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

22 September 2023

Quantitative data – results for sample collection dates up to 15 September 2023 (epidemiological week 37)
Sequencing/Genomics data – results for samples collected up to 2 September 2023 (epidemiological week 35)*
**no new data since last report*

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

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

Wastewater levels

Epidemiological weeks 33-37

- From weeks 33-37, 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 **33-37 increases and/or higher levels have been seen in Gauteng (Daspoort WWTW) and Cape Town (in Borcheds Quarry WWTW and Zandevleit WWTW)**
- 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 31-35

no new data since last report

- No new genomics results were obtained for week 35
- 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 eThekwini (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.

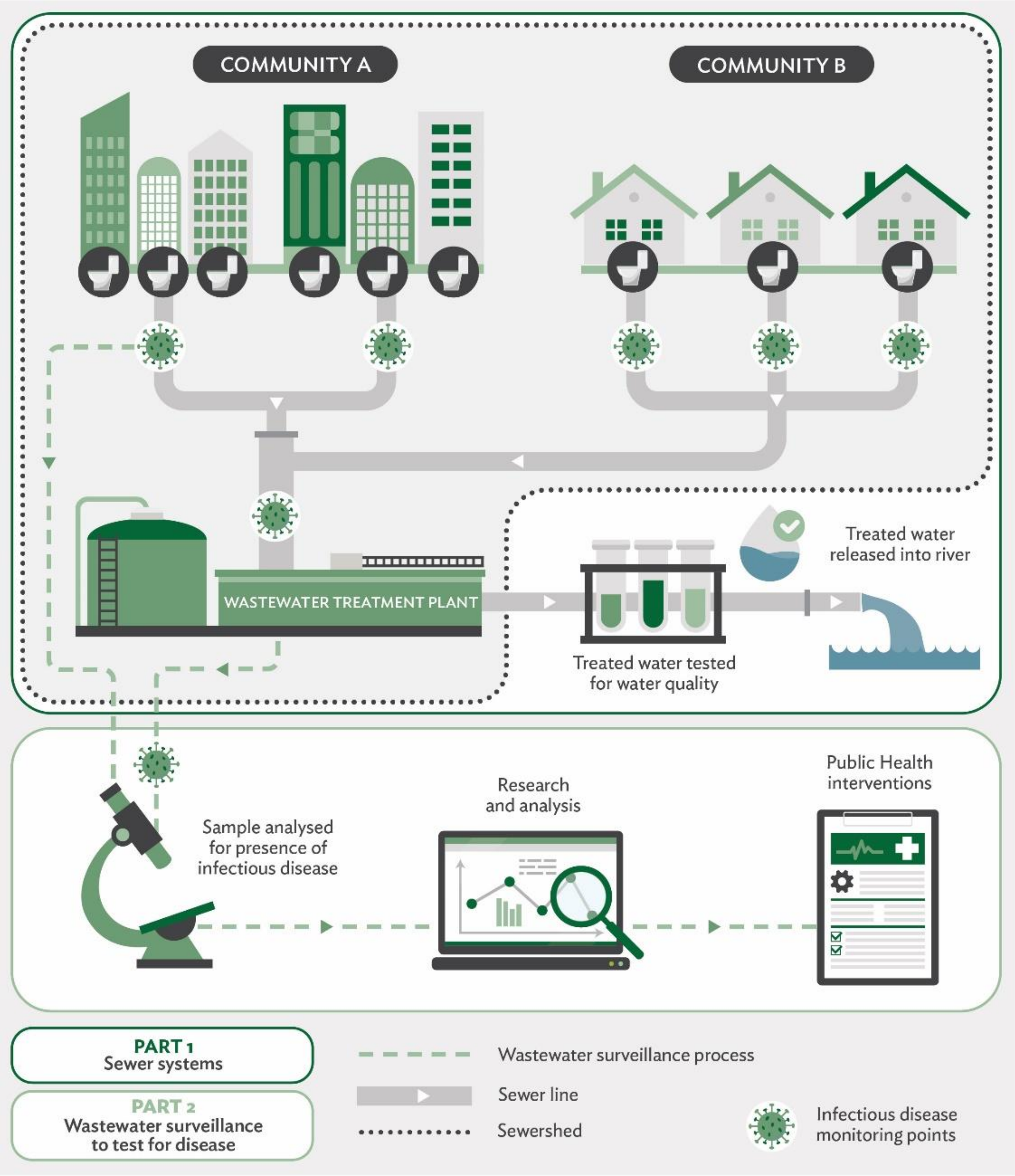
Wastewater-based Epidemiology for COVID-19

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/

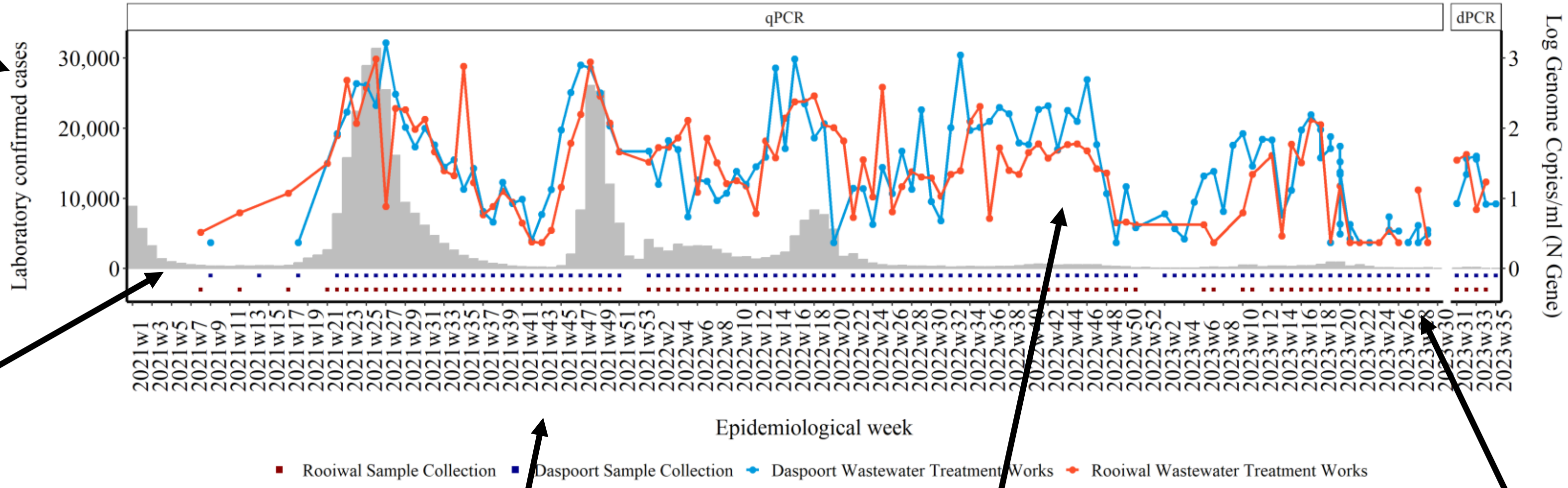
For a technical description and analysis of wastewater levels and results see
https://pubmed.ncbi.nlm.nih.gov/37506905/
https://www.medrxiv.org/content/10.1101/2022.12.15.22283506v1 (accepted by Nature Communications, publication pending)

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?



Facets:

Indicates the platform used to test samples

Left vertical axis:

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

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

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:

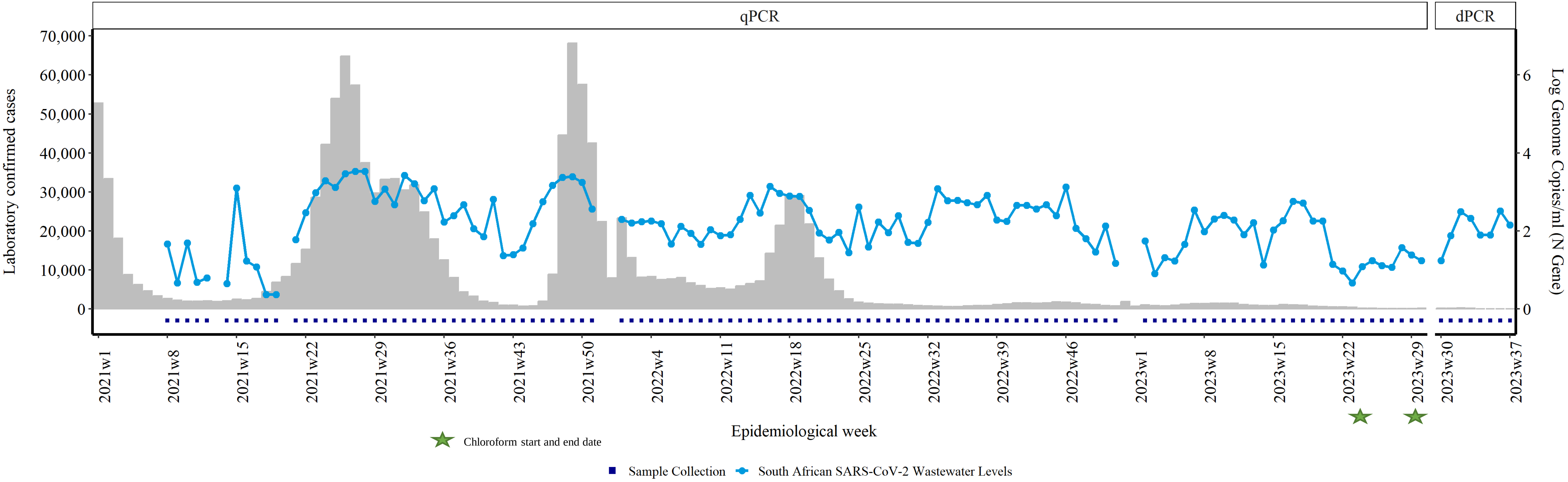
Changes in wastewater SARS-CoV-2 results over time for different treatment facilities

Coloured squares:

Epi weeks during which samples were collected

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, eThekwini, Mangaung, Nelson Mandela, Buffalo City, and City of Cape Town (grey bars), by epidemiological week, 2021-2023.

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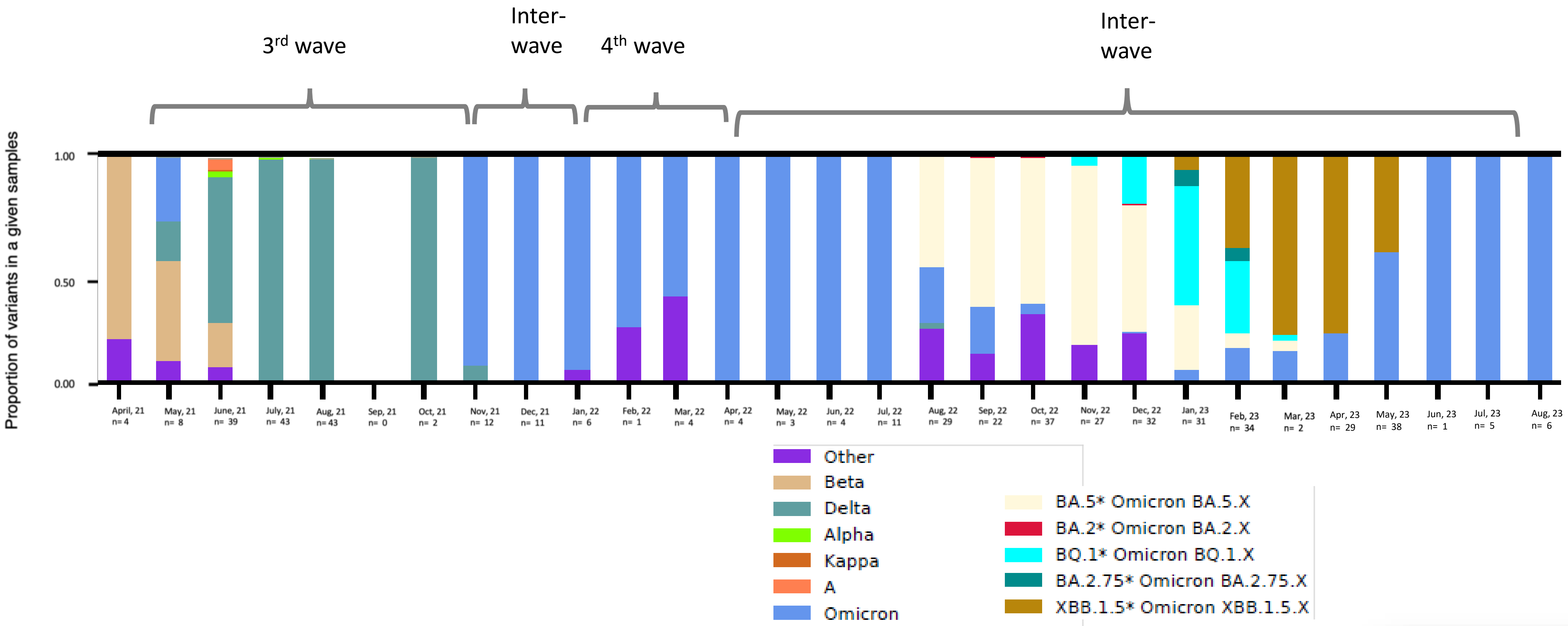
South Africa at a glance:

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

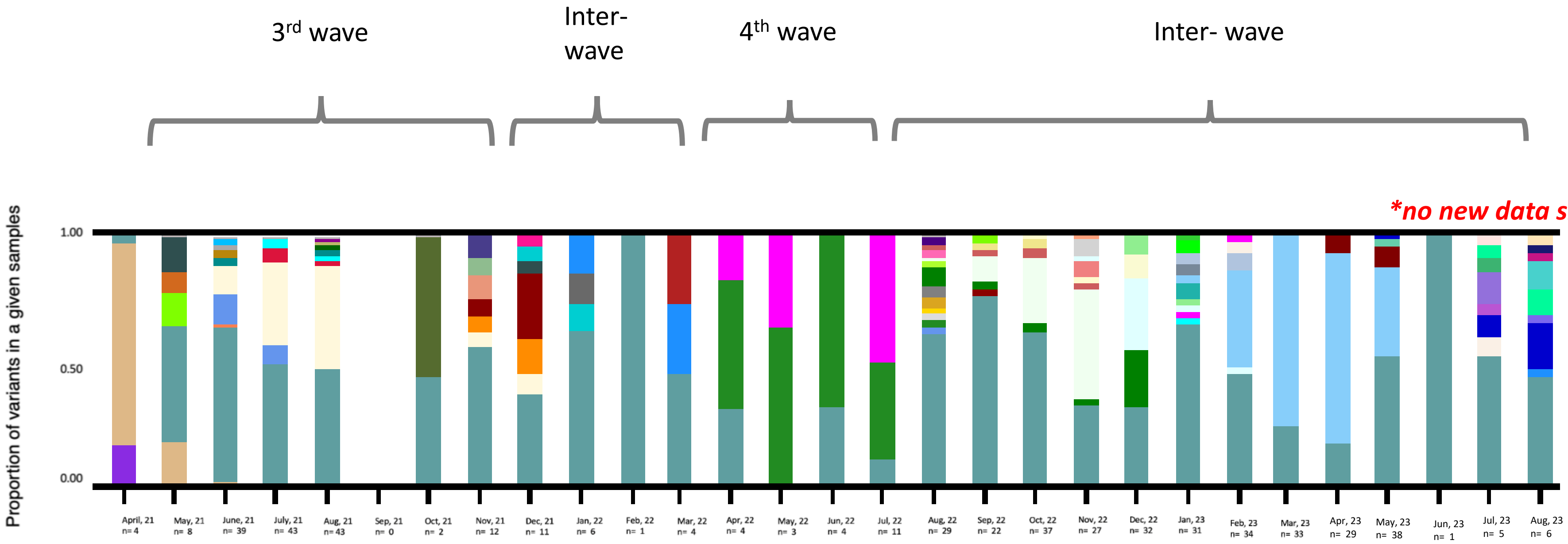
**no new data since last report*



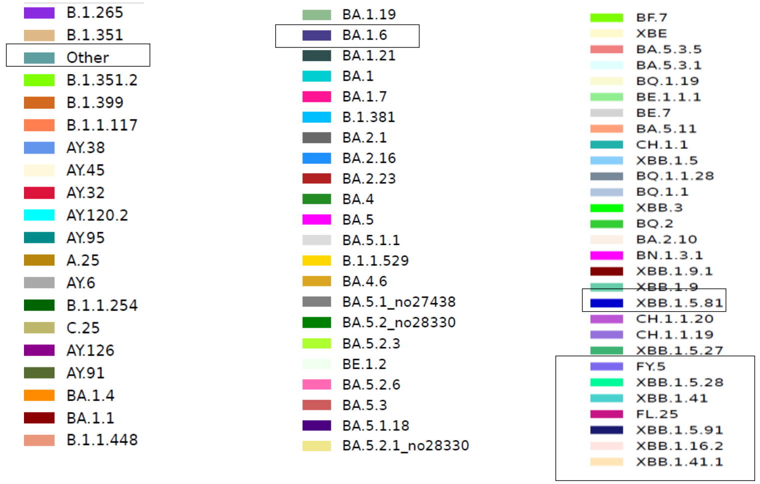
South Africa at a glance:
Circulating lineages as determined by Freyja deconvolution of sequence data

- 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.

no new data since last report



no new data since last report



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

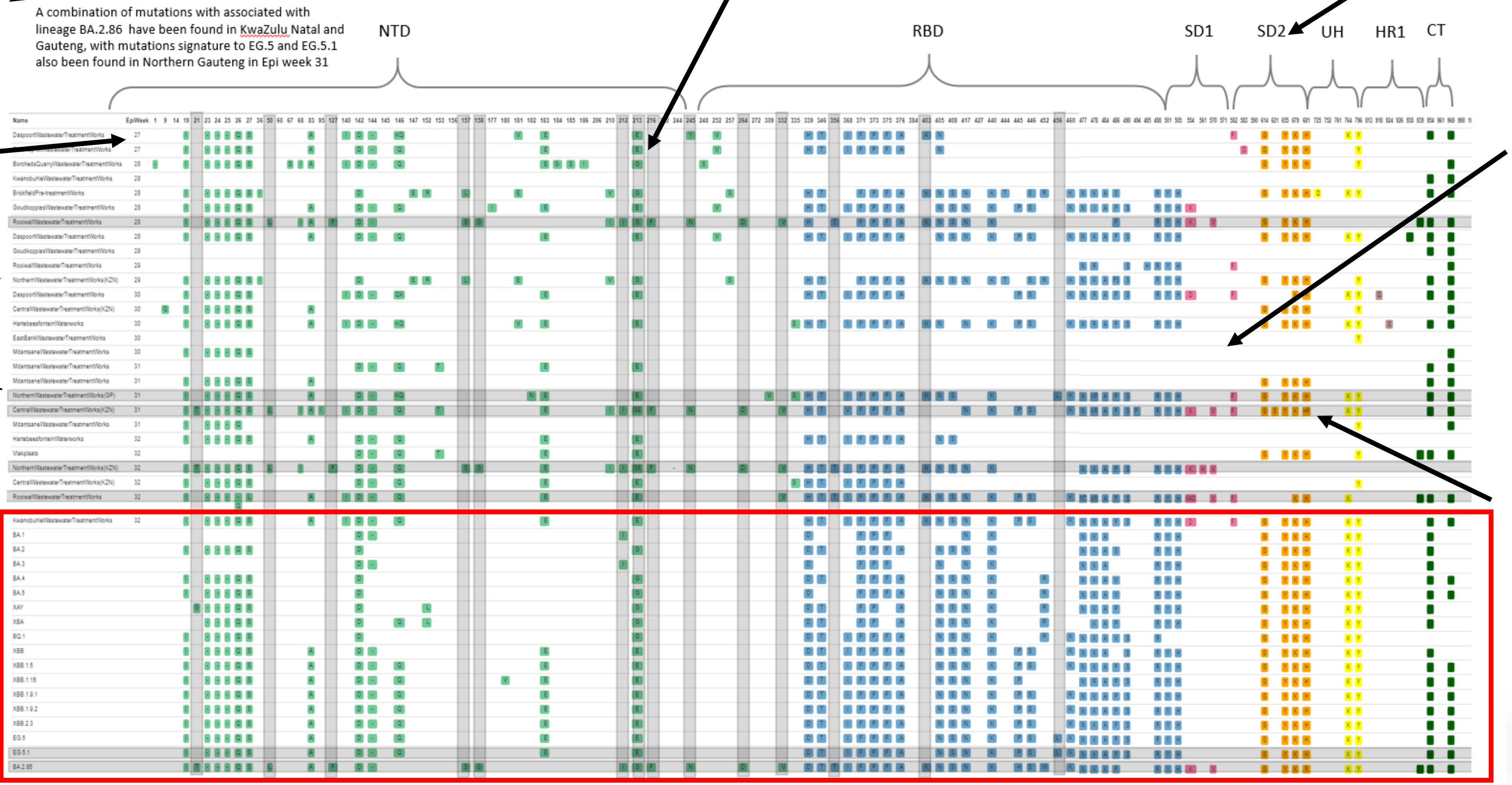
Reference lineages:

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

Highlighted

Highlighting mutations found in wastewater samples that correspond to the reference lineages

Spike mutational profile of samples from sites across South Africa and VOCs/VOIs processed in weeks 30 31 32, 2023



A combination of mutations with associated with lineage BA.2.86 have been found in KwaZulu Natal and Gauteng, with mutations signature to EG.5 and EG.5.1 also been found in Northern Gauteng in Epi week 31

Top x-axis:

Position of the spike protein

Blank rows or columns

Positions along the spike protein in which there are no changes in the spike protein amino acids of the samples compared to the Wuhan reference genome

Coloured blocks

Indicate changes (mutations) in the amino acids of the spike protein

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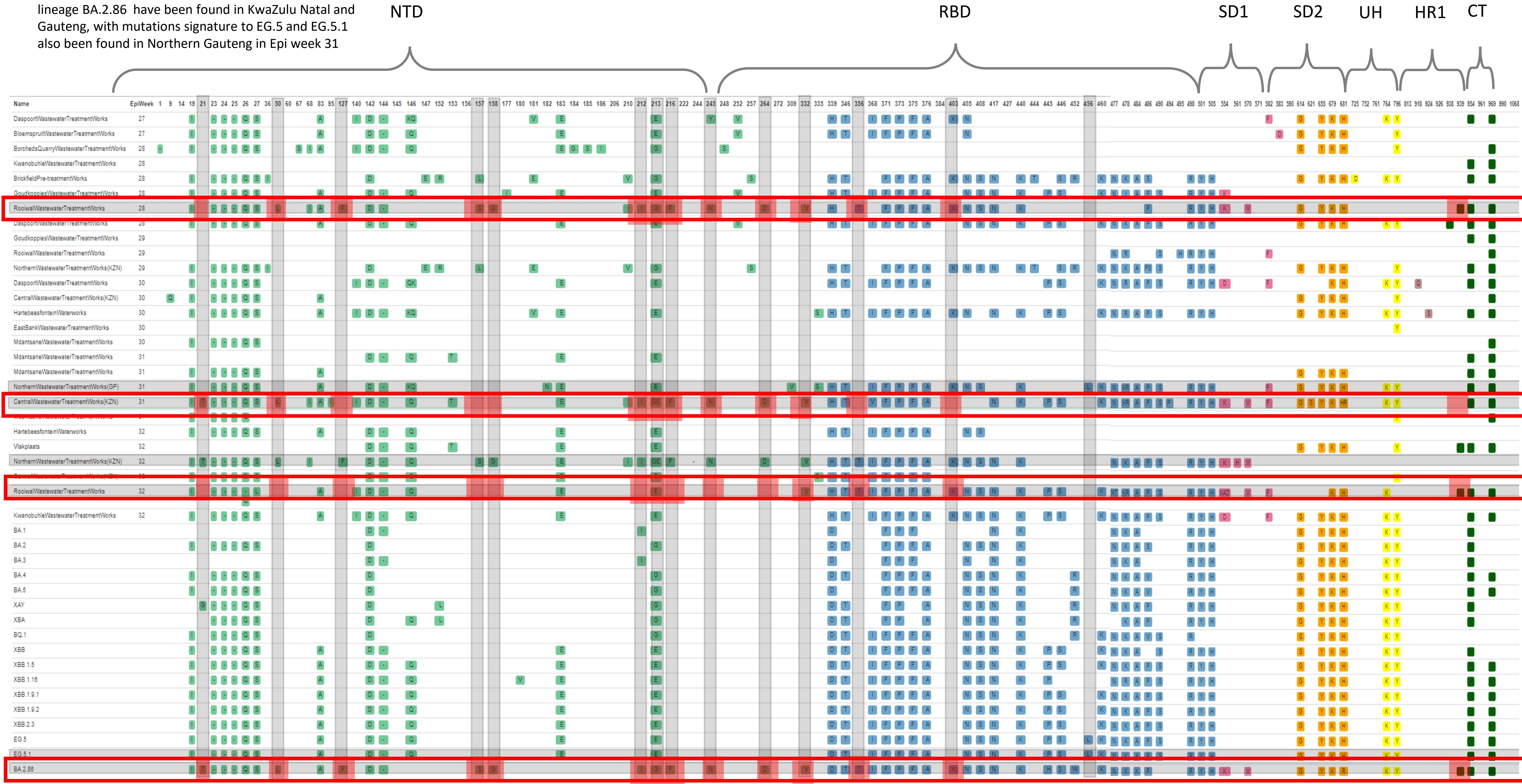
Spike mutational profile of samples from sites across South Africa and VOCs/VOIs processed in weeks 30 31 32, 2023

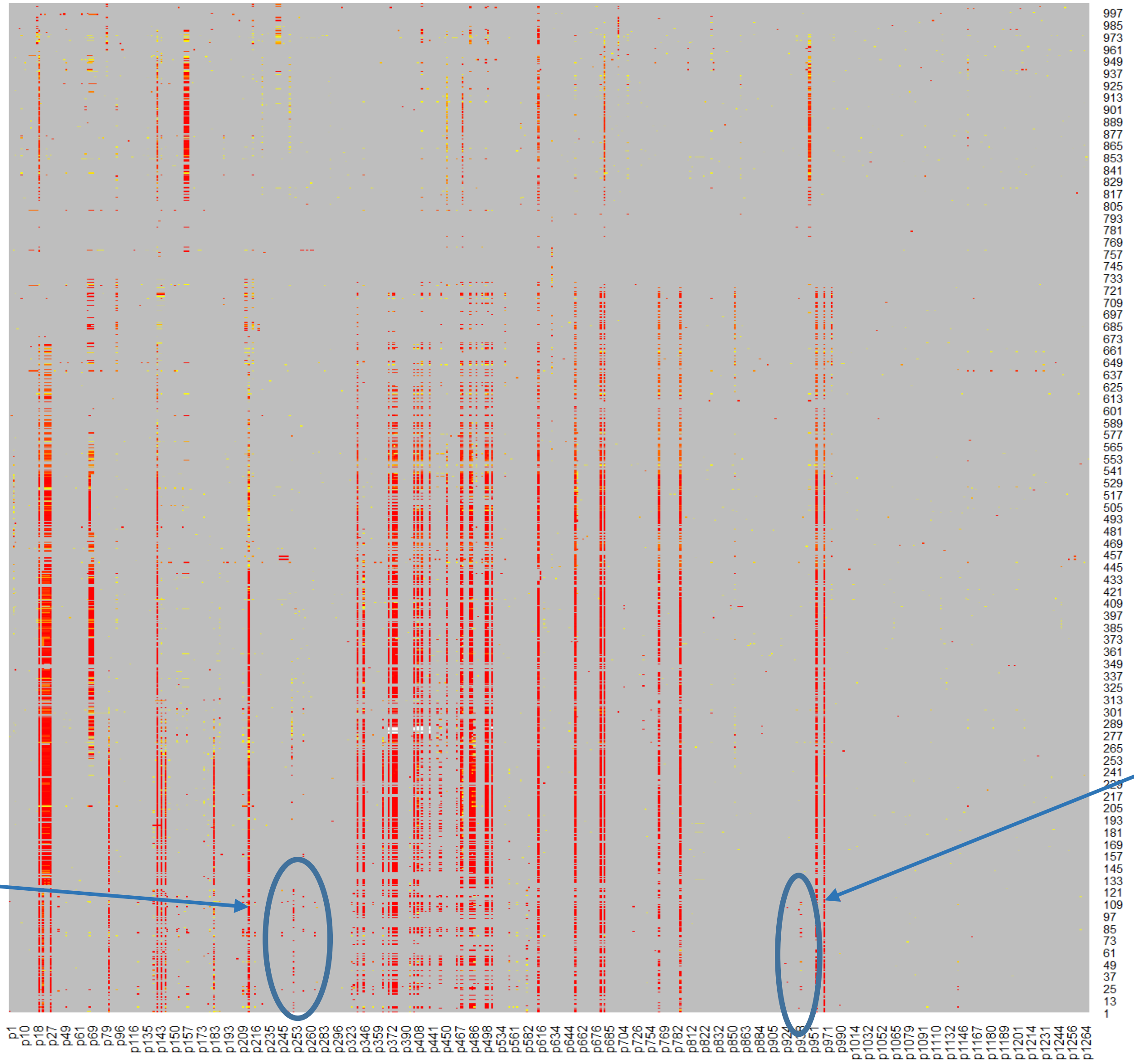
A combination of mutations with associated with lineage BA.2.86 have been found in KwaZulu Natal and Gauteng, with mutations signature to EG.5 and EG.5.1 also been found in Northern Gauteng in Epi week 31



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



G252V

Mutation in spike protein associated with XBB* sub-lineages

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, P6215, 1670V, P681R, S939F, P1143L, Ins16:MPLF*

S939F

Spike protein mutation associated with the BA.2.86 lineage

**no new data since last report*

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.

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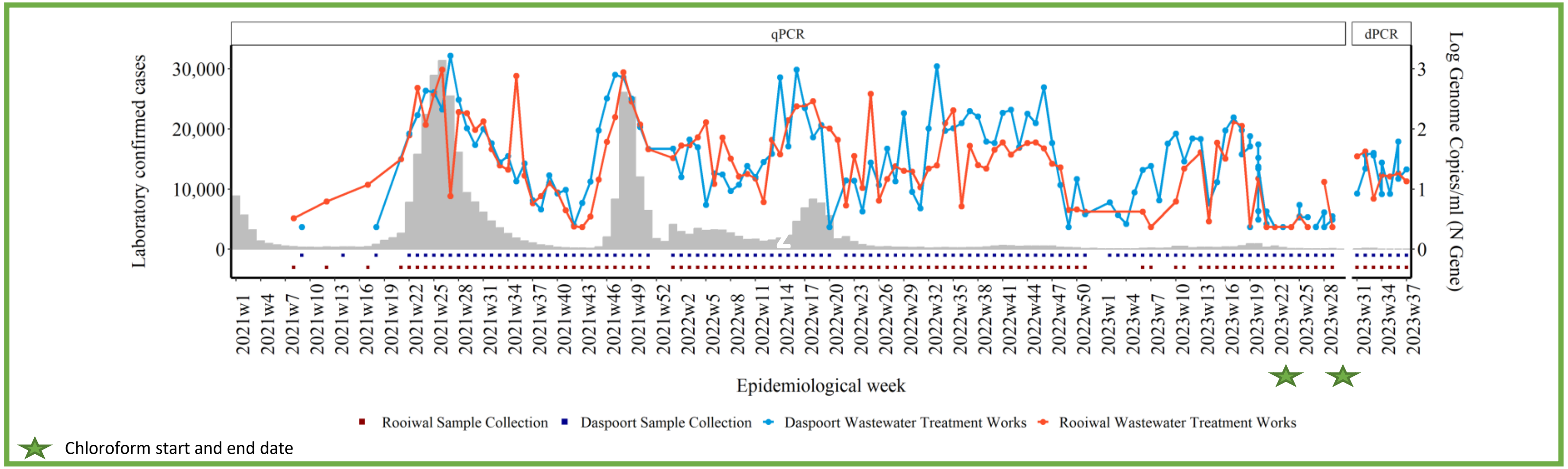
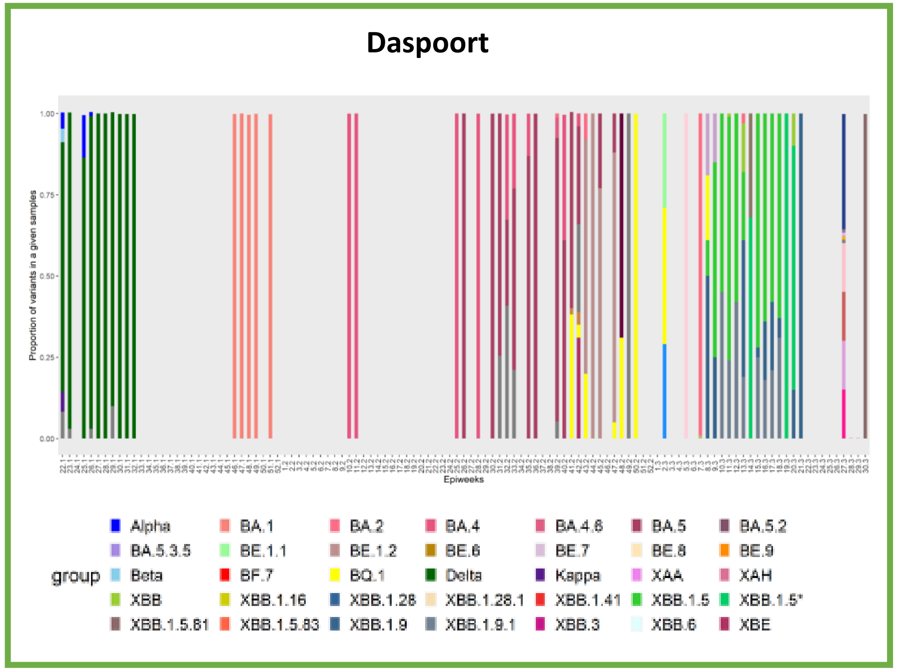
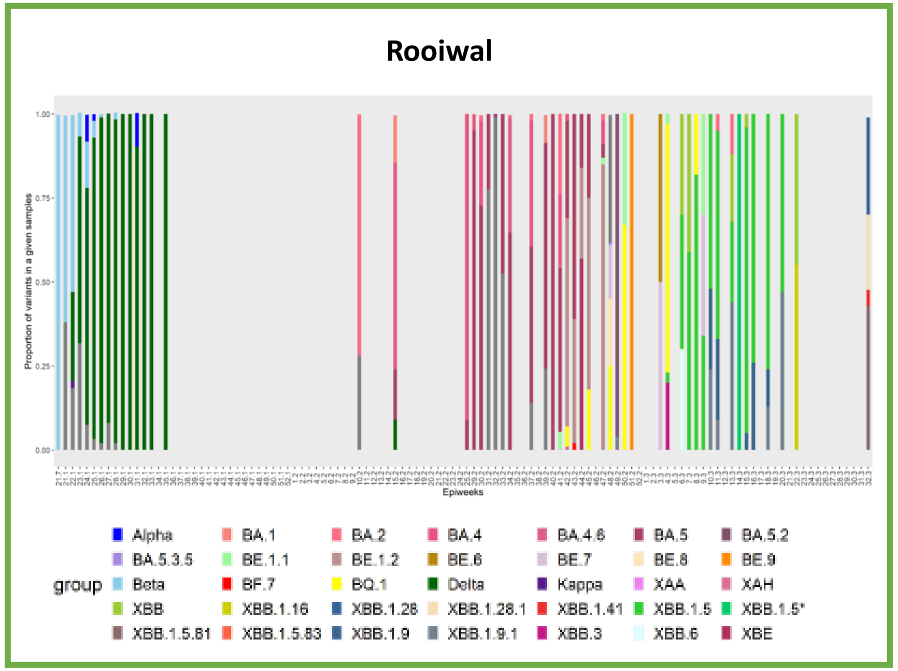
Gauteng - Tshwane




Daspoort


Rooiwal

SARS-CoV-2 levels and Genomic Results in Epi week 37:

- The SARS-CoV-2 levels in Daspoort WWTW increased 2-fold from Epi week 33 to Epi week 36 and decreased in Epi week 37. Levels remain moderate
- Levels in Rooiwal WWTW in Epi week 37 decreased slightly

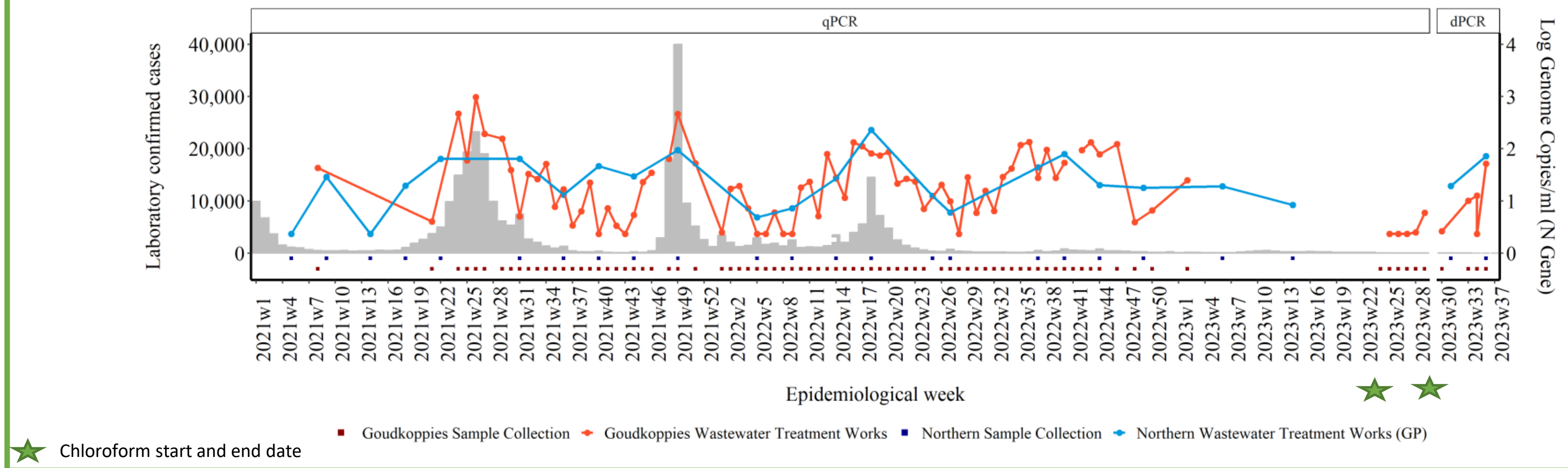
**** Sequencing data ending in Epi week 32 in Rooiwal and 30 in Daspoort WWTW.***

- 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.

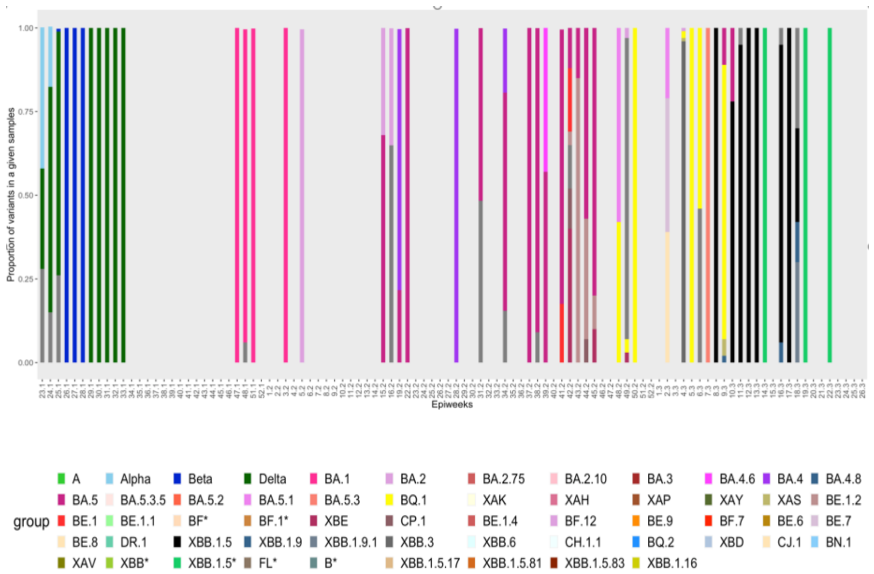
SNP Analysis:

- 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

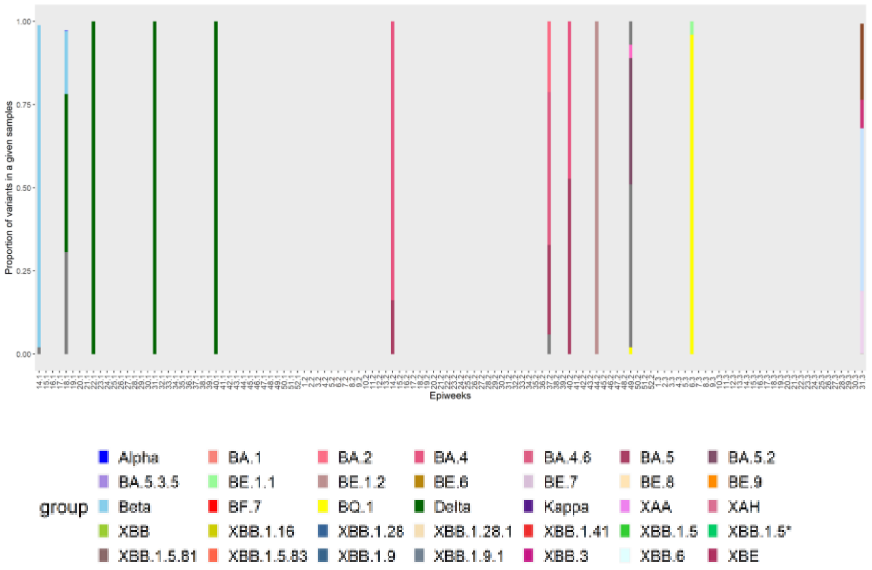
Gauteng - Johannesburg



Goudkoppies



Northern



SARS-CoV-2 levels and Genomic Results in Epi week 37:

- The SARS-CoV-2 levels in Goudkoppies WWTW increased from Epi week 30, and levels were intermediate from Epi week 34. No new results from Epi week 37 are available
- The SARS-CoV-2 levels increased to moderate in Northern WWTW in Epi week 36. No new results from Epi week 37 are available

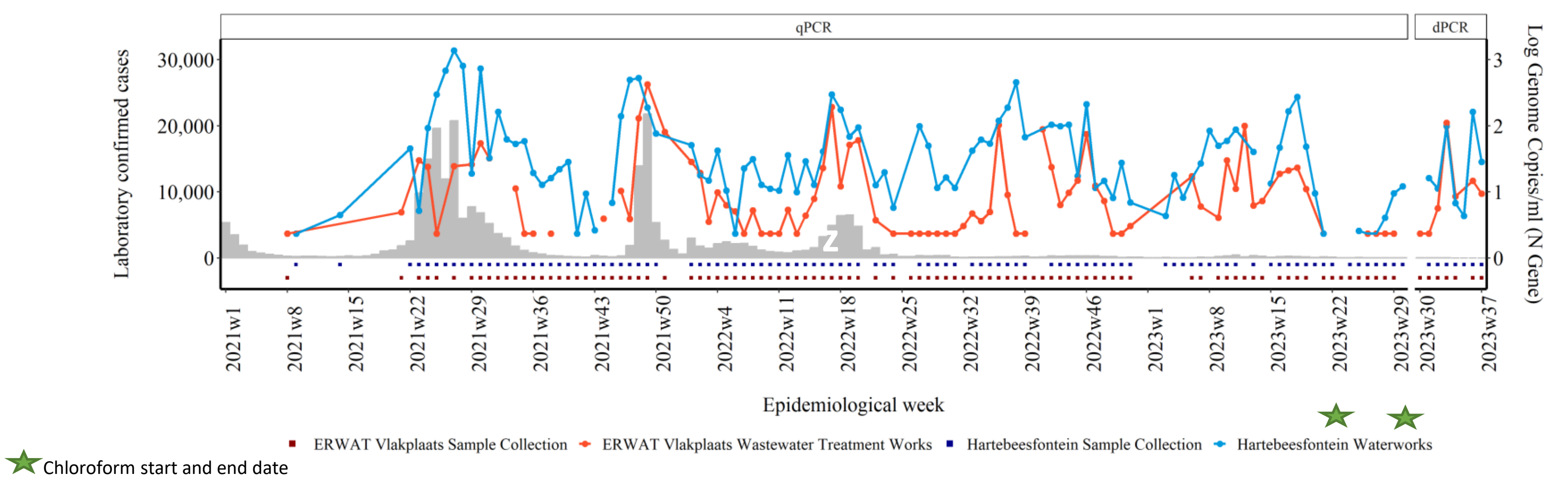
**** Sequencing data ending in Epi week 26 in Goudkoppies and 31 in Northern WWTW.***

- 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.

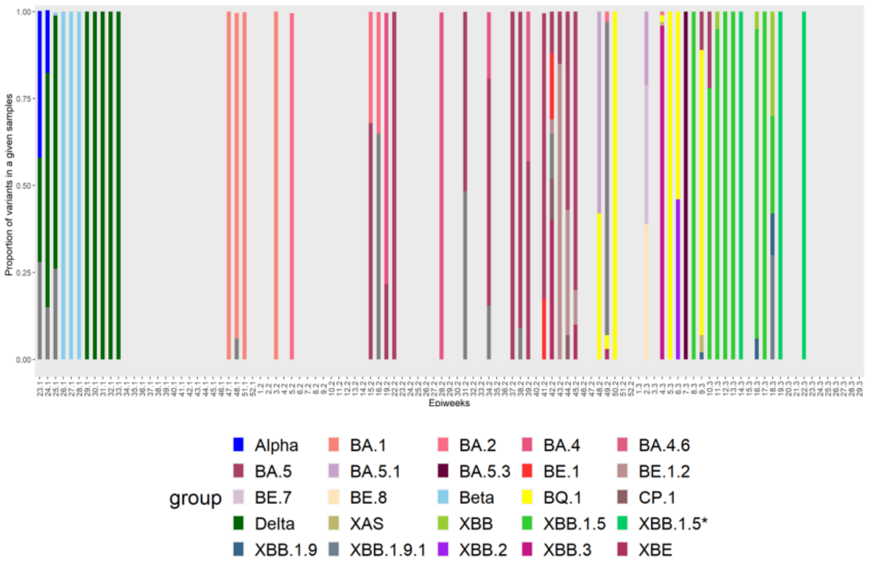
SNP Analysis:

- 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.

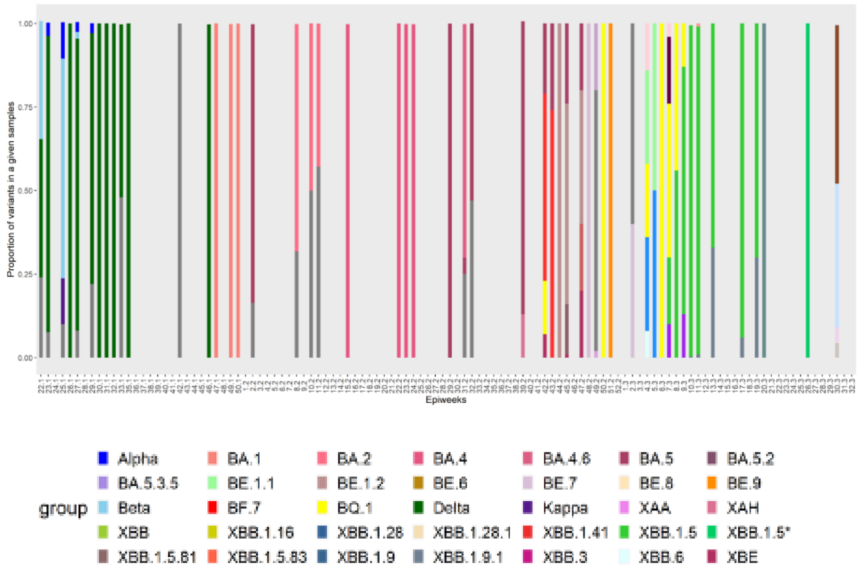
Gauteng - Ekurhuleni



ERWAT Vlakplaat



Hartebeesfontein



SARS-CoV-2 levels and Genomic Results in Epi week 37:

- 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. In Epi week 37, levels decreased and remain at moderate concentrations
- As of Epi week 37, SARS-CoV-2 levels decreased in Vlakplaats WWTW, after a slight increase in Epi week 36

**** Sequencing data ending in Epi week 29 in Vlakplaats and 32 in Hartebeesfontein WWTW.***

- 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 Hartrbeesfontein during Epi weeks 32.

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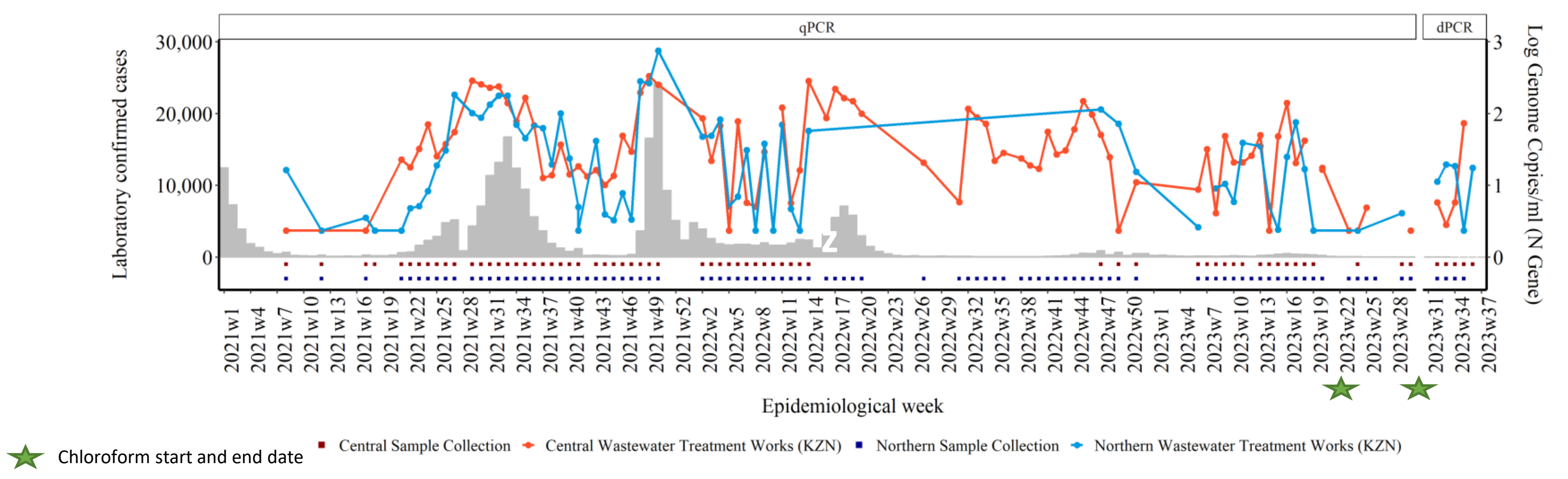
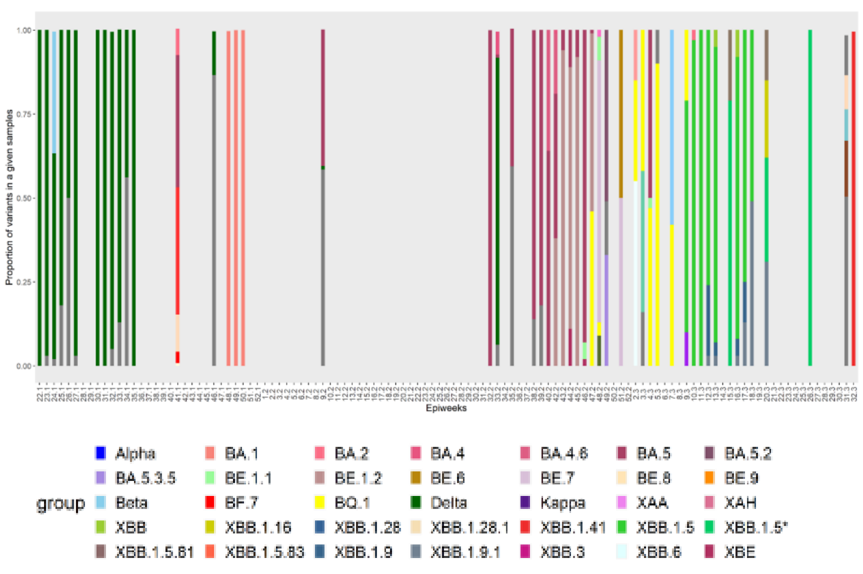
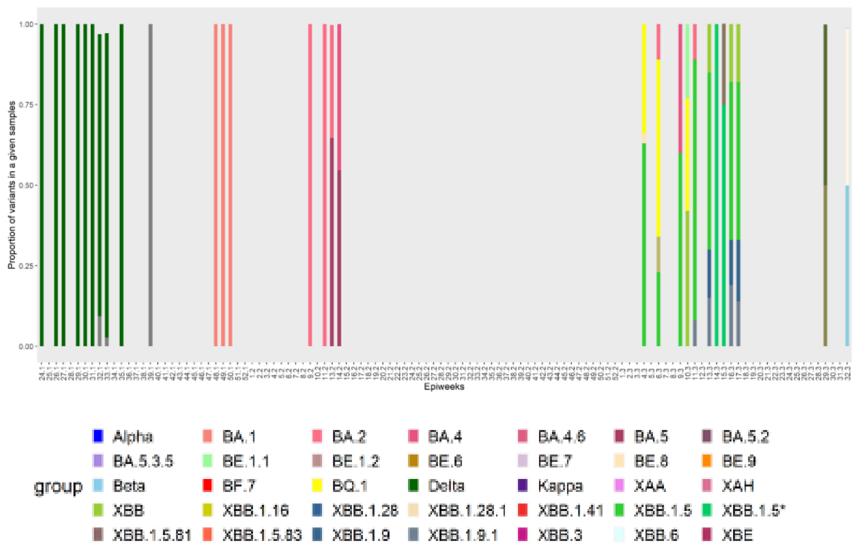
KwaZulu-Natal - eThekwini



Central



North



SARS-CoV-2 levels and Genomic Results in Epi week 37:

- No new results from Central WWTW in Epi week 37 however, the SARS-CoV-2 levels showed a sharp increases 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. No new results from Epi week 37 are available

**** Sequencing data ending in Epi week 32 in Central and Northern WWTW.***

- 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 eThekwini water treatment plant
- In eThekwini North, FL.25, BA.2.16, BA.2.15 were circulating during Epi week 32

SNP Analysis:

- In both Central and Northern eThekwini 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

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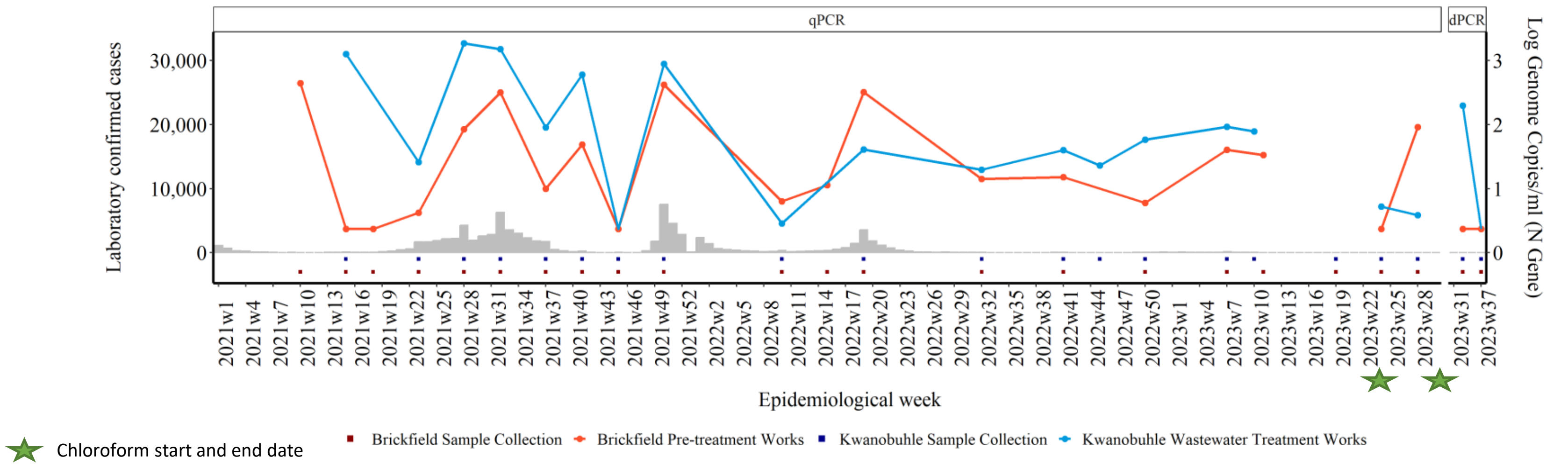
Eastern Cape – Nelson Mandela



SARS-CoV-2 levels and Genomic Results in Epi week 37:

- As of Epi week 37, a sharp decline in levels were seen in Kwanobuhle WWTW
- SARS-CoV-2 levels increased from low to intermediate from Epi week 24 to in Epi week 31 in Brickfield Pre-treatment works. As of Epi week 37, SARS-CoV-2 levels remain low

**** Sequencing data ending in Epi week 29 in Brickfield and 32 in Kwanobuhle WWTW.***

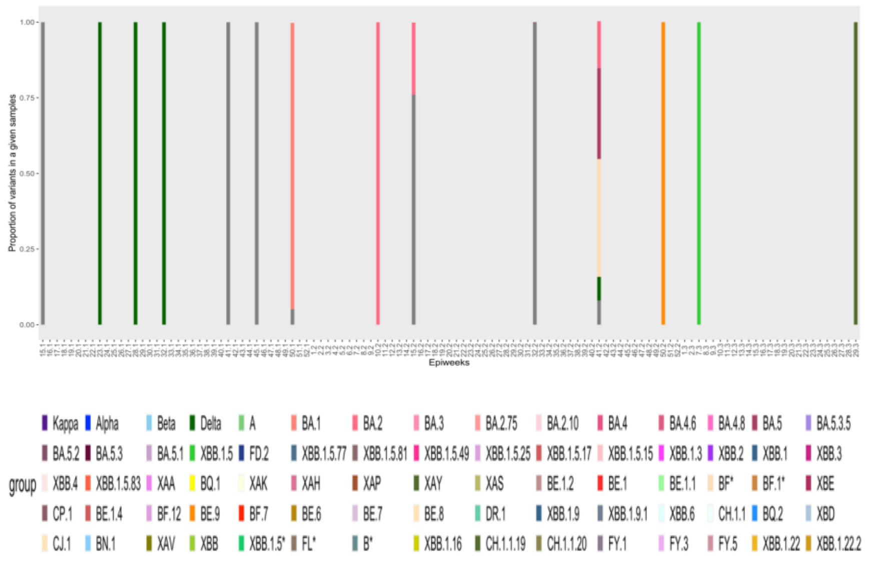
Freyja Output:

- 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.

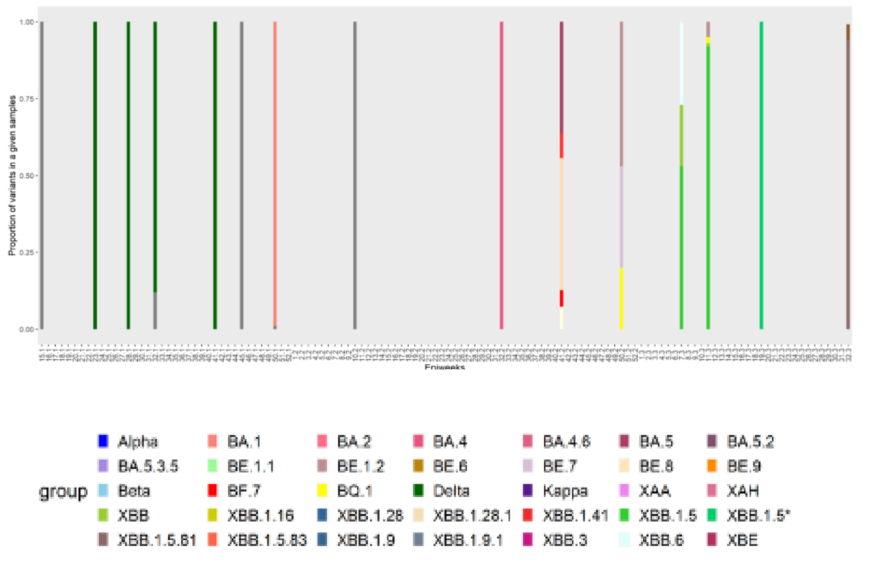
SNP Analysis:

- 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

Brickfield



Kwanobuhle



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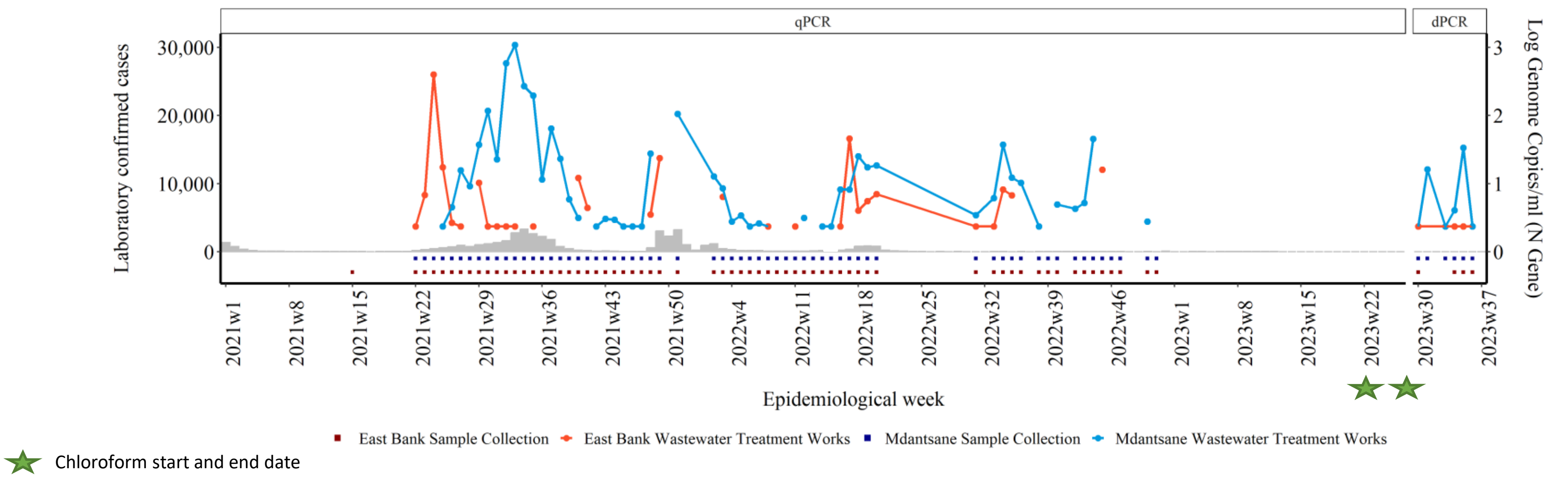
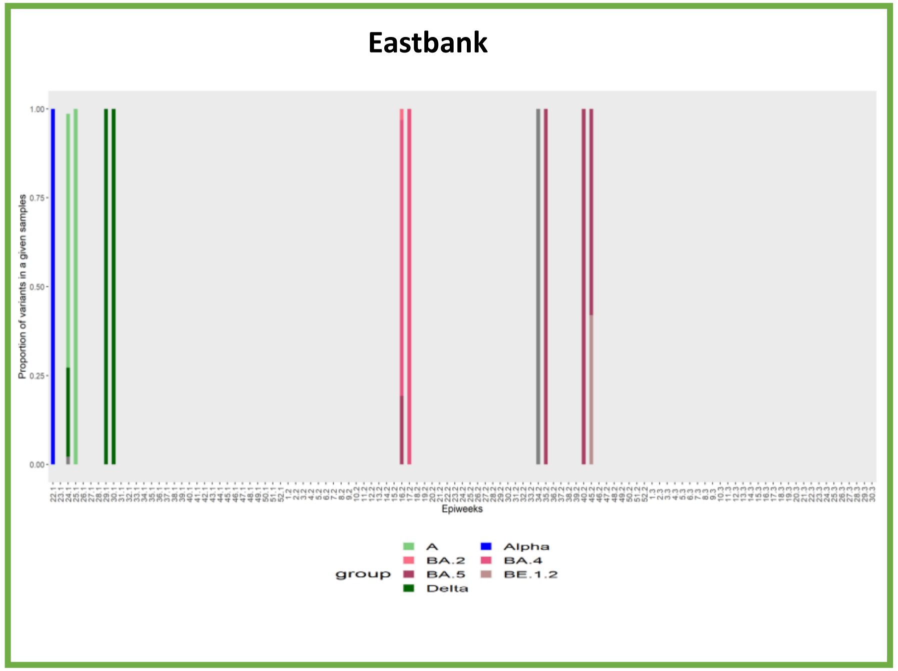
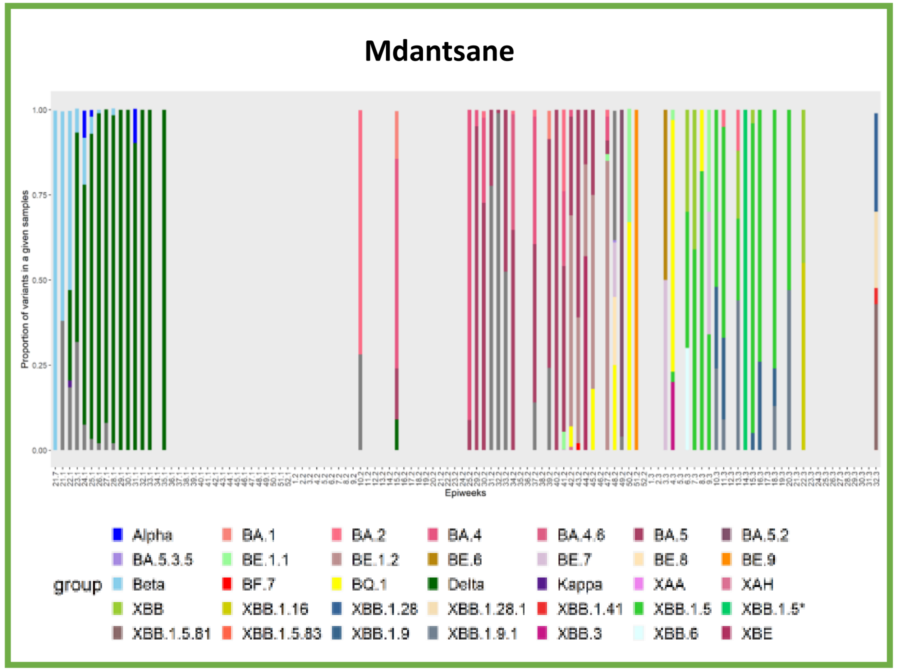
Eastern Cape – Buffalo City







SARS-CoV-2 levels and Genomic Results in Epi week 37:

- No new results from Mdantsane WWTW in week 36 and 37, however, an increase in SARS-CoV-2 levels at was seen in Epi week 35 Levels remain intermediate
- No new results from East Bank WWTW in Epi week 37 however, SARS-CoV-2 levels remained low from week 29 to Epi week 36

**** Sequencing data ending in Epi week 30 in Eastbank and 31 in Mdantsane 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

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.

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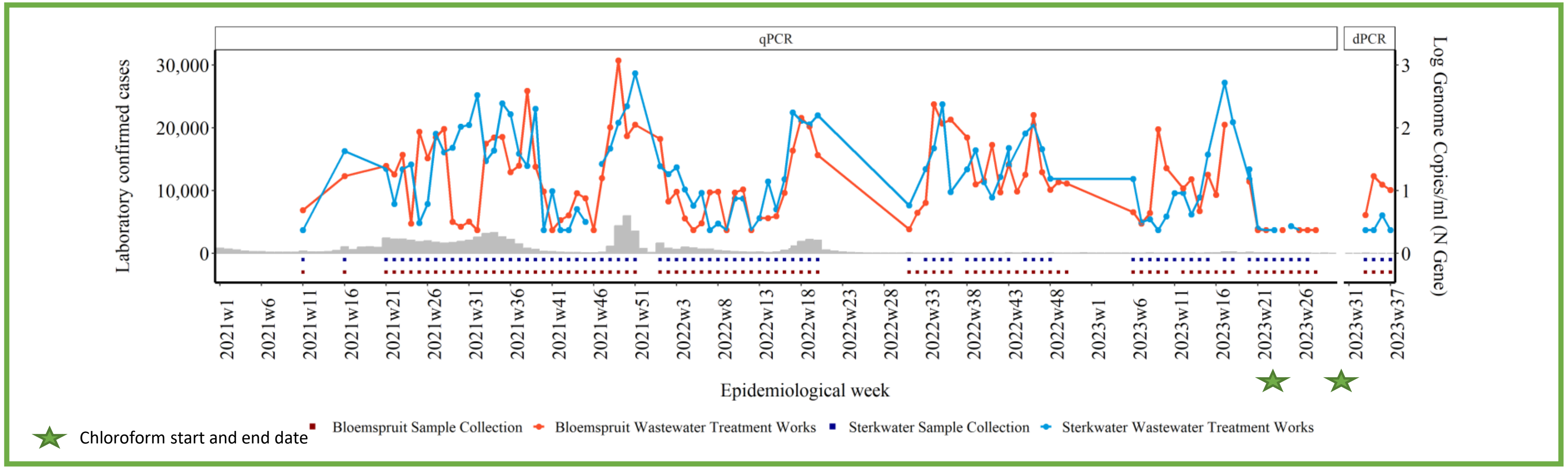
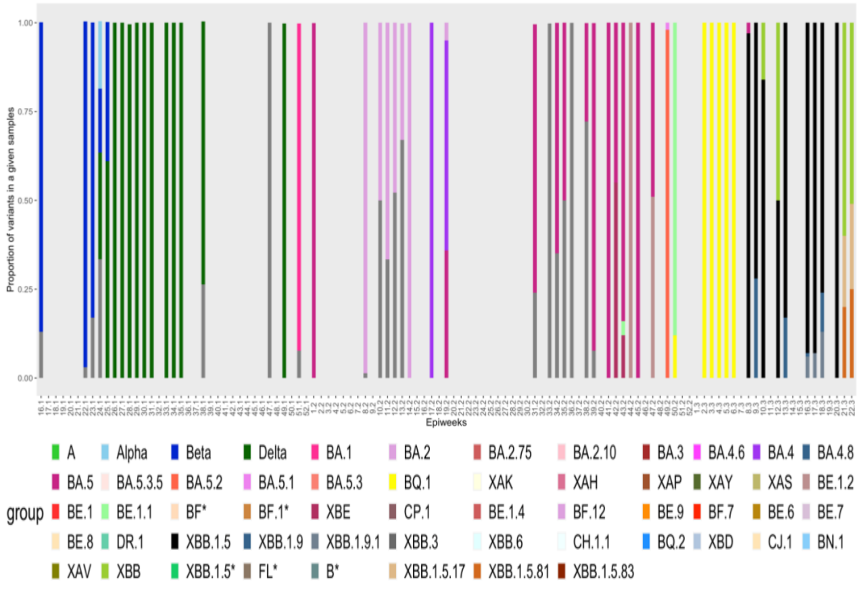
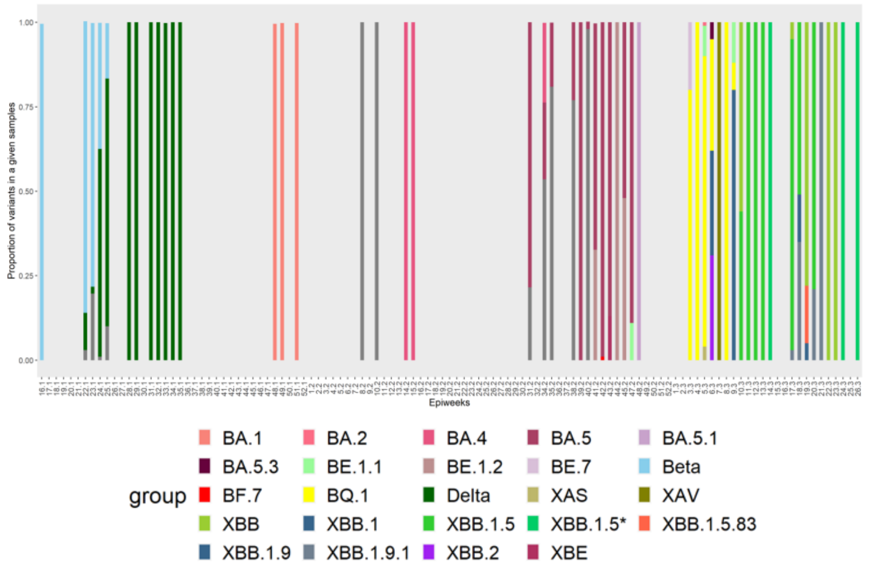
Free State – Mangaung



Bloemspruit



Sterkwater



SARS-CoV-2 levels and Genomic Results in Epi week 37:

- In Bloemspruit WWTW, a small decrease in SARS-CoV-2 levels was observed. Levels remain intermediate to low
- A decrease in SARS-CoV-2 levels were seen in Sterkwater WWTW, and levels remain low in Epi week 37

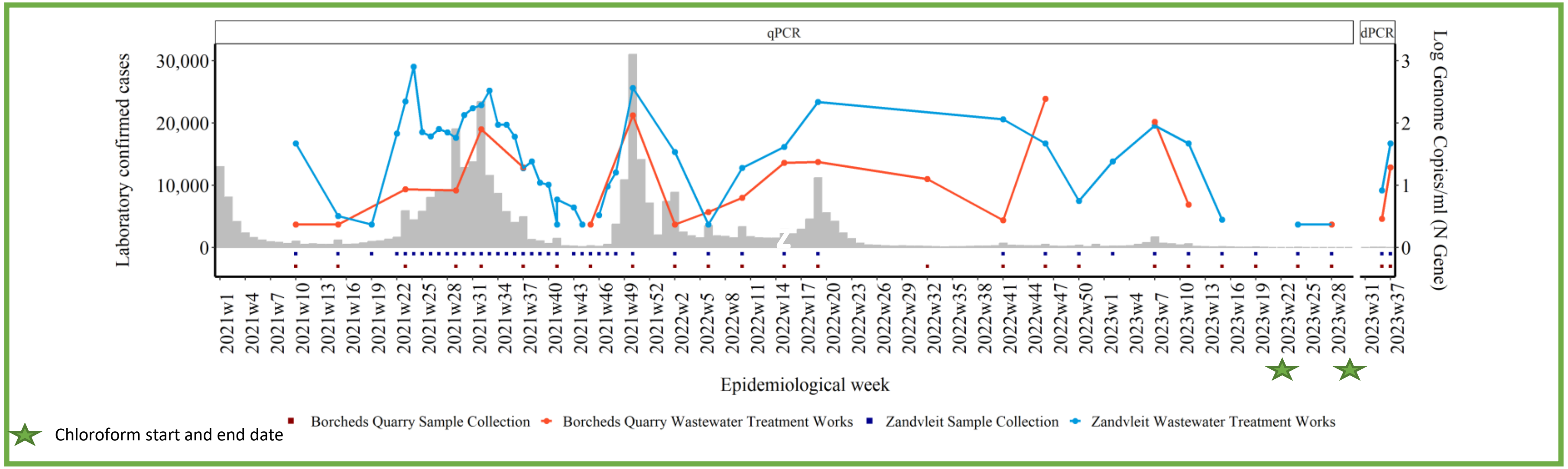
**** Sequencing data ending in Epi week 22 in Bloemspruit and 26 in Sterkwater WWTW.***

- 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

Western Cape – City of Cape Town



SARS-CoV-2 levels and Genomic Results in Epi week 37:

- In Epi week 37, SARS-CoV-2 levels in Borcheds Quarry WWTW increased 2-fold to intermediate concentrations
- A 2-fold increase in SARS-CoV-2 levels were observed in Zandevleit WWTW in Epi week 37

**** Sequencing data ending in Epi week 19 in Borcheds and Zandvleit WWTW.***

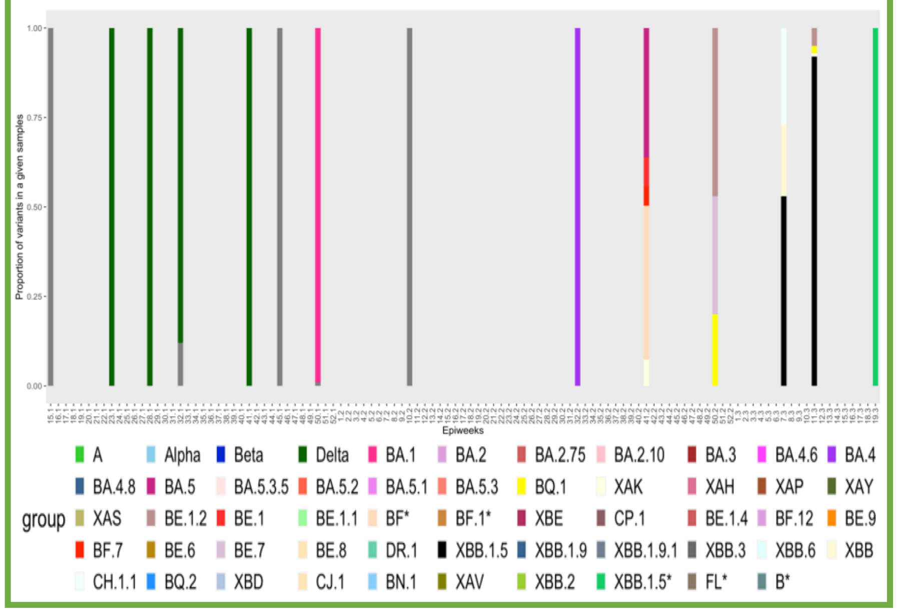
Freyja output:

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

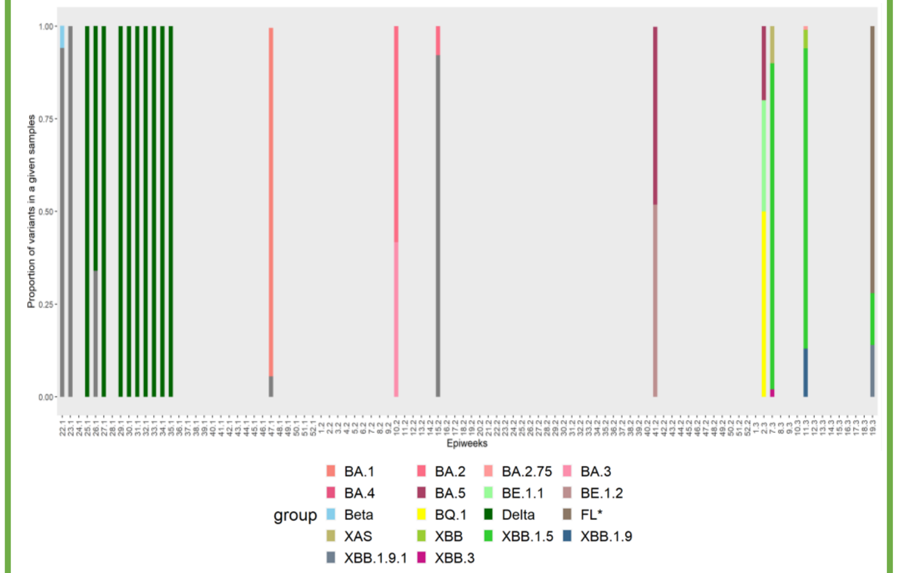
SNP Analysis:

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

Borcheds Quarry



Zandvleit



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Division of the National Health Laboratory Service

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TEAM