



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

15 September 2023

Sample collection dates up to 8 September 2023
(Epidemiological week 36)

F. Els*, N.S Msomi*, N.L Ndlovu, V. Mabasa, E. Phalane, M.E Macheke, S. Gwala, T. Mangena, 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

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

Wastewater levels

Epidemiological weeks 33-36

- From weeks 33-36, the cumulative SARS-CoV-2 levels measured at wastewater treatment works (WWTW) **has remained around 2 log (100) genome copies/ml of wastewater**. This has followed on from the increases observed in weeks 31-33, 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).
- In weeks 33-36, increases and/or higher levels have been seen in Gauteng (Daspoort, Rooiwal, Goudkoppies, Northern and Hartebeesfontein WWTP) KwaZulu-Natal (Northern WWTP) and Free State (Sterkwater WWTP)
- 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. <https://www.nicd.ac.za/diseases-a-z-index/disease-index-covid-19/surveillance-reports/weekly-respiratory-pathogens-surveillance-report-week/>

Wastewater genomics

Epidemiological weeks 31-33

- No new genomics results were obtained for week 34
- From weeks 31-33, samples from 9 of 16 WWTP have genomic sequencing results, as SARS-CoV-2 sequencing coverage in the remaining samples are too low for meaningful interpretation
- **Omicron lineages XBB.1.5 followed by XBB.1.16 and XBB sub-lineages** remain the dominant lineages circulating in clinical samples in June, July and August 2023
- The **Omicron lineage BA.2.86** is evidently circulating in KwaZulu-Natal in eThekweni (in the catchments of Northern and Central WWTPs), and in Gauteng, in the City of Johannesburg (catchment of Northern WWTP) and the City of Tshwane (in Rooiwal WWTP catchment).
- The **Omicron lineages EG.5 and EG.5.1** are evidently present in City of Johannesburg (Northern WWTP) as the mutation F456L, which is signature to this lineage has been found in Northern Gauteng in epi week 31 (03 August, 2023).

Interpretation: Ongoing transmission of SARS-CoV-2 due to Omicron lineages including the new lineages BA.2.86 and EG.5, and EG.5.1 at higher levels than June 2023.

Wastewater-based Epidemiology for COVID-19

How is wastewater tested for SARS-CoV-2?

For a full description of this process, see the photoessay developed in collaboration with the Gauteng City Region Observatory

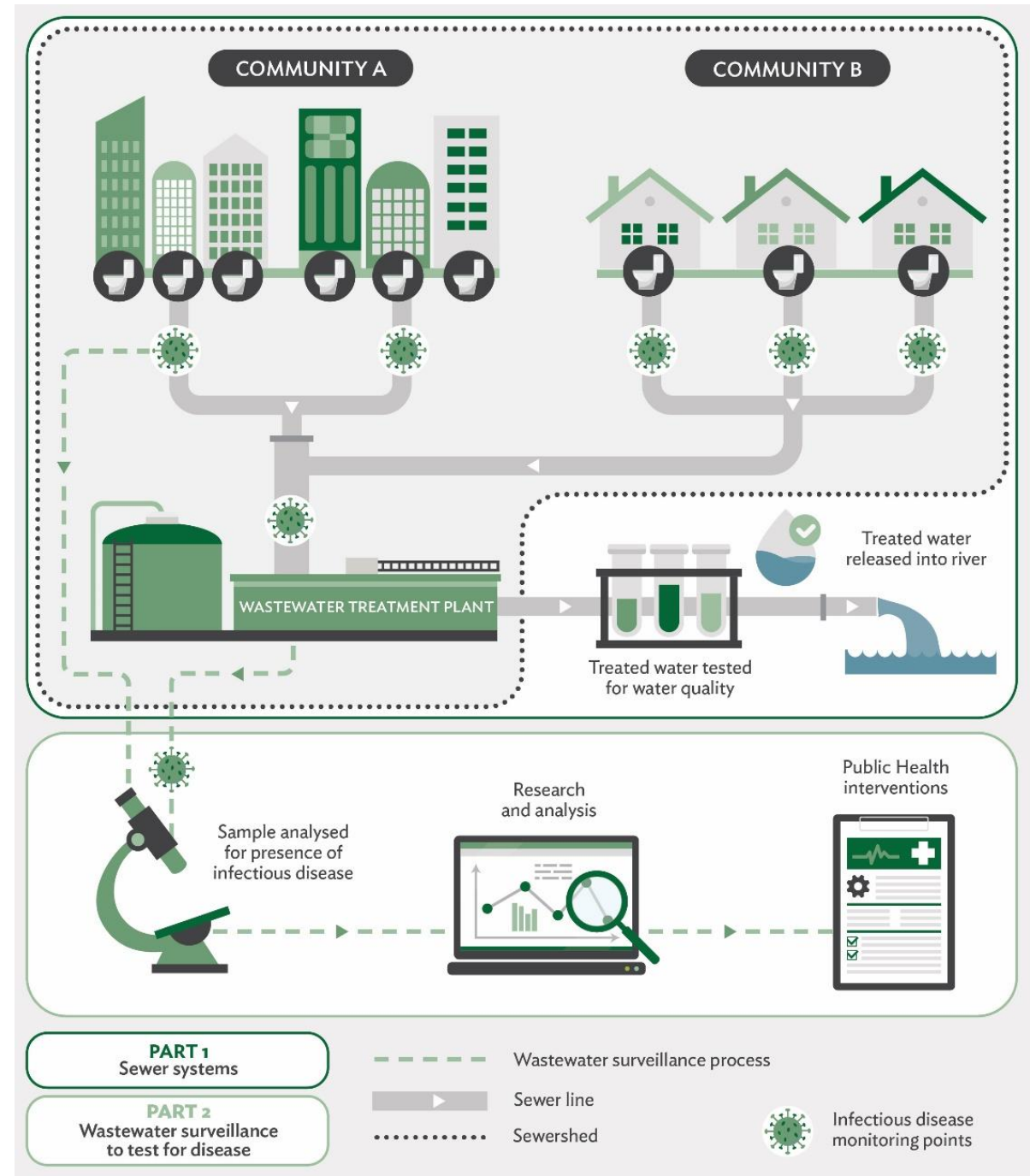
<https://www.gcro.ac.za/outputs/photo-essays/detail/photo-essay-sewersheds-what-can-wastewater-tell-us-about-community-health/>

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?

Left vertical axis:

Number of lab confirmed cases in *the metro or district where the water treatment plant is located*

Bars:

Number of lab confirmed clinical cases in specimens submitted to NICD from persons in the metro/subdistrict where the plant is located

Horizontal axis:

Epidemiological weeks from 2021 to 2023

Coloured lines:

Changes in wastewater SARS-CoV-2 results over time for different treatment facilities

Coloured squares:

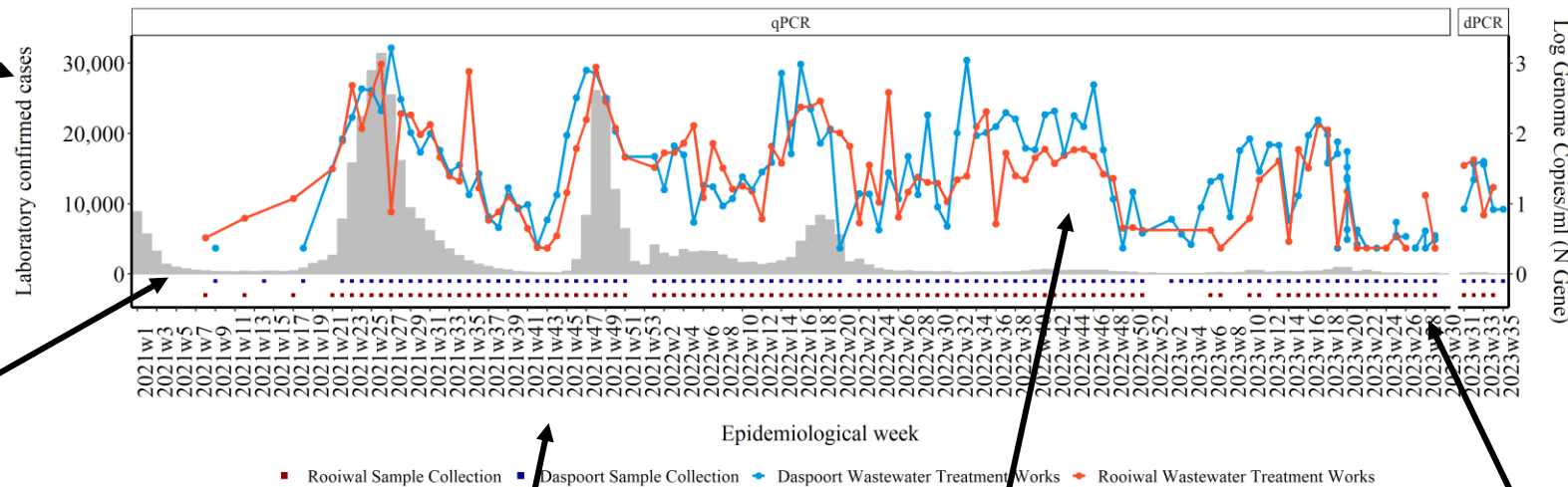
Epi weeks during which samples were collected

Facets:

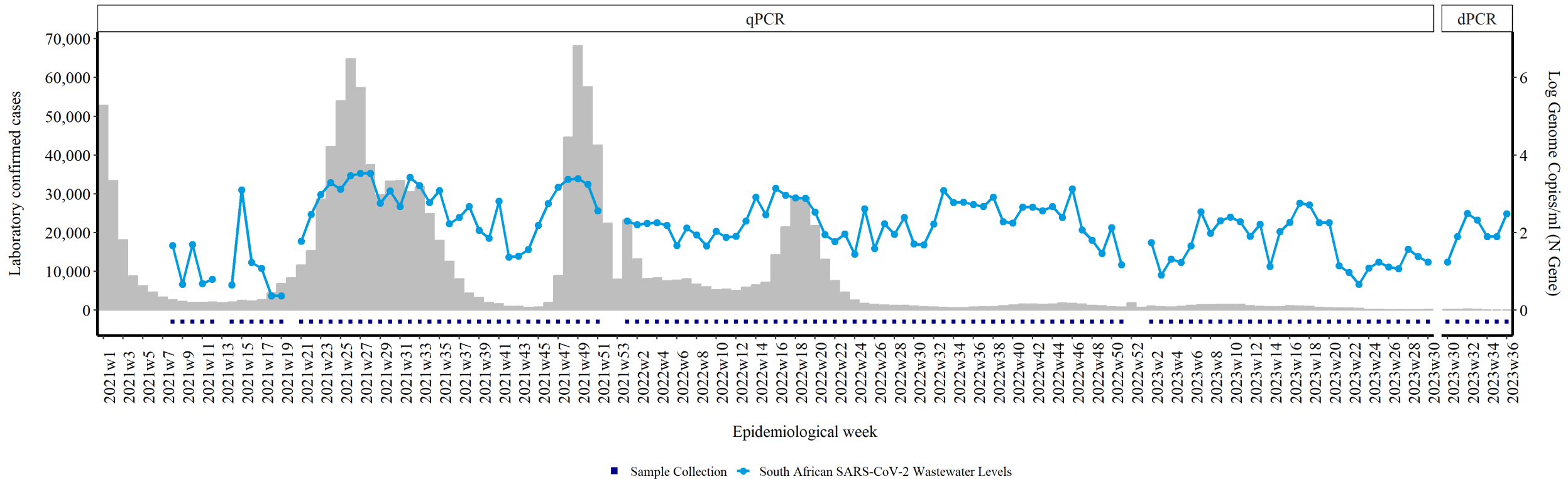
Indicates the platform used to test samples

Right vertical axis:

Log (ie 10 to the power x) copies of SARS-CoV-2 genome per ml of wastewater. So $\log 2=10^2=100$ copies per millilitre, $\log 3=10^3=1000$ copies per millilitre



South Africa at a glance: Summed total of clinical and genome copies

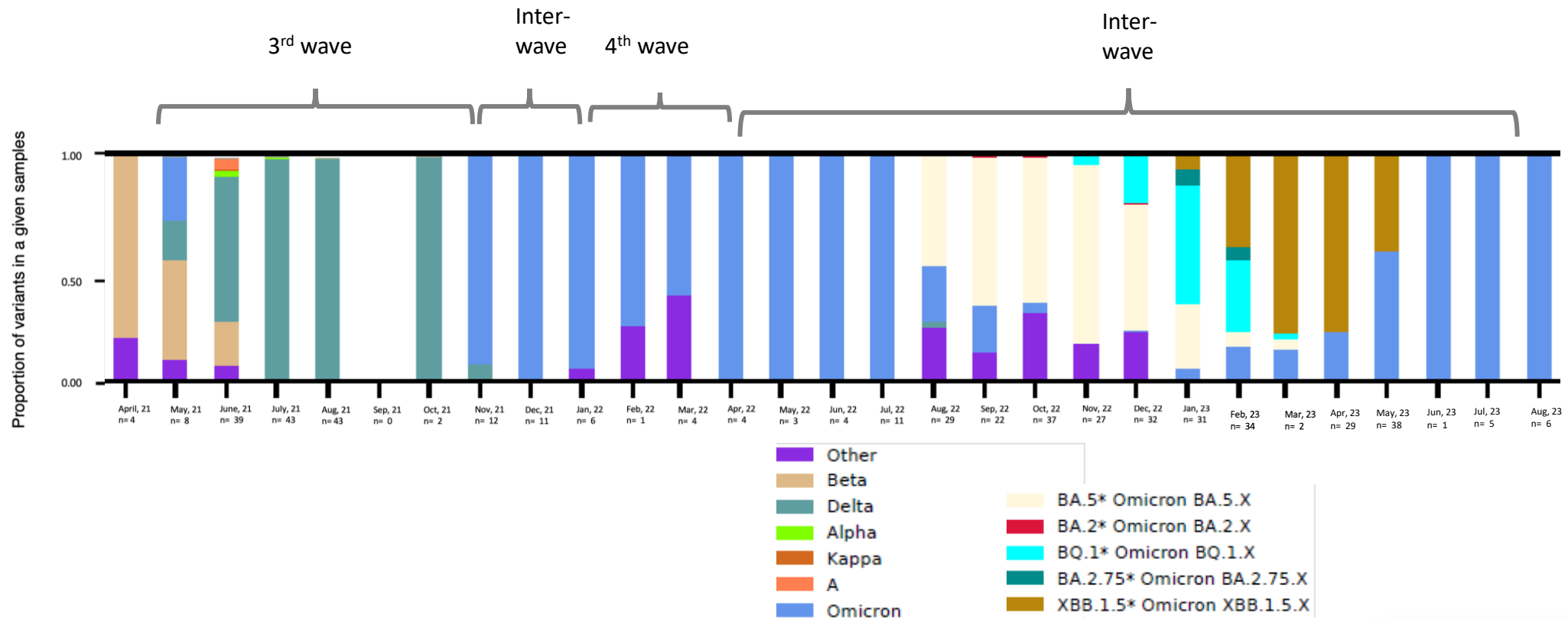


Changes in levels of SARS-Cov-2 (line graph) in in-flowing untreated wastewater from plants tested by NICD, compared with laboratory-confirmed cases from [Tshwane, Johannesburg, Ekurhuleni, eThekweni, Mangaung, Nelson Mandela, Buffalo City, and City of Cape Town](#) (grey bars), by epidemiological week, 2021-2023.

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

- 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 32 (10 August 2023)
 - Omicron lineages XBB.1.5 and Omicron were circulating from June to August, with Omicron dominance in August.

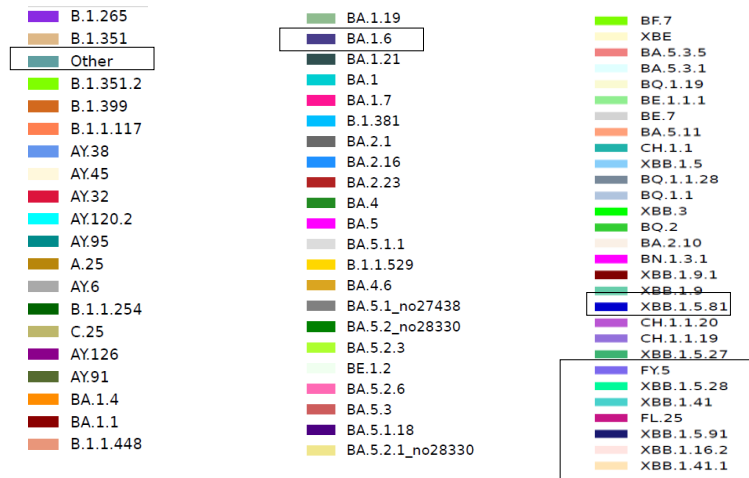
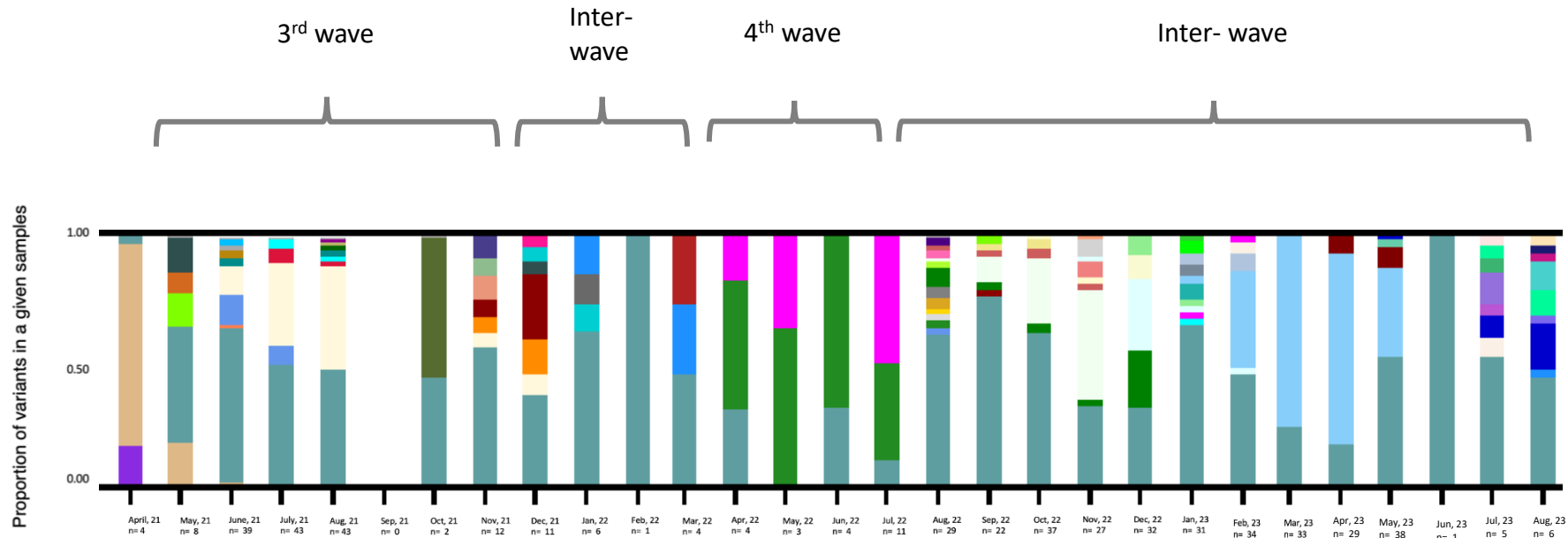
Inferred variants in wastewater samples from South African wastewater treatment plants by month, between April 2021- August 2023



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

- Results from sequencing data ending in epi week 32 (10 August 2023)

- Omicron lineages XBB.1.5 and Omicron were circulating from June to August, with Omicron dominance in August.
- Omicron XBB sub-lineages were in circulation throughout August.
- The predominant lineages circulating in clinical samples in the recent week are XBB.1.5 followed by XBB.1.16 and XBB sub-lineages.



Lineages detected by Freyja in week 32:

- XBB.1.41.1
- XBB.1.5.91
- FL.25
- XBB.1.41
- XBB.1.5.28
- FY.5
- XBB.1.5.81
- BA.2.16

BA.2.86 is likely contained in 'other' as numbers are small

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

Summary:

Summary of lineages associated with most common mutations associated

Epi Week:

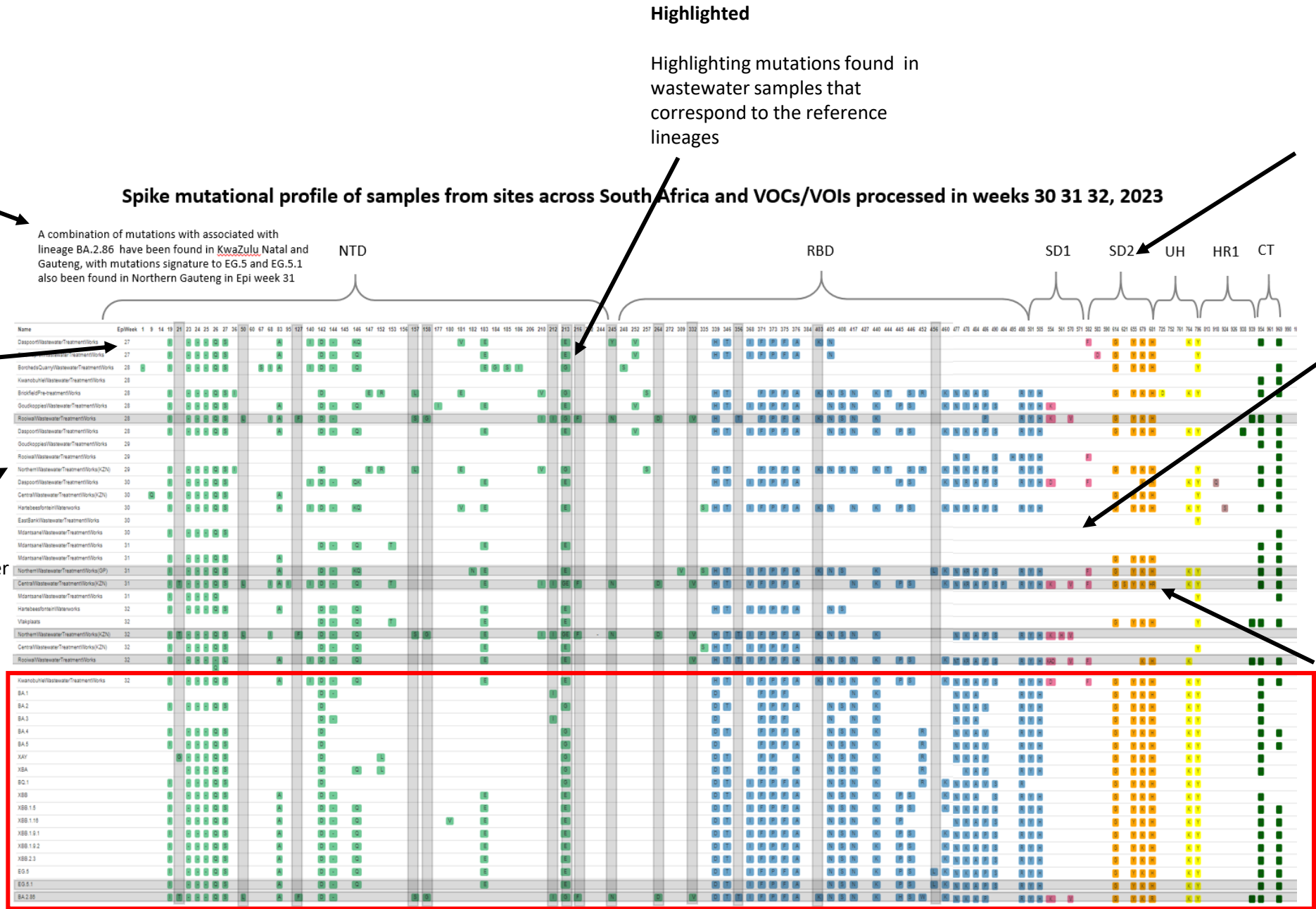
Epidemiological week during which samples were collected

Site Names:

Sites from which wastewater samples were collected

Reference lineages:

Reference lineages with signature mutations with which wastewater samples are compared for lineage determination



Amino acid mutations and frequency – Spike protein

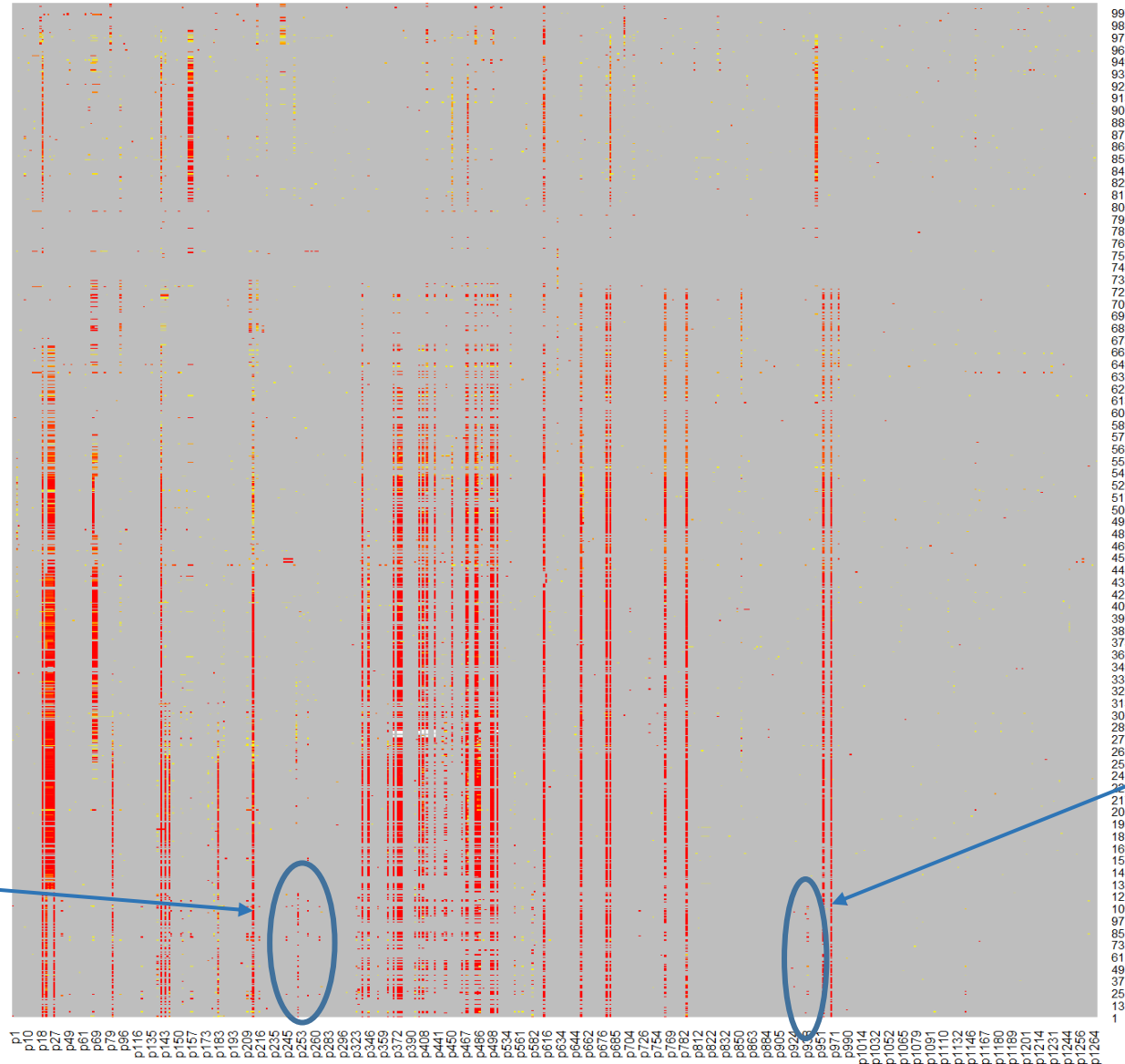
XBB* is a recombinant of BA.2.10.1 and BA.2.75 that is characterised by one or more of the following mutations in the spike protein: V83A, Y144-, H146Q, Q183E, V213E, G252V, G339H, R346T, L368I, V445P, G446S, N460K, F486S, F490S

List of variants and sub-lineages of interest and concern
(<https://www.who.int/en/activities/tracking-SARS-CoV-2-variants>)

BA.2.86 is a highly mutated sub-lineage of BA.2, recently circulating in Denmark, Israel and the United States of America and is characterised by one or more of the following mutations in the spike protein: R21T, S50L, H69-, V70-, V127F, F157S, R158G, N211-, L212I, V213G, L216F, H245N, A264D, I332V, K356T, R403K, V445H, N450D, L452W, N481K, V483-, E484K, E554K, A570V, P621S, 1670V, P681R, S939F, P1143L, Ins16:MPLF*

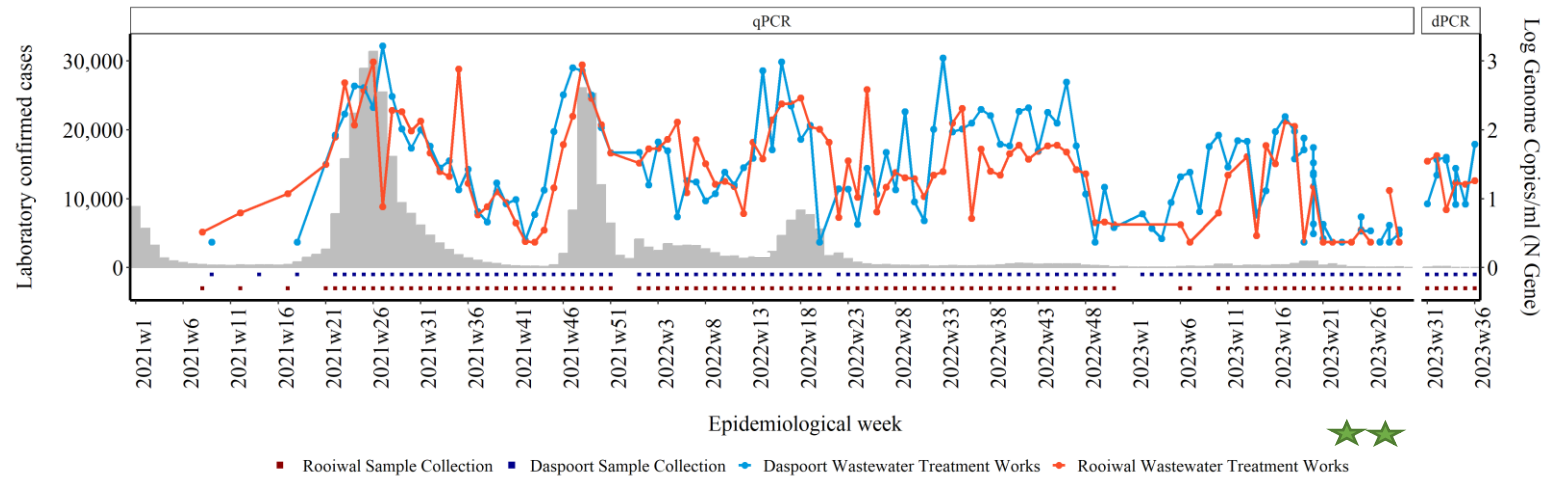
G252V
Mutation in spike protein associated with XBB* sub-lineages

S939F
Spike protein mutation associated with the BA.2.86 lineage



Heatmap showing patterns of emerging mutations in the spike region of SARS-CoV-2, collected from April, 2021 - August, 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.

Gauteng - Tshwane



★ Chloroform start and end date

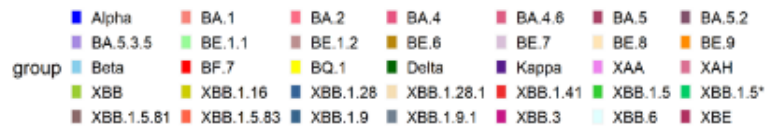
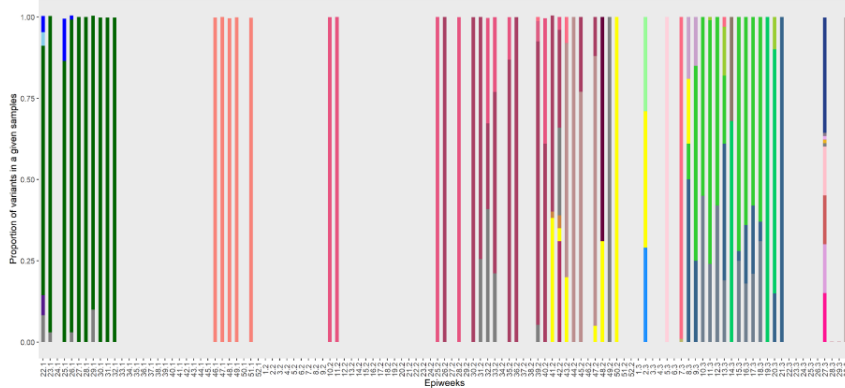
SARS-CoV-2 levels and Genomic Results in Epi week 36:

- The SARS-CoV-2 levels in Daspoort WWTW increased 2-fold from Epi week 33 to Epi week 36 to moderate levels
- Levels in Rooiwal WWTW in Epi week 36 increased slightly

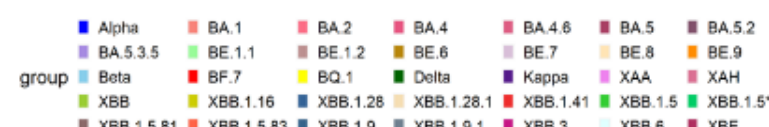
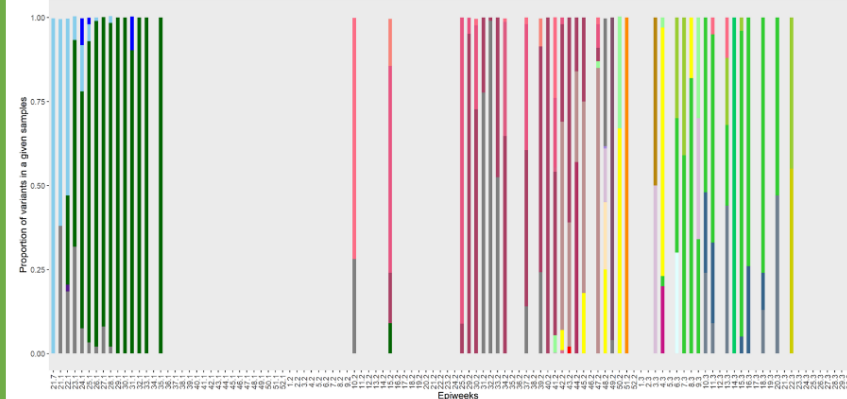
Sequencing data ending in Epi week 32:

- Omicron lineages FD.2, XBB.1.5.49, XBB.1.5.17, XBB.1.5.15, XBB.1.5.25, FY.3, FY.1, XBB.1.22.1, XBB.1.22, FY.5 were circulating in Daspoort during Epi week 27. With XBB.1.5.81 dominating in Epi week 30.
- Omicron lineages XBB.1.5.81, XBB.1.28, XBB.1.28.1, XBB.1.4, XBB.1.5.83 were circulating in Rooiwal during epi week 32.

Daspoort



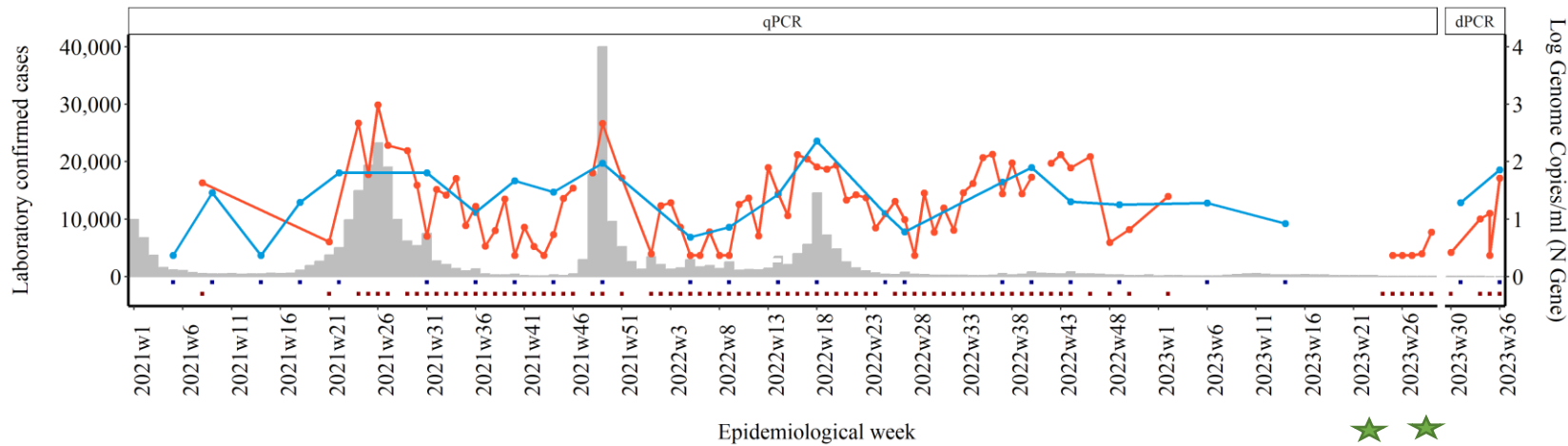
Rooiwal



SNP Analysis:

- In Daspoort, mutations associated with XBB.1.5 (V83A, Q183E, R346T, L368I, F486P, F490S) were detected during Epi week 30.
- A combination of mutations (V127F, F157S, R158G, L212I, V213G, L216F, H245N, A264D, I332V, K356T, R403K, V483-, E554K, A570V, P621S, 1670V, S939F) associated with lineage BA.2.86 were found in Rooiwal, Gauteng

Gauteng - Johannesburg



SARS-CoV-2 levels and Genomic Results in Epi week 36:

- The SARS-CoV-2 levels in Goudkoppies WWTW increased from Epi week 30, and levels are intermediate from Epi week 34, and remain low
- The SARS-CoV-2 levels increased moderately from Epi week 30. SARS-CoV-2 levels are moderate in Northern WWTW in Epi week 36

Sequencing data ending in Epi week 32:

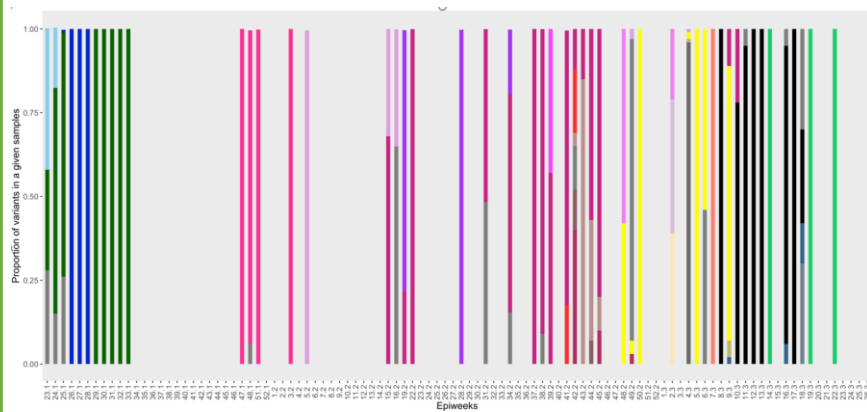
- SARS-CoV-2 sequencing coverage in the Goudkoppies samples collected during Epi weeks 30-32 are too low for meaningful interpretation
- Omicron lineages XBB.1.5.28, XBB.1.41.1, XBB.2.4, XBB.1.42.1 and XBB.2.9 were circulating during Epi week 31 in Northern Gauteng.

SNP Analysis:

- SNP analysis could not be performed as the SARS-CoV-2 sequencing coverage in the Goudkoppies samples collected during Epi weeks 30-32 are too low for meaningful interpretation
- In Northern Gauteng, mutations associated with EG.5 and EG.5.1 (F456L) and XBB.1.5 (V83A, Q183E, R346T, L368I, F486P, F490S) were detected during Epi week 31.

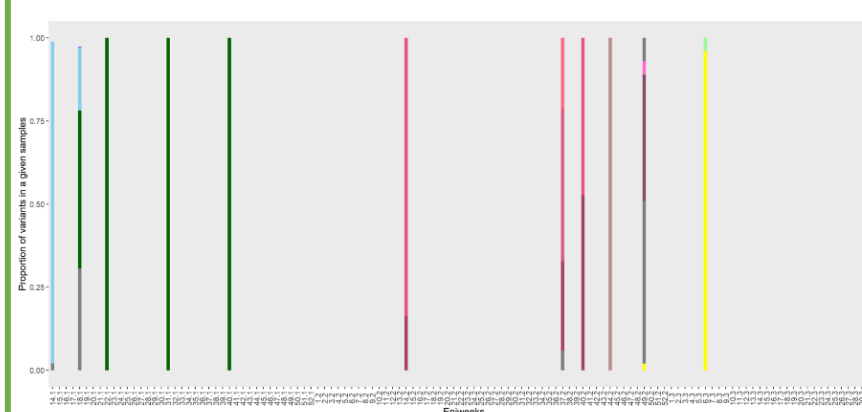
★ Chloroform start and end date ■ Goudkoppies Sample Collection ◆ Goudkoppies Wastewater Treatment Works ■ Northern Sample Collection ◆ Northern Wastewater Treatment Works (GP)

Goudkoppies



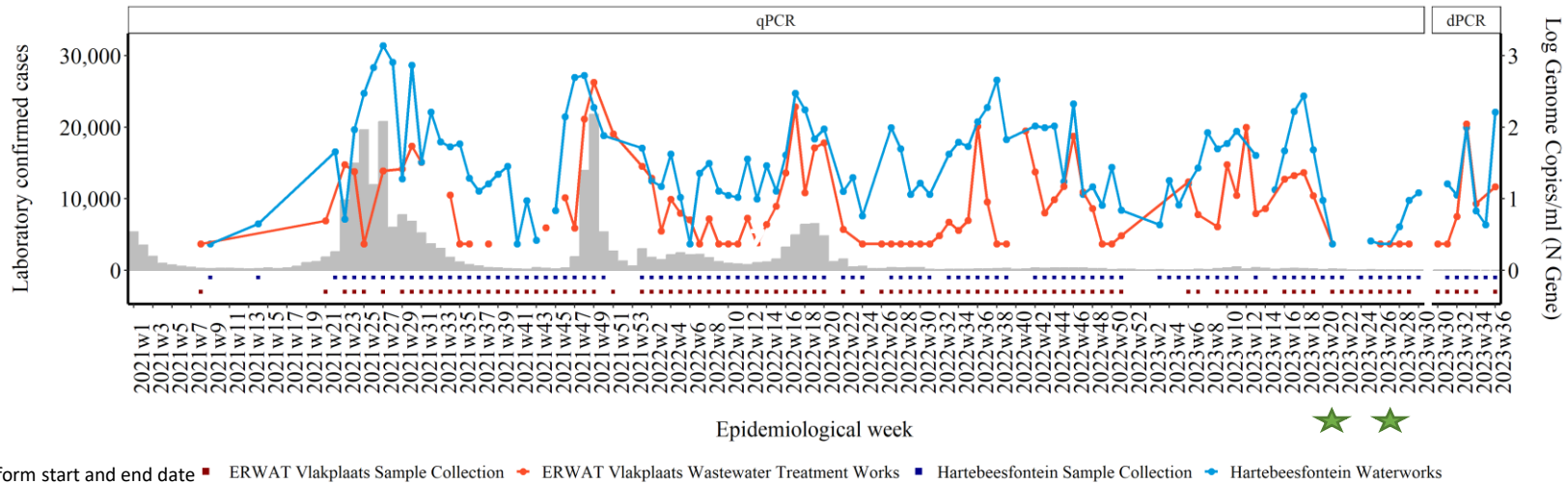
■ A ■ Alpha ■ Beta ■ Delta ■ BA.1 ■ BA.2 ■ BA.2.75 ■ BA.2.10 ■ BA.3 ■ BA.4.6 ■ BA.4 ■ BA.4.8
■ BA.5 ■ BA.5.3.5 ■ BA.5.2 ■ BA.5.1 ■ BA.5.3 ■ BQ.1 ■ XAK ■ XAH ■ XAP ■ XAY ■ XAS ■ BE.1.2
■ BE.8 ■ DR.1 ■ XBB.1.5 ■ XBB.1.9 ■ XBB.1.9.1 ■ XBB.3 ■ XBB.6 ■ CH.1.1 ■ BQ.2 ■ BE.9 ■ BF.7 ■ BE.6 ■ BE.7
■ XAV ■ XBB* ■ XBB.1.5* ■ FL* ■ B* ■ XBB.1.5.17 ■ XBB.1.5.81 ■ XBB.1.5.83 ■ XBB.1.5

Northern



■ Alpha ■ BA.1 ■ BA.2 ■ BA.4 ■ BA.4.6 ■ BA.5 ■ BA.5.2
■ BA.5.3.5 ■ BE.1.1 ■ BE.1.2 ■ BE.6 ■ BE.7 ■ BE.8 ■ BE.9
■ XBB ■ XBB.1.16 ■ BQ.1 ■ Delta ■ Kappa ■ XAA ■ XAH
■ XBB.1.5.81 ■ XBB.1.5.83 ■ XBB.1.9 ■ XBB.1.9.1 ■ XBB.3 ■ XBB.6 ■ XBE

Gauteng - Ekurhuleni



SARS-CoV-2 levels and Genomic Results in Epi week 36:

- The SARS-CoV-2 levels in Hartebeesfontein WWTW increased significantly from low levels in Epi week 33, to moderately high levels in Epi week 36
- As of Epi week 36, SARS-CoV-2 levels decreased from intermediate to low in Epi week 34 in Vlakplaats WWTW, and increased slightly in Epi week 36

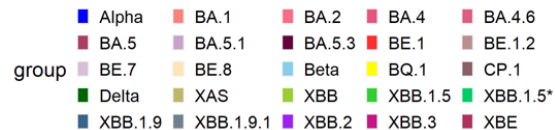
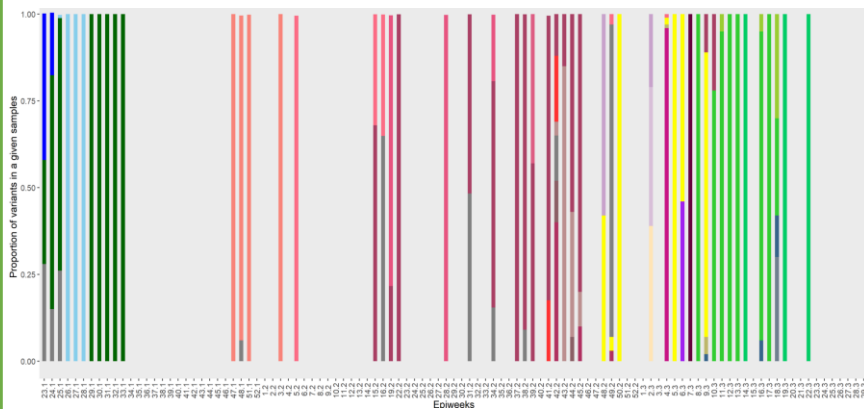
Sequencing data ending in Epi week 32:

- SARS-CoV-2 sequencing coverage in the Vlakplaats samples collected during Epi weeks 30-32 are too low for meaningful interpretation
- Omicron lineages XBB.1.5.28, XBB.2.9, XBB.2.4, XBB.2.8 and XBB.1.27 were circulating in Epi weeks 30 at the Hartebeesfontein water treatment plant

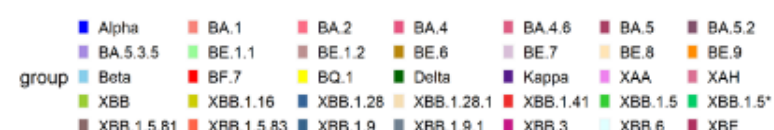
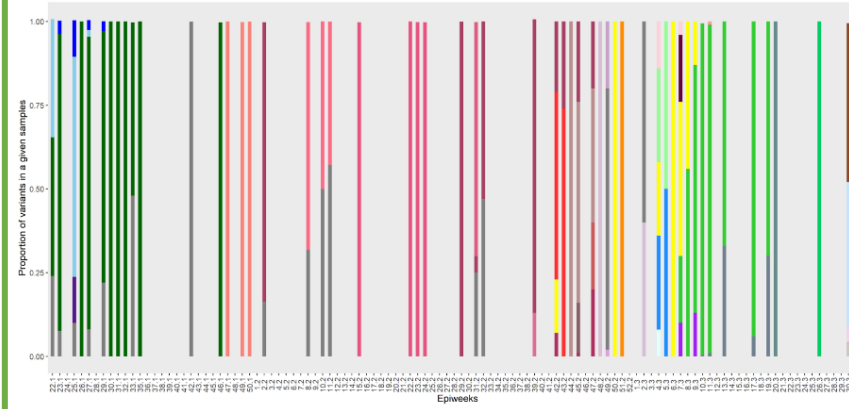
SNP Analysis:

- SNP analysis could not be performed as the SARS-CoV-2 sequencing coverage in the Vlakplaats samples collected during Epi weeks 30-32 are too low for meaningful interpretation
- A combination of mutations associated with the XBB 1.15 lineage (V83A, Q183E, R346T, L368I, F486P, F490S) were detected in Hartebeesfontein during Epi weeks 32.

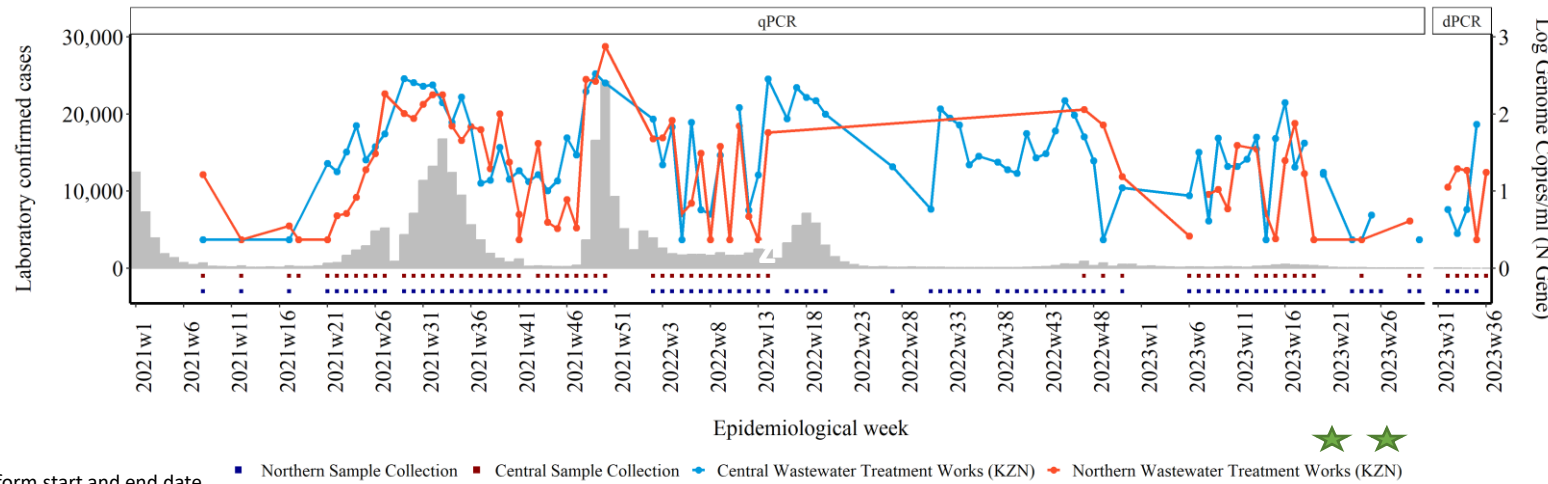
ERWAT Vlakplaats



Hartebeesfontein



KwaZulu-Natal - eThekweni



SARS-CoV-2 levels and Genomic Results in Epi week 36:

- No new results from Central WWTW in Epi week 36 however, the SARS-CoV-2 levels showed a sharp increase from low levels (1 log copy/ml) to intermediate levels 2 log copies/ml in Epi week 35
- SARS-CoV-2 levels increase from low to intermediate in week Epi 36 in Northern WWTW

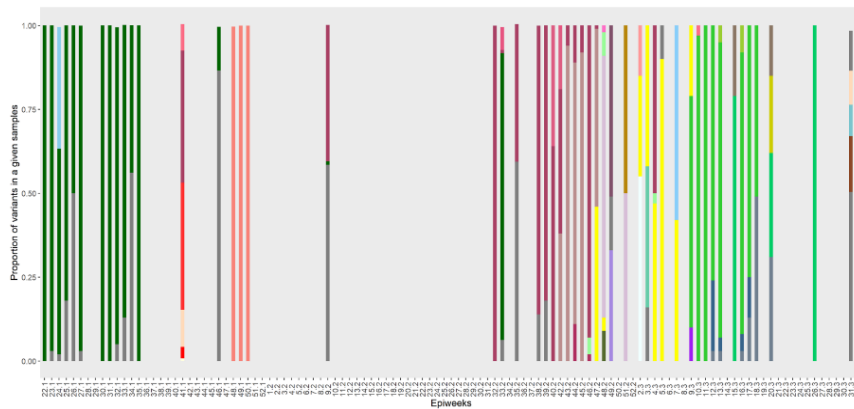
Sequencing data ending in Epi week 32:

- XBB.1.5.91, XBB.1.41.1, BA.2.1, EG.2, FL.2.4, XBB.1.41 were circulating in Epi week 31, with XBB.1.41 dominating in Epi week 32 at the Central eThekweni water treatment plant
- In eThekweni North, FL.25, BA.2.16, BA.2.15 were circulating during Epi week 32

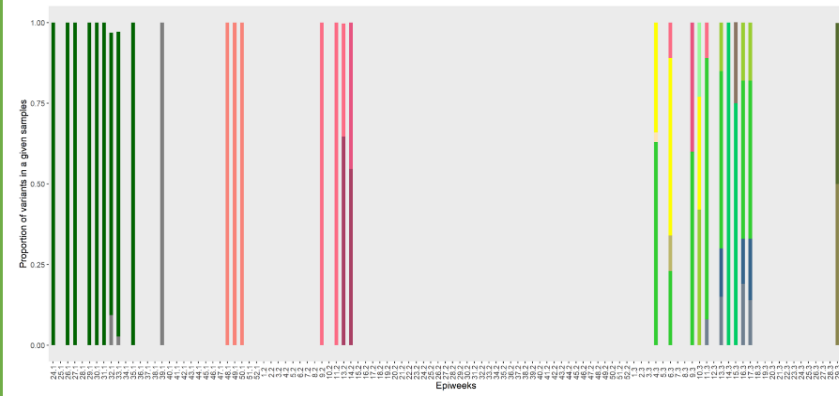
SNP Analysis:

- In both Central and Northern eThekweni mutations associated with BA.2.86 (R21T, S50L, V127F, F157S, R158G, L212I, V213G) and XBB sub-lineages (V83A, Q183E, R346T, L368I, F486P, F490S) were detected during Epi weeks 31 and 32

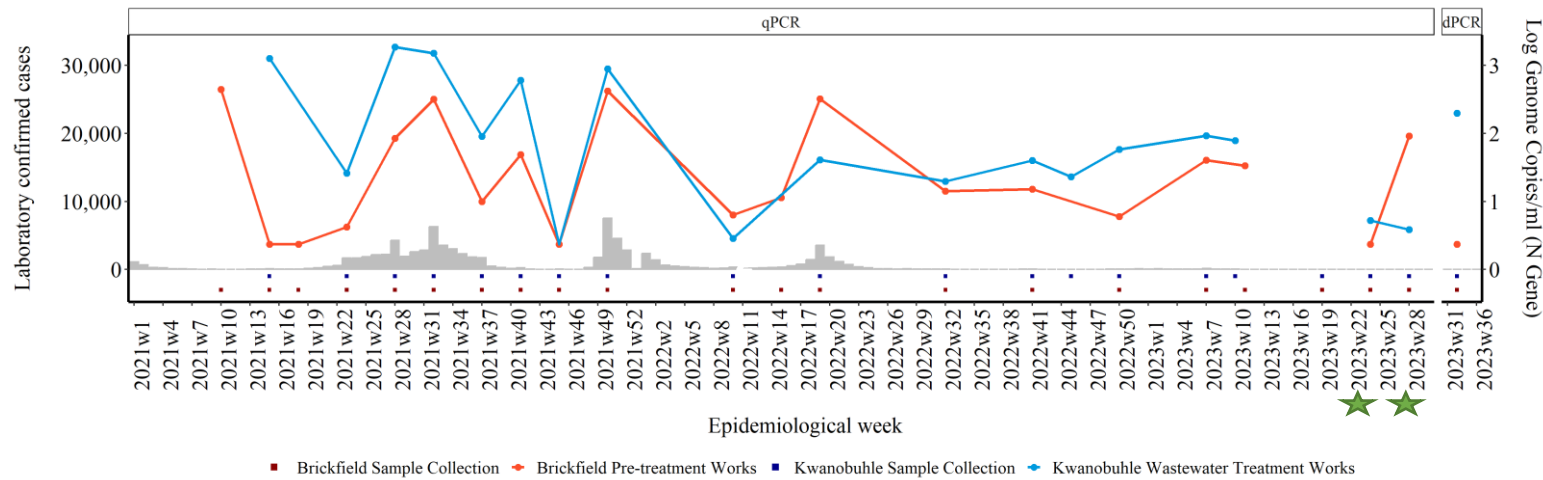
Central



North



Eastern Cape – Nelson Mandela



★ Chloroform start and end date

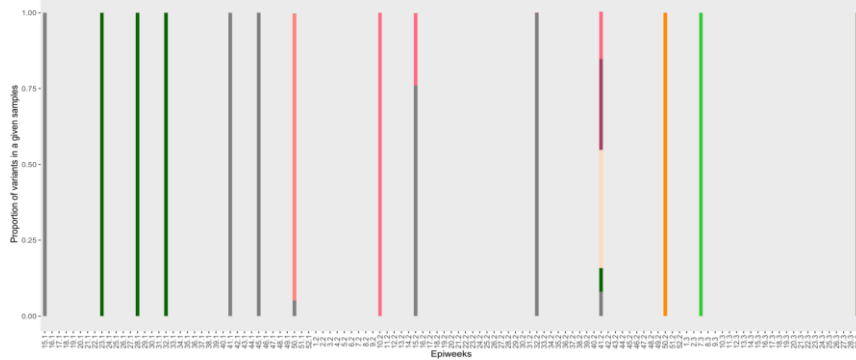
SARS-CoV-2 levels and Genomic Results in Epi week 36:

- No new results from Epi week 36, however, and increase was seen in Epi week 31 in Kwanobuhle WWTP
- No new results from Epi week 36 however, SARS-CoV-2 levels decreased from intermediate to low in Epi week 31 in Brickfield Pre-treatment works

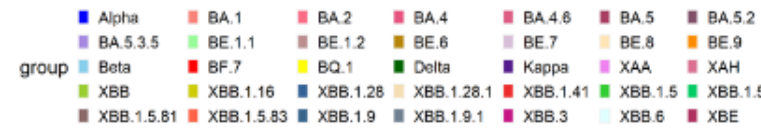
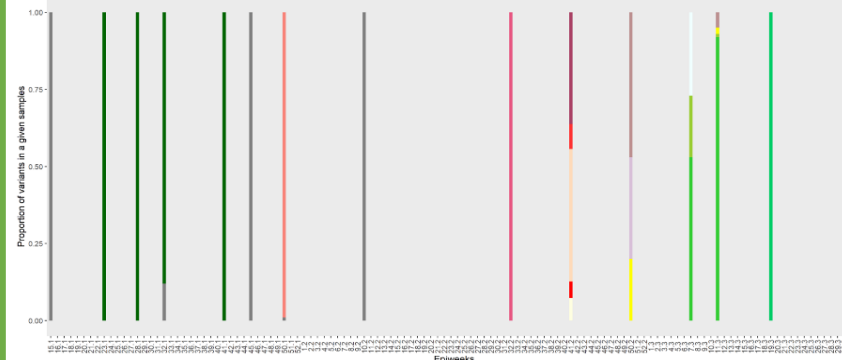
Freyja Output:

- SARS-CoV-2 sequencing coverage in the Brickfield samples collected during Epi weeks 30-32 are too low for meaningful interpretation
- Omicron lineages XBB.1.5.81, XBB.1.5.32 and XBB.1.5.27 were circulating in Kwanobuhle during epi week 32.

Brickfield



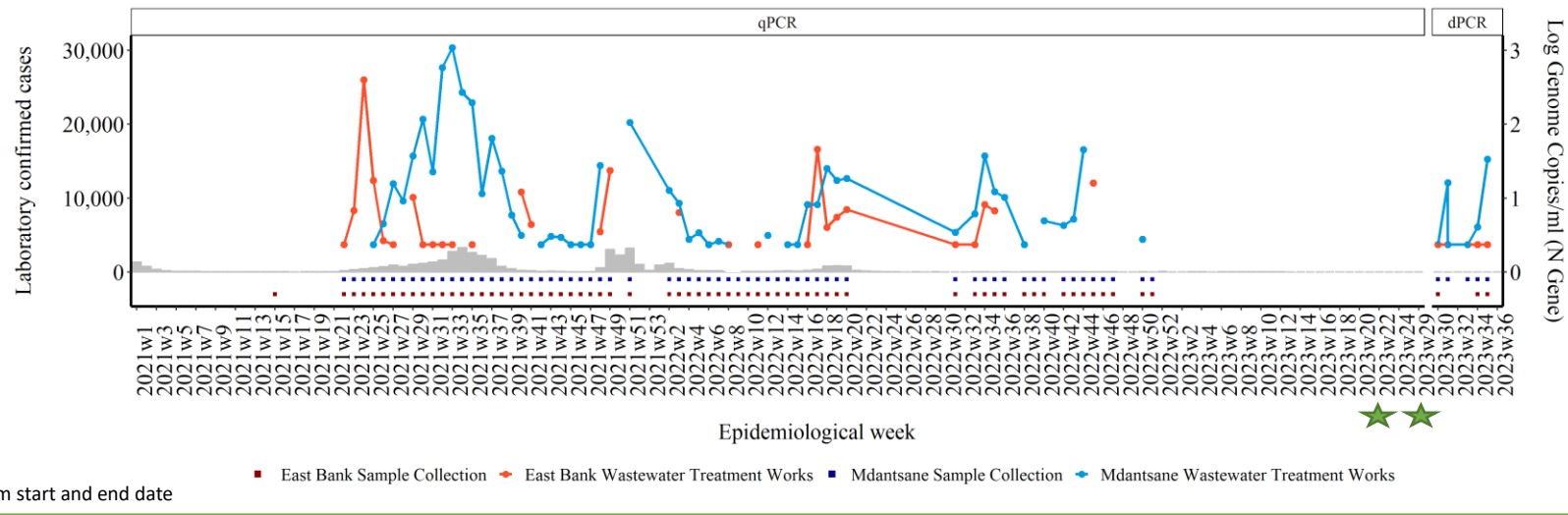
Kwanobuhle



SNP Analysis:

- SNP analysis could not be performed as the SARS-CoV-2 sequencing coverage in the Brickfield samples collected during Epi weeks 30-32 are too low for meaningful interpretation
- Mutations associated with XBB.1.5 (V83A, Q183E, R346T, L368I, F486P, F490S) have been found in Kwanobuhle during Epi weeks 30 and 31. A mutation (R403K) associated with BA.2.86 was also detected in Kwanobuhle during Epi week 32

Eastern Cape – Buffalo City



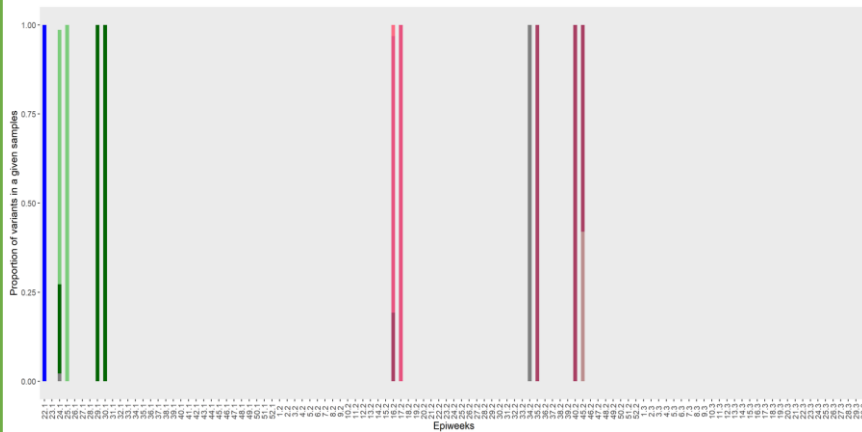
SARS-CoV-2 levels and Genomic Results in Epi week 36:

- No new results from Mdantsane WWTW in week 36, however, an increase in SARS-CoV-2 levels at was seen in Epi week 35 Levels remain intermediate
- SARS-CoV-2 levels remain low in East Bank WWTW

Freyja output:

- SARS-CoV-2 sequencing coverage in the Eastbank samples collected during Epi weeks 30-32 are too low for meaningful interpretation .
- Omicron lineage XBB.1.5.88 was the dominant lineage circulating in Mdantsane during Epi week 31, with XBB.1.5.32, XBB.1.5.56, and XBB sublineages also in circulation

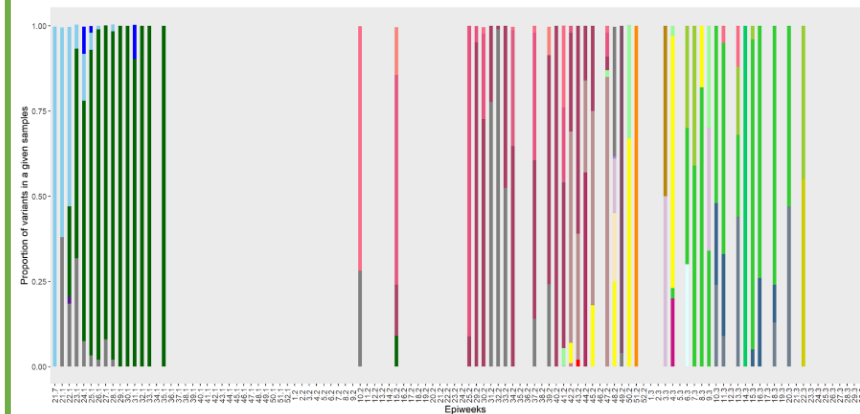
Eastbank



group

- A
- Alpha
- BA.2
- BA.4
- BA.5
- BE.1.2
- Delta

Mdantsane



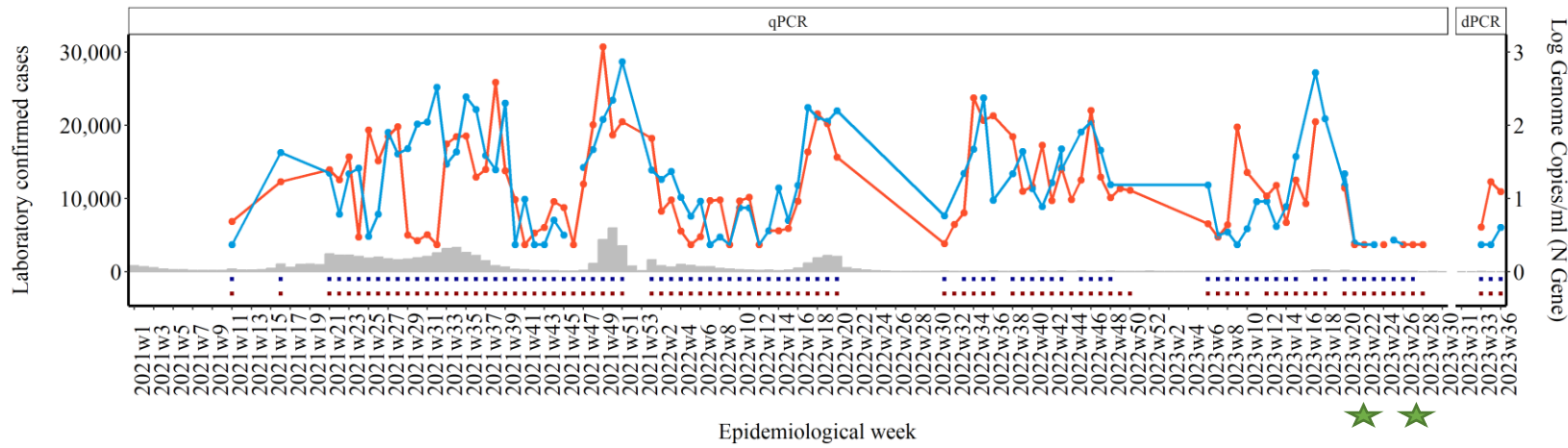
group

- Alpha
- Beta
- XBB
- XBB.1.5.81
- XBB.1.5.83
- XBB.1.9
- XBB.1.9.1
- XBB.3
- XBB.6
- XBB.9
- BA.1
- BA.2
- BA.4
- BA.4.6
- BA.5
- BA.5.2
- BE.1.1
- BE.1.2
- BE.6
- BE.7
- BE.8
- BE.9
- BF.7
- BQ.1
- Delta
- Kappa
- XAA
- XAH
- XBB.1.16
- XBB.1.28
- XBB.1.28.1
- XBB.1.41
- XBB.1.5
- XBB.1.5*
- XBB.1.5.81
- XBB.1.5.83
- XBB.1.9
- XBB.1.9.1
- XBB.3
- XBB.6
- XBB.9

SNP Analysis:

- SNP analysis could not be performed as the SARS-CoV-2 sequencing coverage in the Eastbank samples collected during Epi weeks 30-32 are too low for meaningful interpretation
- A combination of mutations (V83A, Q183E, R346T, L368I, F486P, F490S) associated with lineage XBB 1.15 have been found in Mdantsane during Epi weeks 30 and 31.

Free State – Mangaung



★ Chloroform start and end date ■ Bloemspruit Sample Collection ◆ Bloemspruit Wastewater Treatment Works ■ Sterkwater Sample Collection ◆ Sterkwater Wastewater Treatment Works

SARS-CoV-2 levels and Genomic Results in Epi week 36:

- In Bloemspruit WWTW, a small decrease in SARS-CoV-2 levels was observed. Levels remain intermediate
- A small increase in SARS-CoV-2 levels were seen in Sterkwater WWTW, and levels remain low

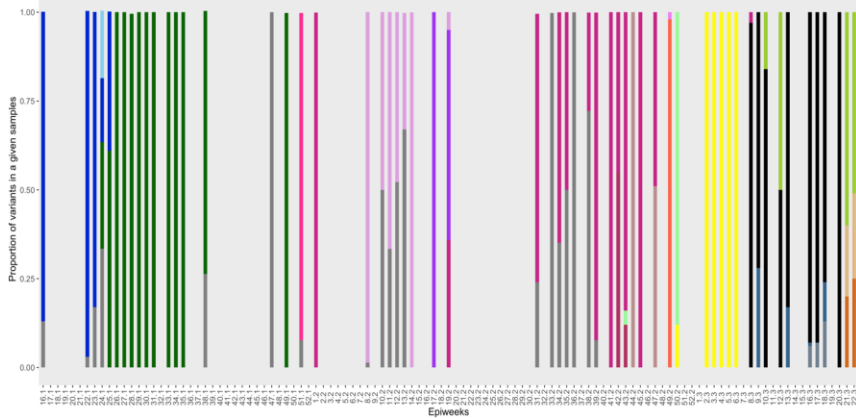
Freyja output:

- SARS-CoV-2 sequencing coverage in the Bloemspruit and Sterkwater samples collected during Epi weeks 30-32 are too low for meaningful interpretation

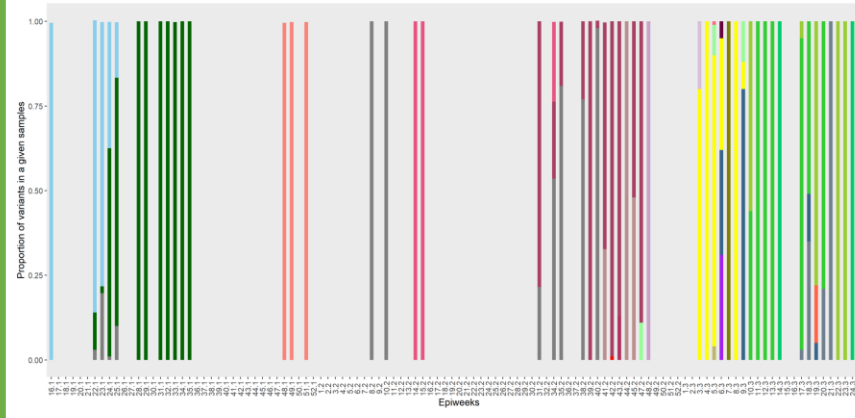
SNP Analysis:

- SNP analysis could not be performed as the SARS-CoV-2 sequencing coverage in the Bloemspruit and Sterkwater samples collected during Epi weeks 30-32 are too low for meaningful interpretation

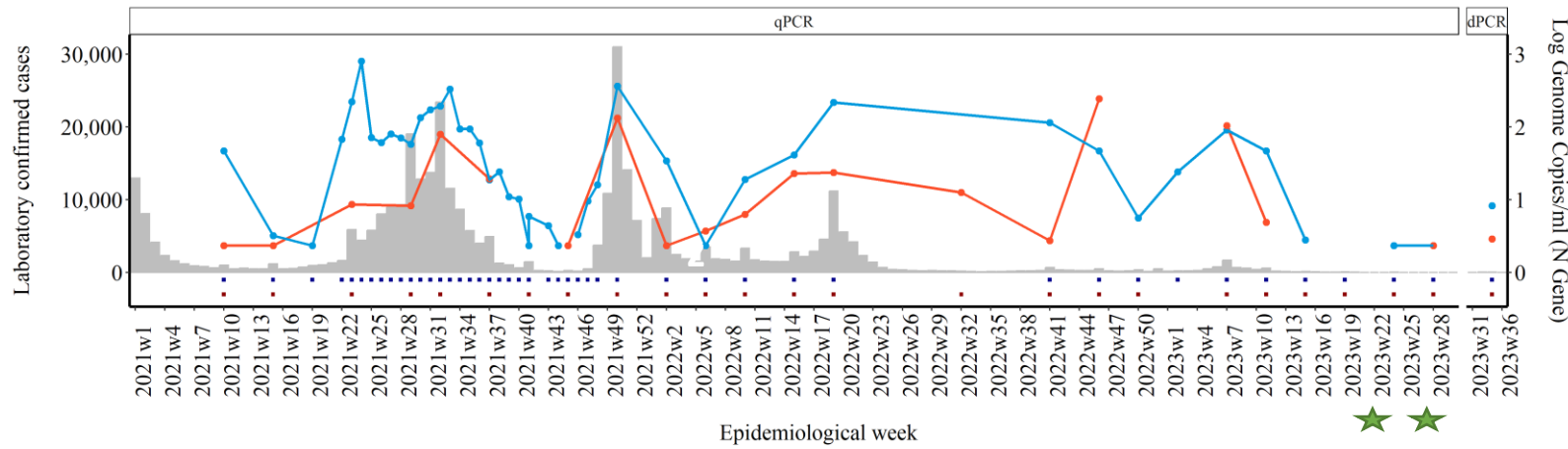
Bloemspruit



Sterkwater



Western Cape – City of Cape Town



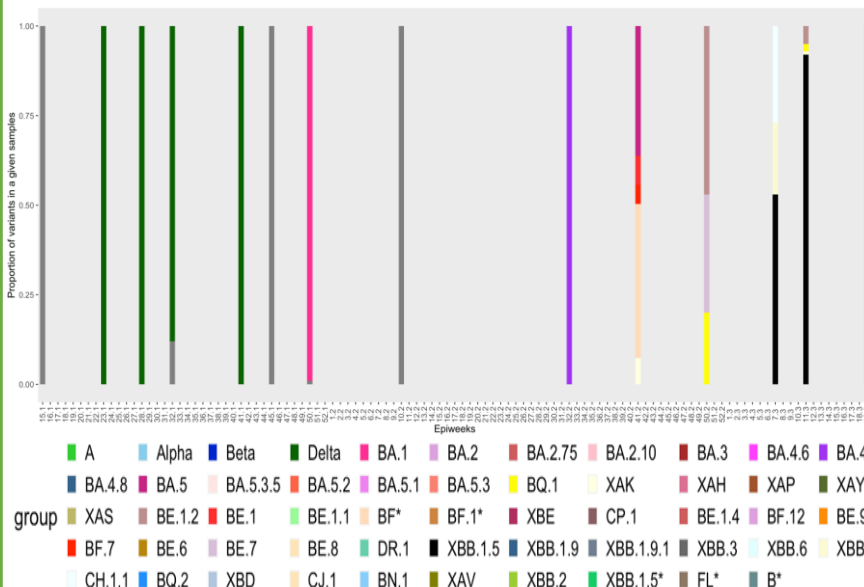
SARS-CoV-2 levels and Genomic Results in Epi week 36:

- In Epi week 34, SARS-CoV-2 in wastewater had minimal increases Borcheds Quarry WWTP
- Minimal increased SARS-CoV-2 levels were observed in Zandvleit WWTP in Epi week 34

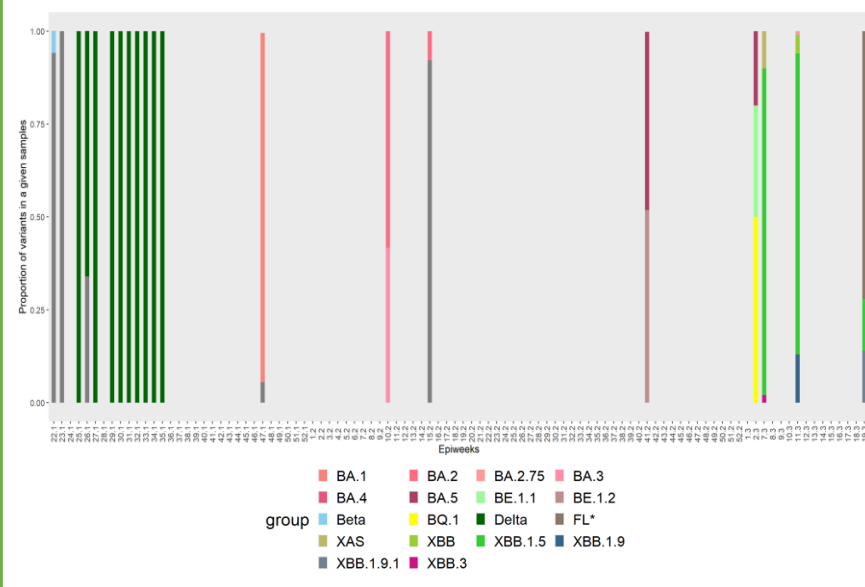
Freyja output:

- Freyja lineage determination could not be performed as no samples were collected from both Cape Town wastewater treatment plants during Epi weeks 30-32.

Borcheds Quarry



Zandvleit



SNP Analysis:

- SNP analysis could not be performed as no samples were collected from both Cape Town wastewater treatment plants during Epi weeks 30-32.

COLLABORATORS



FUNDERS



TEAM

