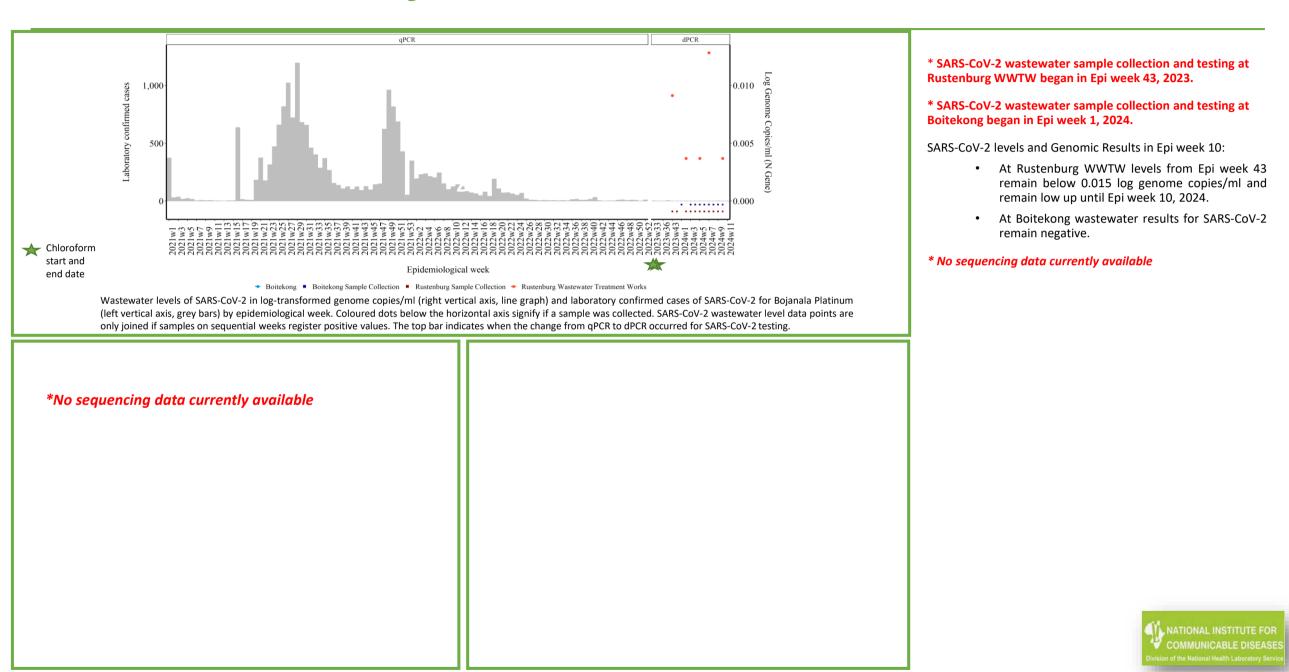
- During epiweek 43, Omicron lineages BA.2.86, XBB.1.9 lineages were circulating in Borcheds
- During Epi week 49, lineages BA.2.86.X WAS dominantly circulating in Zandvlet.
- In epiweek 4 BA.2.86 and JN.1.X lineages were circulating in Zandvlet, with JN.1.X lineages at highest proportion

SNP Analysis:

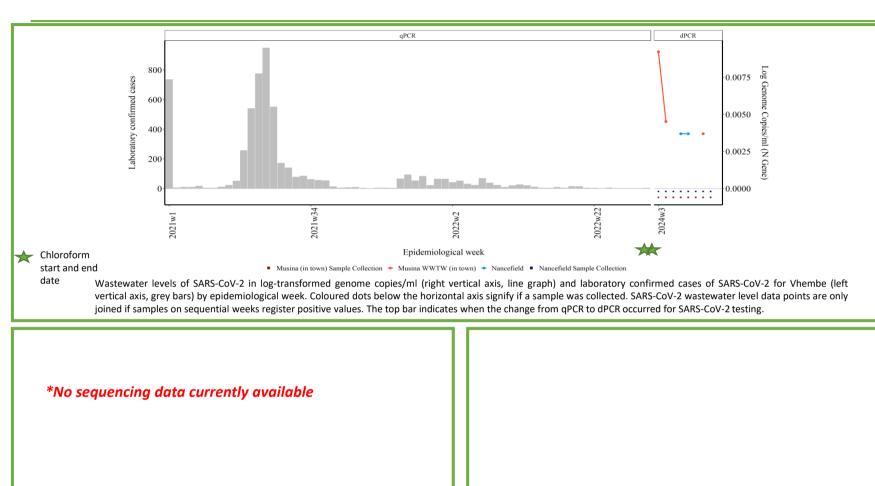
 A combination of mutations (V127F, L212I, V213G, L216F, H245N, A264D, I332V, K356T) associated with lineage BA.2.86 were found in both Borcheds Quarry and Zandevleit.

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North West – Bojanala Platinum



Limpopo – Vhembe



* SARS-CoV-2 wastewater sample collection and testing at Musina WWTW began in Epi week 3, 2024.

* SARS-CoV-2 wastewater sample collection and testing at Nancefield began in Epi week 3, 2024.

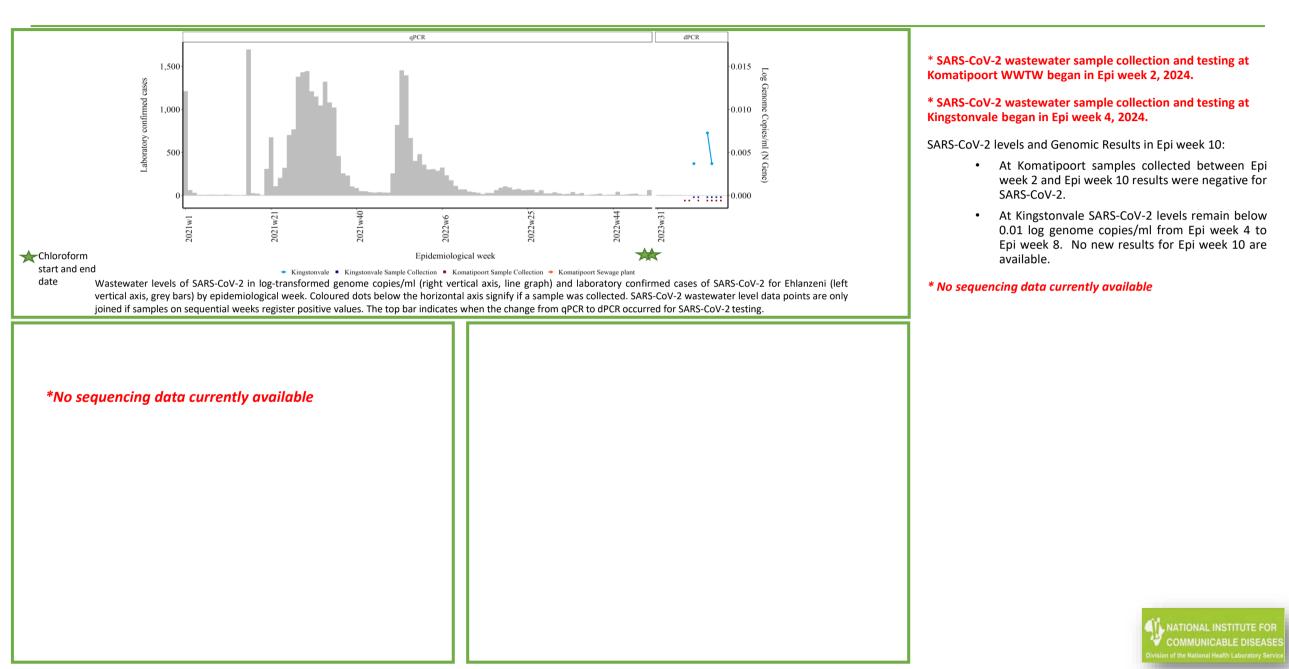
SARS-CoV-2 levels and Genomic Results in Epi week 10:

- At Musina WWTW levels from Epi week 3 remain below 0.01 log genome copies/ml and remain low up until Epi week 4, 2024. Levels remain low in Epi week 9. No new results for Epi week 10 are available.
- At Nancefield levels were low in Epi weeks 6 and 7, below 0.005 log genome copies/ml. No new results for Epi week 10 are available.

* No sequencing data currently available



Mpumalanga – Ehlanzeni



COLLABORATORS

Contemporary Scripps Research



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BILL& MELINDA GATES foundation





