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

3 November 2023

Sample collection dates up to 27 October 2023
(Epidemiological week 43)

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 33-43

- From weeks 33-43, 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 **33-43 increases and/or higher levels have been seen in Gauteng (Rooiwal WWTW, Goudkoppies WWTW and Vlakplaats WWTW), Eastern Cape (Mdantsane WWTW), Free State (Sterkwater WWTW) and Cape Town (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 33-43

- No new genomics results were obtained for week 43
- Genomics results were obtained for weeks 33-39 for the heat map and mutational profile.
- **Omicron lineage BA.2.86 followed by JB.2, XBB.1.4, XBB.1.5.81 and XBB sub-lineages** were the dominant lineages circulating in wastewater samples between August and September 2023
- In clinical samples, **BA.2.86** was also the dominant lineage circulating between August and September 2023, followed by **XBB.1.5** and **XBB.1.5.81.**
- The **Omicron lineage BA.2.86** 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 lineage BA.2.86.

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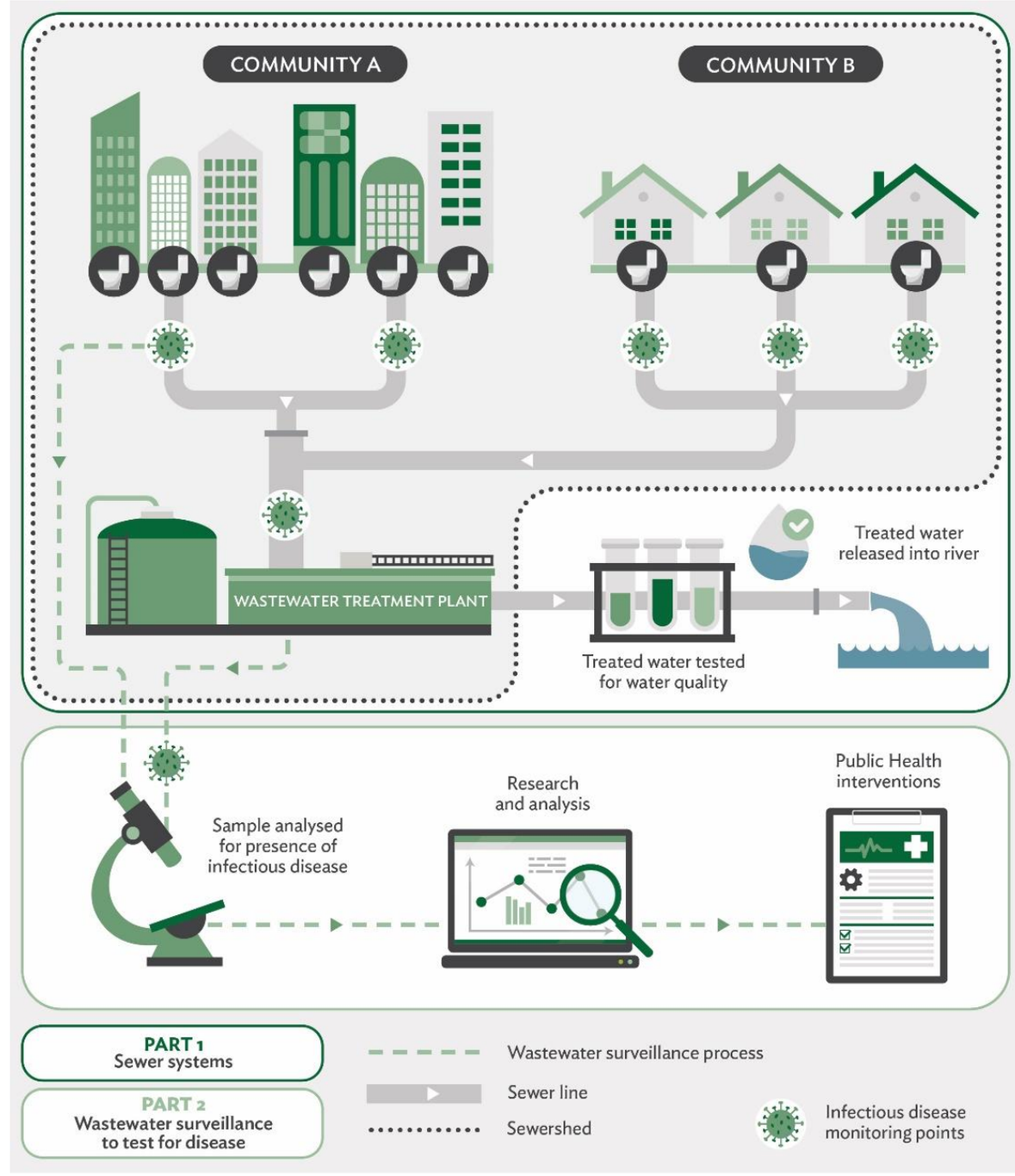
Wastewater-based Epidemiology for COVID-19

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

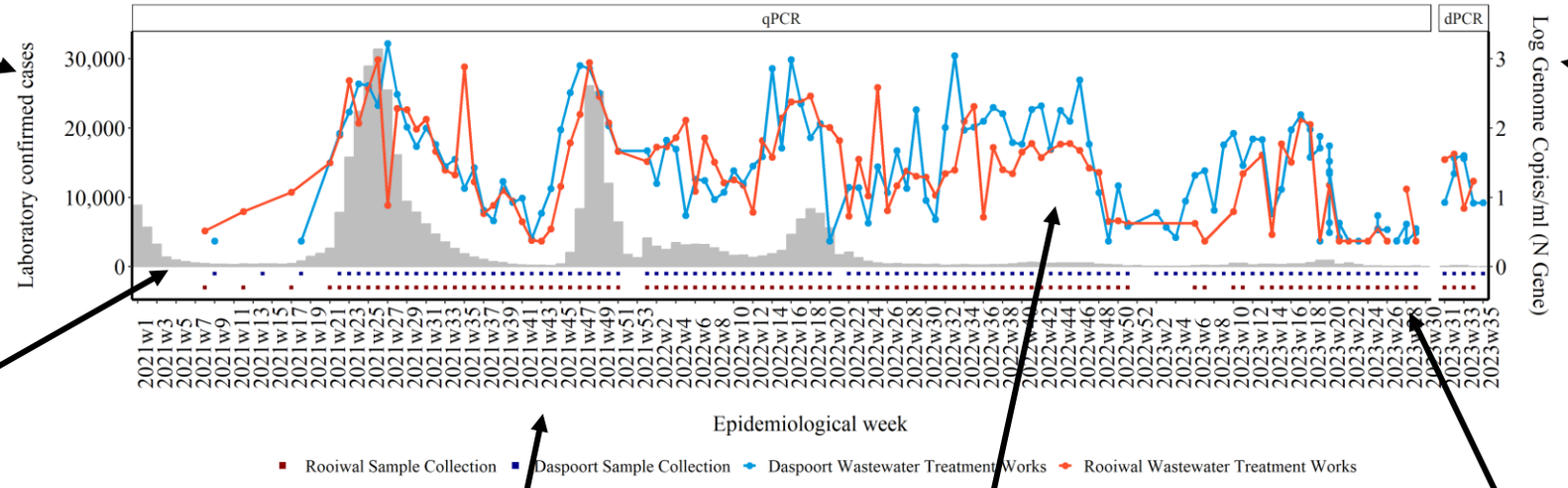


Wastewater-based Epidemiology for COVID-19

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*



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

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:

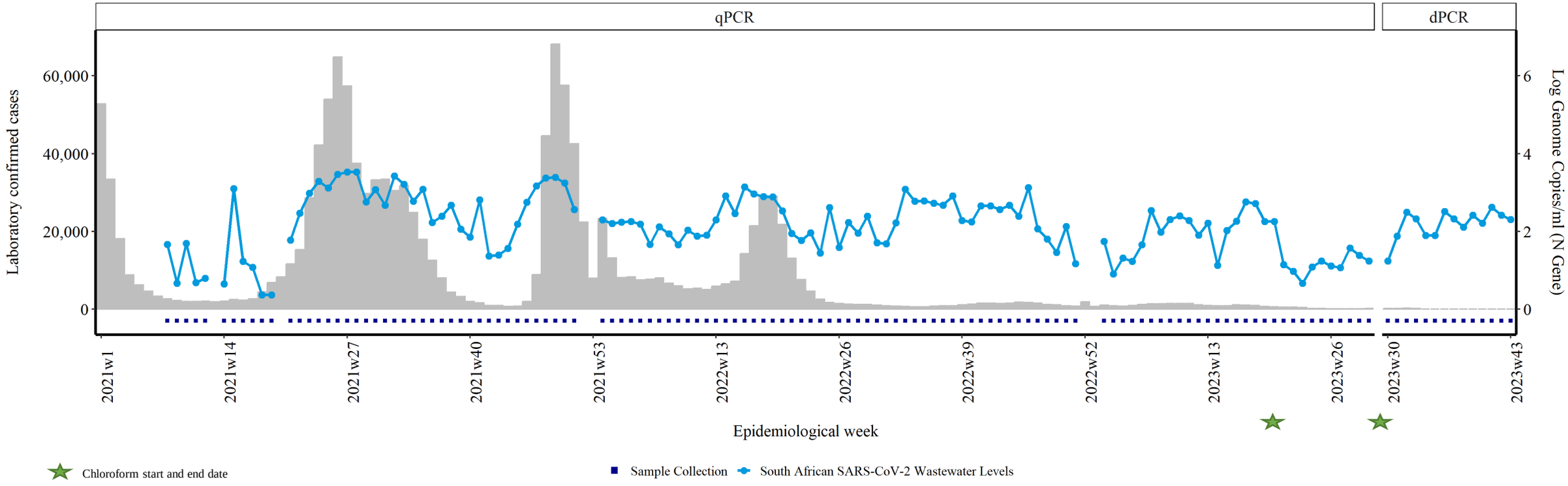
Changes in wastewater SARS-CoV-2 results over time for different treatment facilities

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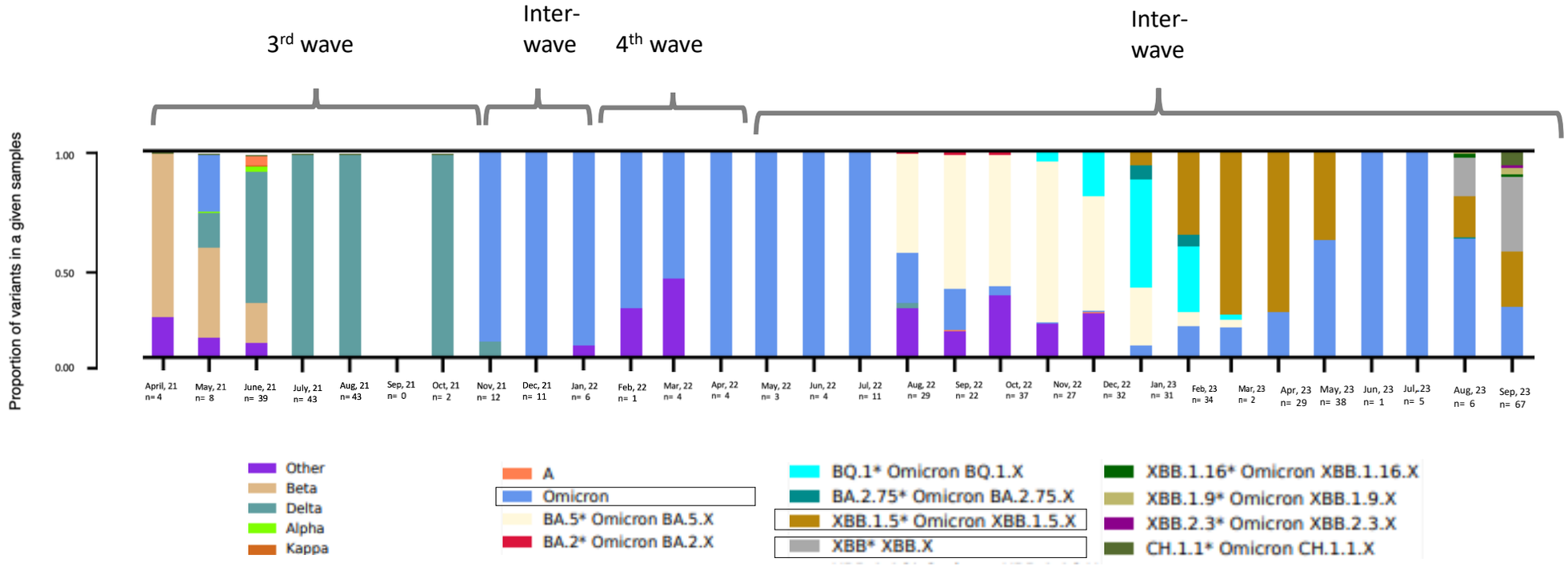
South Africa at a glance:
Circulating variants as determined by Freyja deconvolution of sequence data

- 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 39 (29 September 2023)
  - Omicron lineages XBB.1.5 and Omicron were circulating from August to September, with XBB* dominance in September.

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



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

- Results from sequencing data ending in epi week 39 (29 September 2023)
  - Omicron lineages XBB.1.41 and BA.2.86 were circulating from August to September with BA.2.86 dominance in September.
  - Omicron XBB sub-lineages were in circulation throughout August.
  - The predominant lineages circulating in clinical samples in the recent week are BA.2.86 followed by XBB.1.5, XBB.1.9 and XBB sub-lineages.

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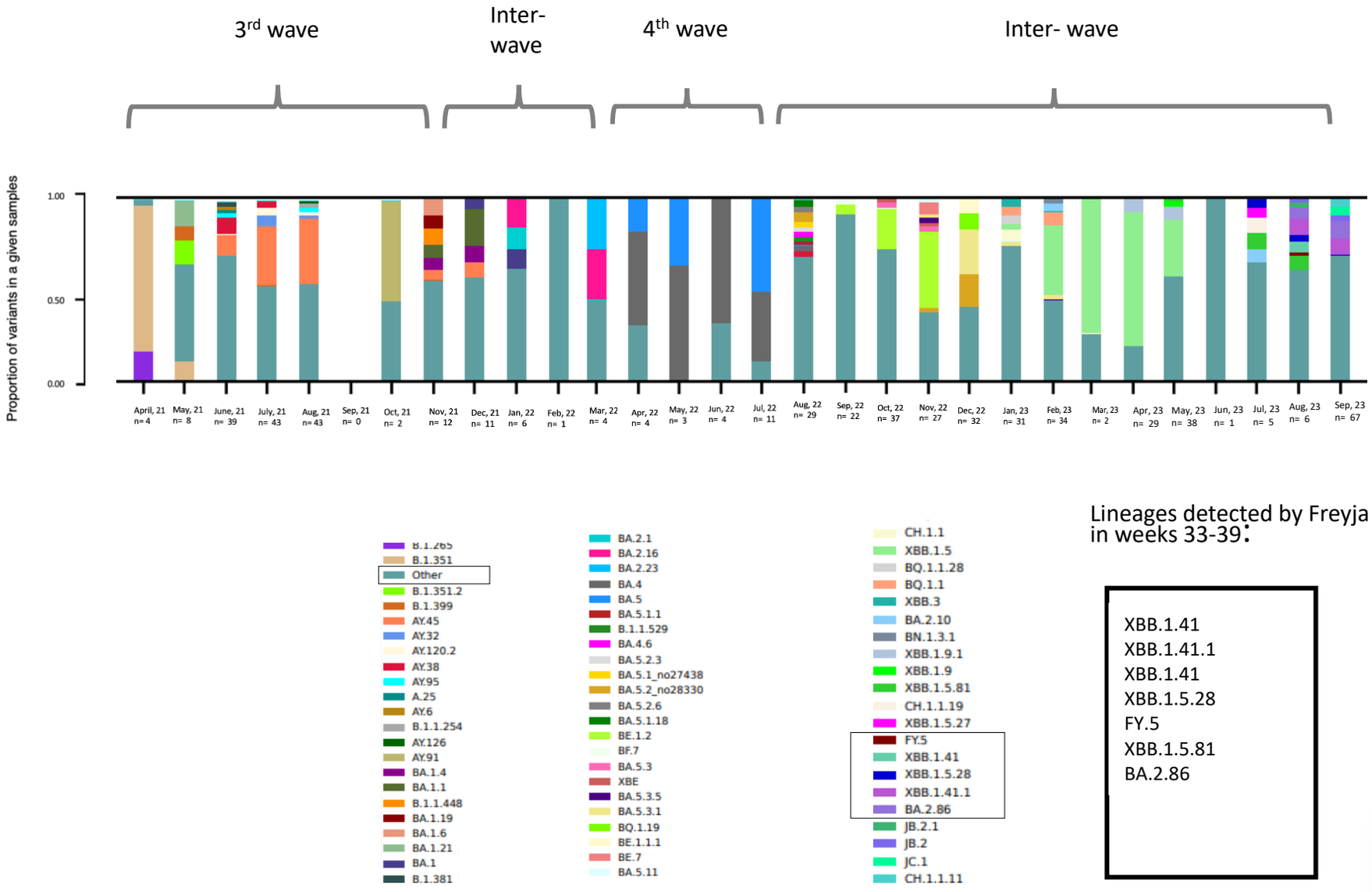

Lineages detected by Freyja in weeks 33-39:

XBB.1.41
XBB.1.41.1
XBB.1.41
XBB.1.5.28
FY.5
XBB.1.5.81
BA.2.86

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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

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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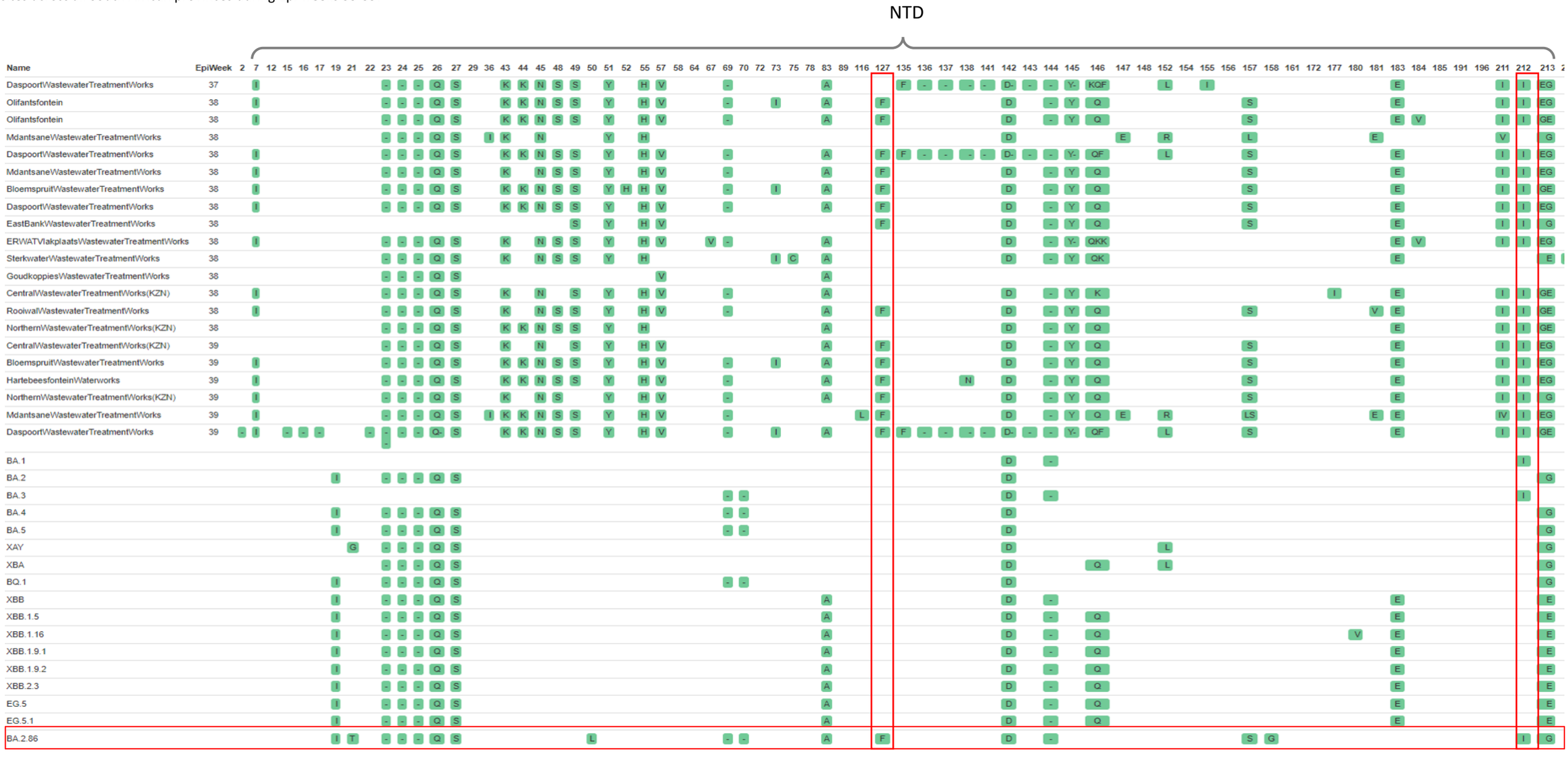
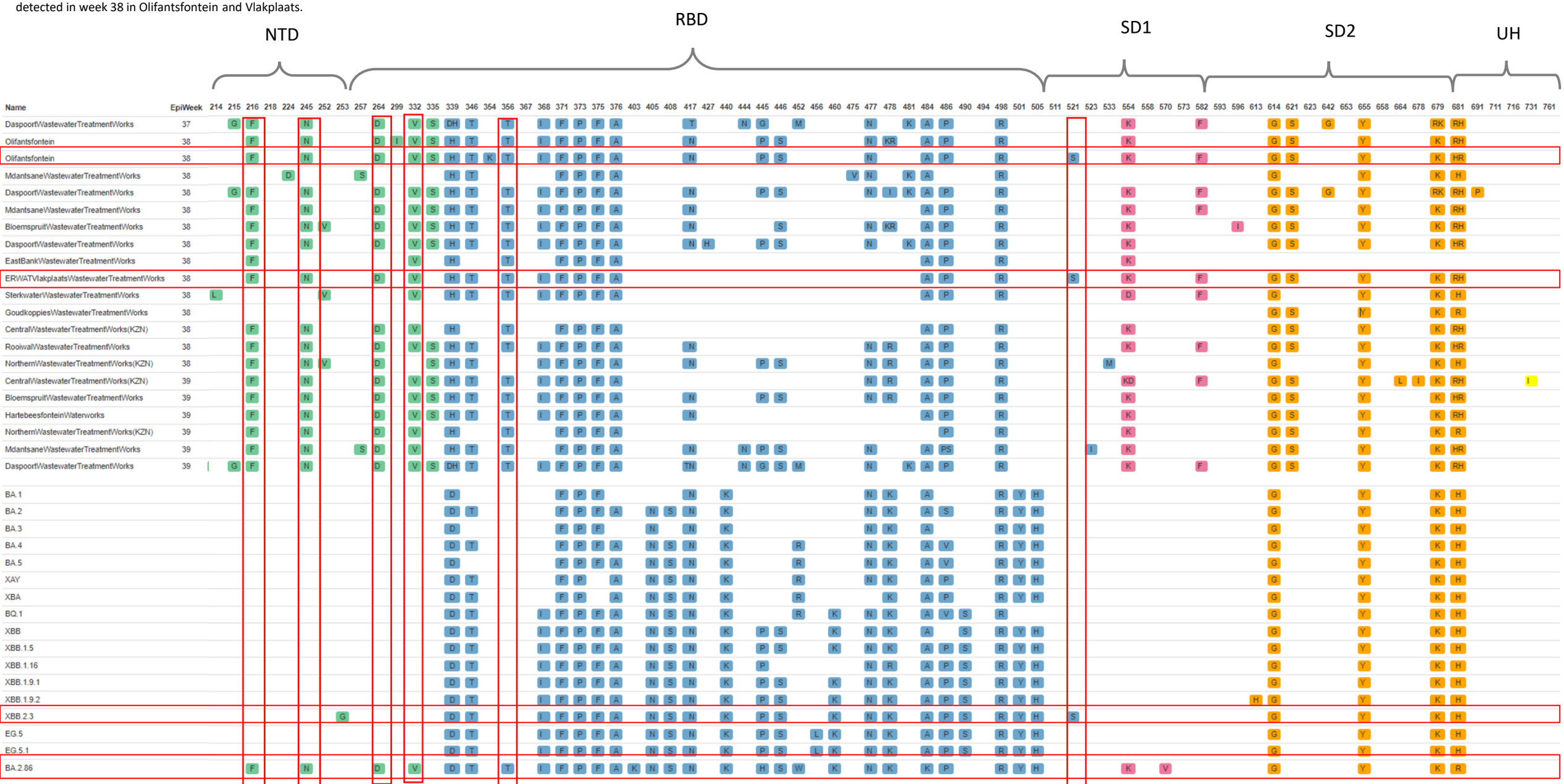
Spike mutational profile of samples from sites across South Africa and VOCs/VOIs processed in weeks 33 - 39, 2023

A combination of mutations associated with lineage BA.2.86 have been found in sites across all South African provinces during Epi weeks 33-39.



Spike mutational profile of samples from sites across South Africa and VOCs/VOIs processed in weeks 33 - 39, 2023

A combination of mutations associated with lineage BA.2.86 have been found in sites across all South African provinces during Epi weeks 33-39. XBB2.3 was also detected in week 38 in Olifantsfontein and Vlakplaats.



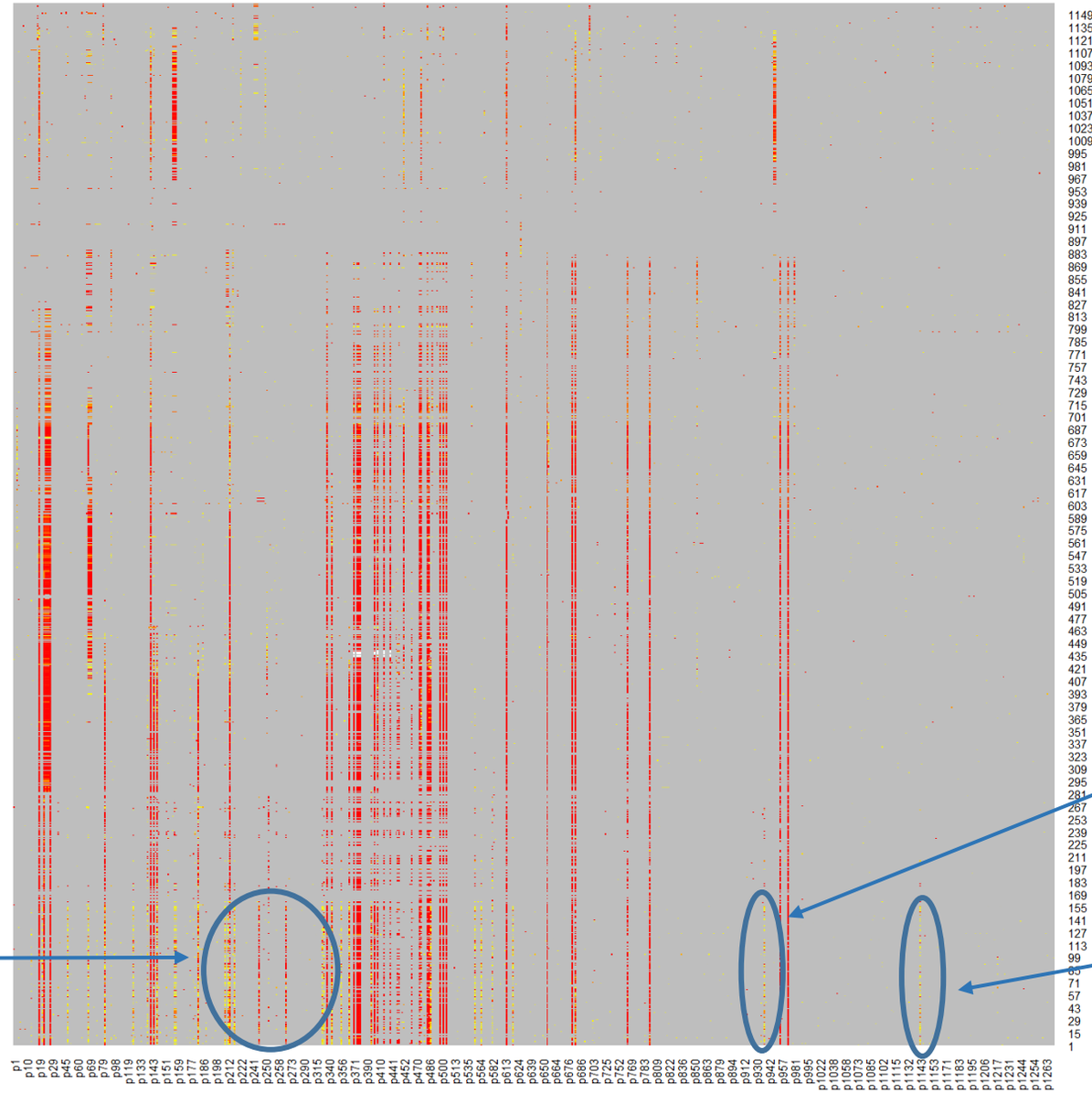
Spike mutational profile of samples from sites across South Africa and VOCs/VOIs processed in weeks 33 - 39, 2023

A combination of mutations associated with lineage BA.2.86 have been found in KwaZulu Natal, Gauteng, Free State, Eastern Cape, and Western Cape in Epi weeks 34 -36.

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

S939F
Spike protein mutation associated with the BA.2.86 lineage

P143L
Spike protein mutation associated with the BA.2.86 lineage

V213E, R346T
Mutations in spike protein associated with XBB* sub-lineages

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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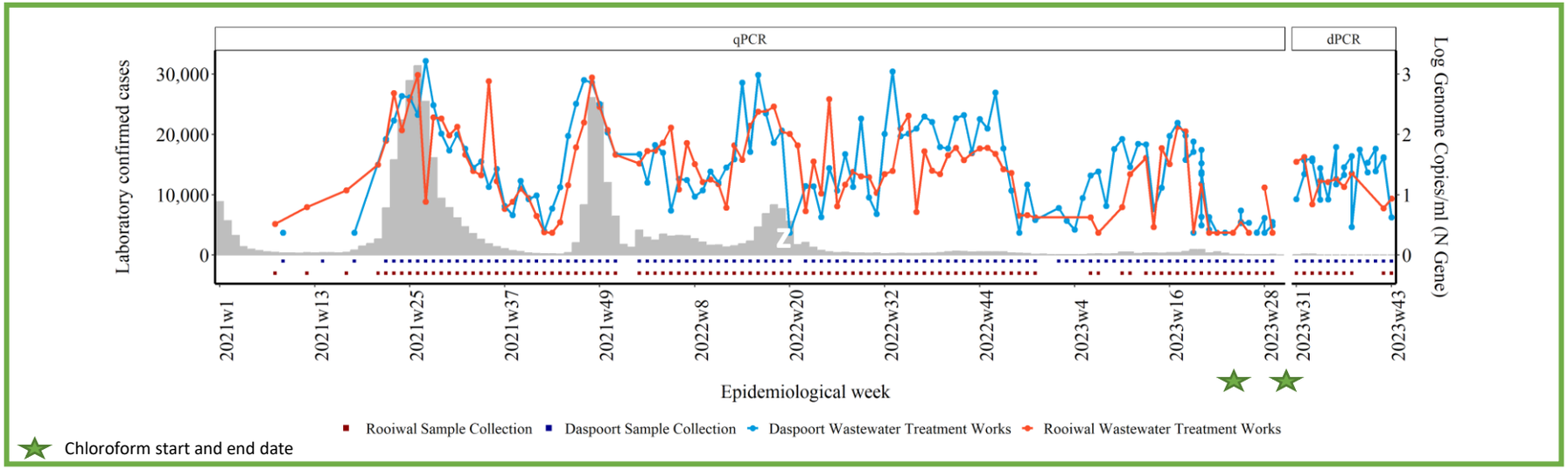
Gauteng - Tshwane



SARS-CoV-2 levels and Genomic Results in Epi week 43:

- In Epi week 43, SARS-CoV-2 levels in Daspoort WWTW decreased and are low.
- SARS-CoV-2 levels in Rooiwal WWTW have increased slightly in Epi week 43. Levels remain low.

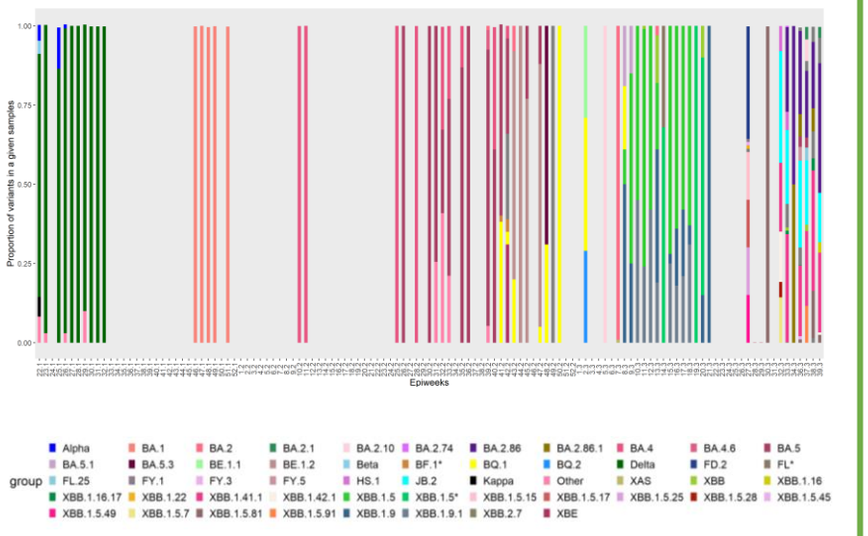
**** Sequencing data ending in Epi week 38 in Rooiwal and 39 in Daspoort. No new sequencing data available.***

- Omicron lineages XBB.1.5.49, XBB.1.16, XBB.1.9.1 and JB.2, were circulating in Daspoort during Epi week 39, with BA.2.86 dominating.
- BA.2.86 was also the dominant lineage detected in Rooiwal during epiweek 38. Other lineages including JB.2, XBB.3 and FY.5 were also in circulation.

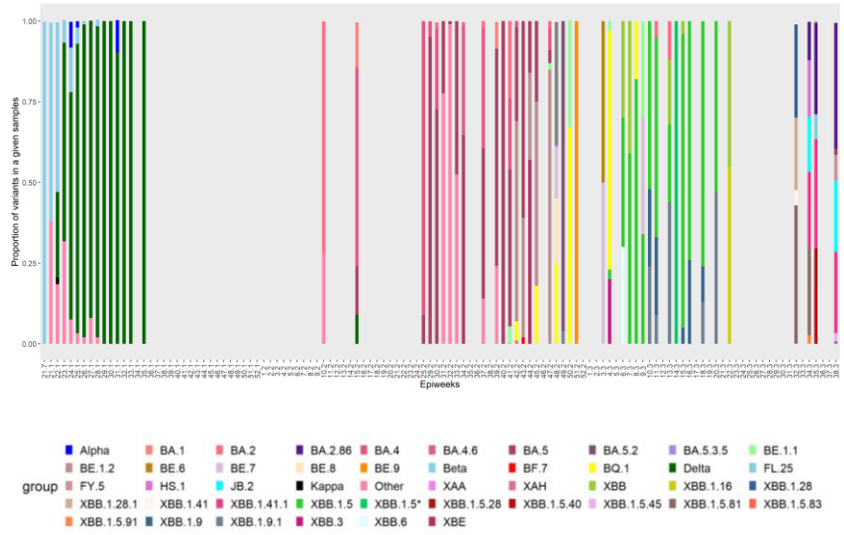
SNP Analysis:

- 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.

Daspoort Wastewater Treatment Works



Rooiwal Wastewater Treatment Works



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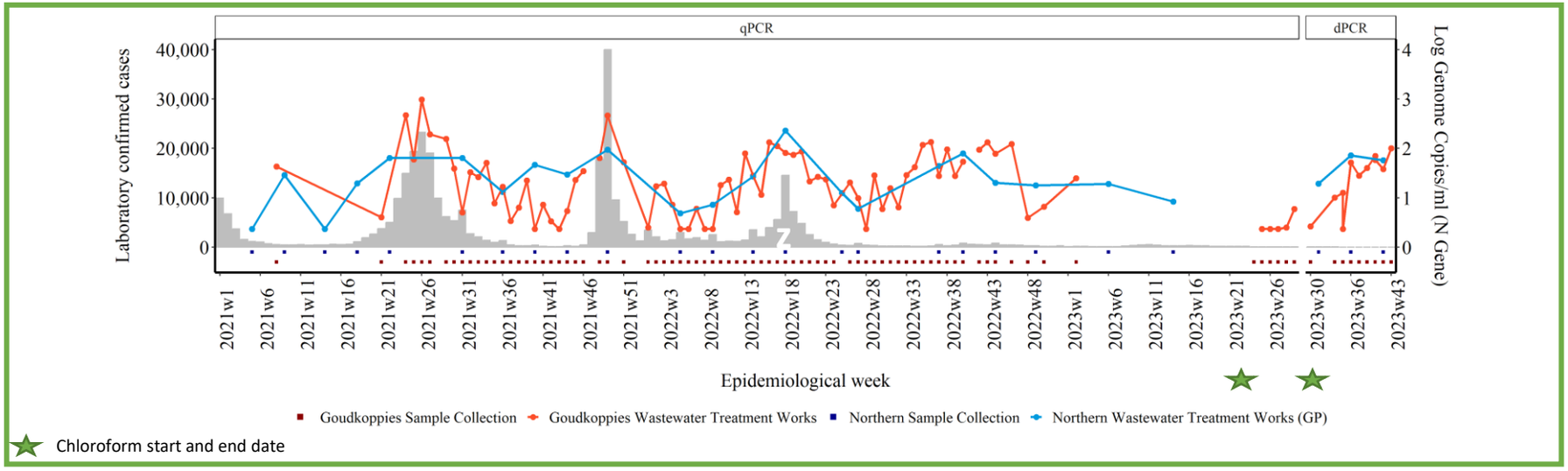
Gauteng - Johannesburg

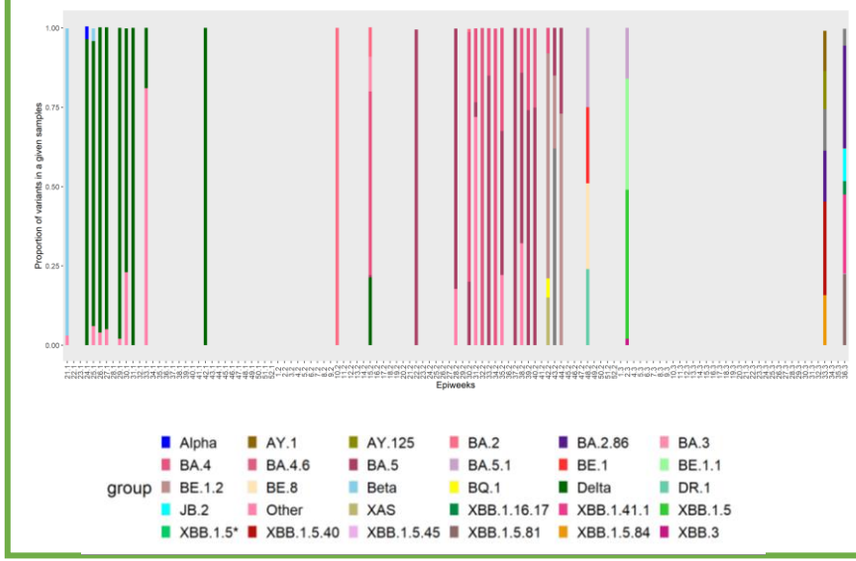


SARS-CoV-2 levels and Genomic Results in Epi week 43:

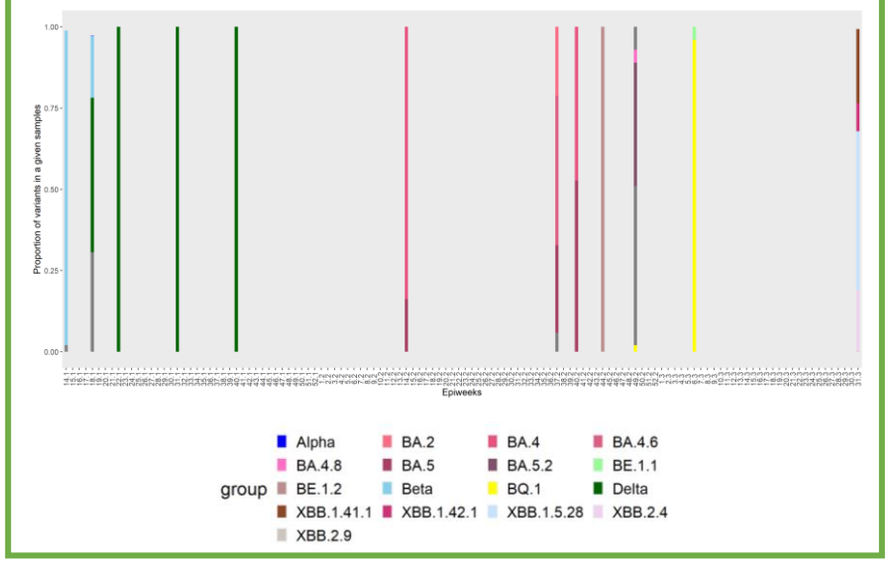
- As of Epi week 43, SARS-CoV-2 levels in Goudkoppies WWTW have increased slightly. Levels remain moderate (2 log (100) genome copies/ml of wastewater).
- In Northern WWTW, SARS-CoV-2 levels decreased slightly, but remain moderate in Epi week 42. No new results for Epi week 43 are available

**** Sequencing data ending in Epi week 36 in Goudkoppies and 31 in Northern. No new sequencing data available.***

Goudkoppies Wastewater Treatment Works



Northern Wastewater Treatment Works



- During epiweek 36, Omicron lineage BA.2.86 was dominating in epiweek 36. Other lineages in circulation included XBB.1.5.81, XBB.1.41.1, JB.2 as well as XBB.1.16.17
- 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:

- A combination of mutations (V127F, L212I, V213G, L216F, H245N, A264D, I332V, K356T) associated with lineage BA.2.86 were found in Goudkoppies.
- SNP analysis could not be performed as the SARS-CoV-2 sequencing coverage in the Northern Johannesburg samples collected during Epi week 34 was too low for meaningful interpretation

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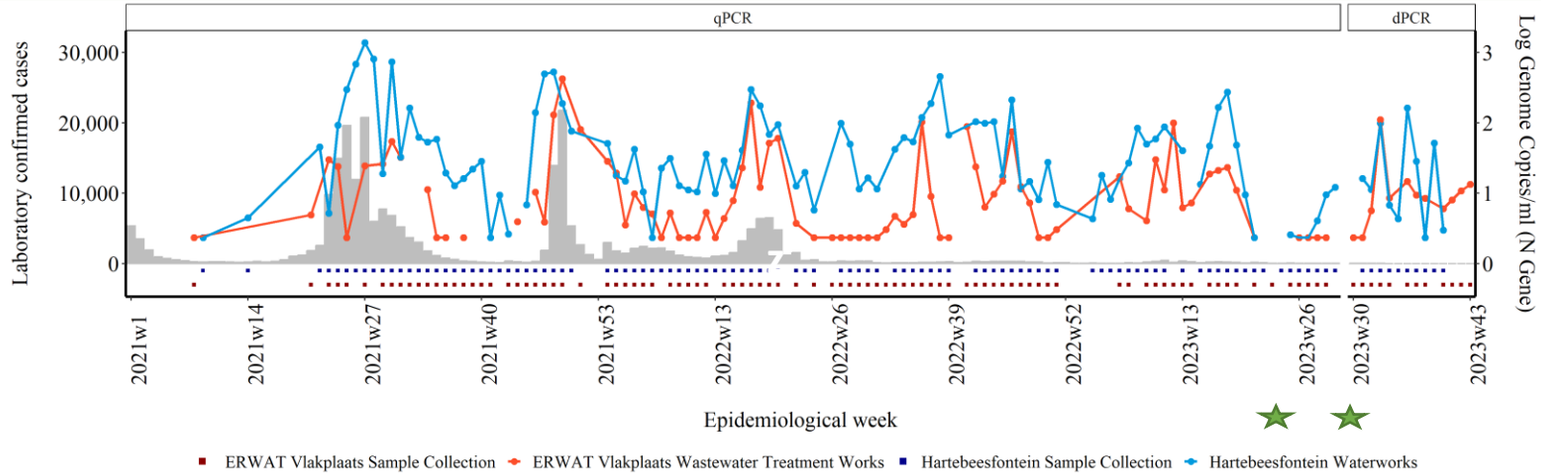
Gauteng - Ekurhuleni



★ Chloroform start and end date

SARS-CoV-2 levels and Genomic Results in Epi week 43:

- No new results were obtained in Epi week 43 in the Hartebeesfontein WWTW. The SARS-CoV-2 levels in Hartebeesfontein WWTW decreased significantly from moderate levels in Epi week 33, to low levels in Epi week 40.
- 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.

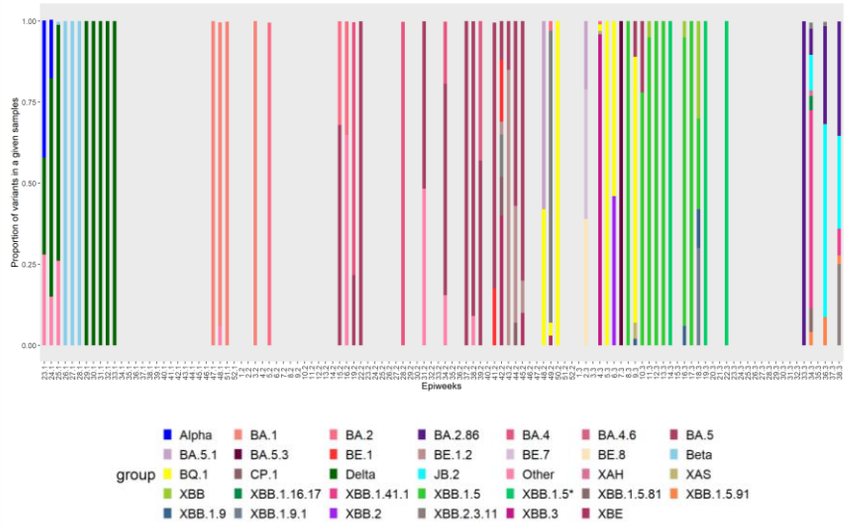
**** Sequencing data ending in Epi week 38 in Vlakplaats and 39 in Hartebeesfontein. No new sequencing data available***

- Omicron lineages BA.2.86, JB.2, XBB.1.41.1 and XBB.1.5.81 were circulating in Vlakplaats during epiweek 38
- Lineages JB.2, XBB.1.41.1 and XBB.1.5.81 were circulating during Epi week 39 at the Hartebeesfontein water treatment plant, with BA.2.86 dominating during week 39.

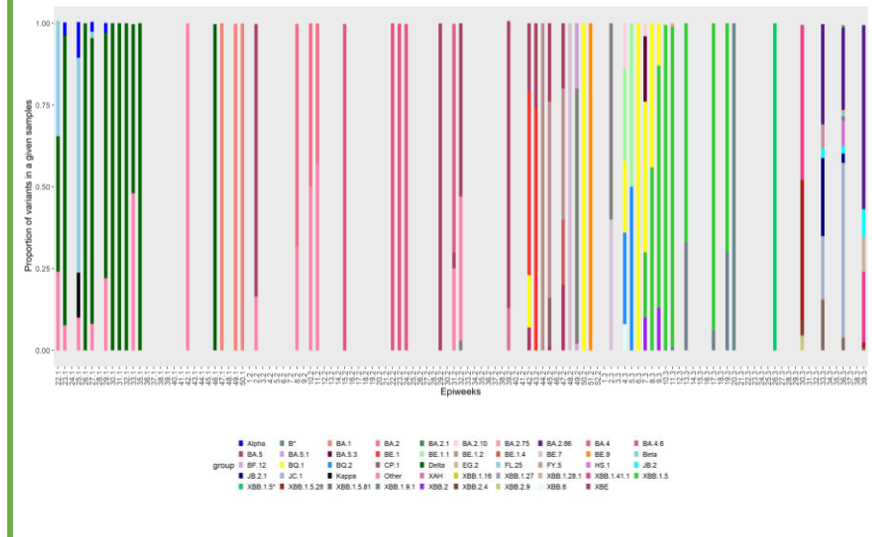
SNP Analysis:

- 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.

ERWAT Vlakplaats Wastewater Treatment Works



Hartebeesfontein Wastewater Treatment Works



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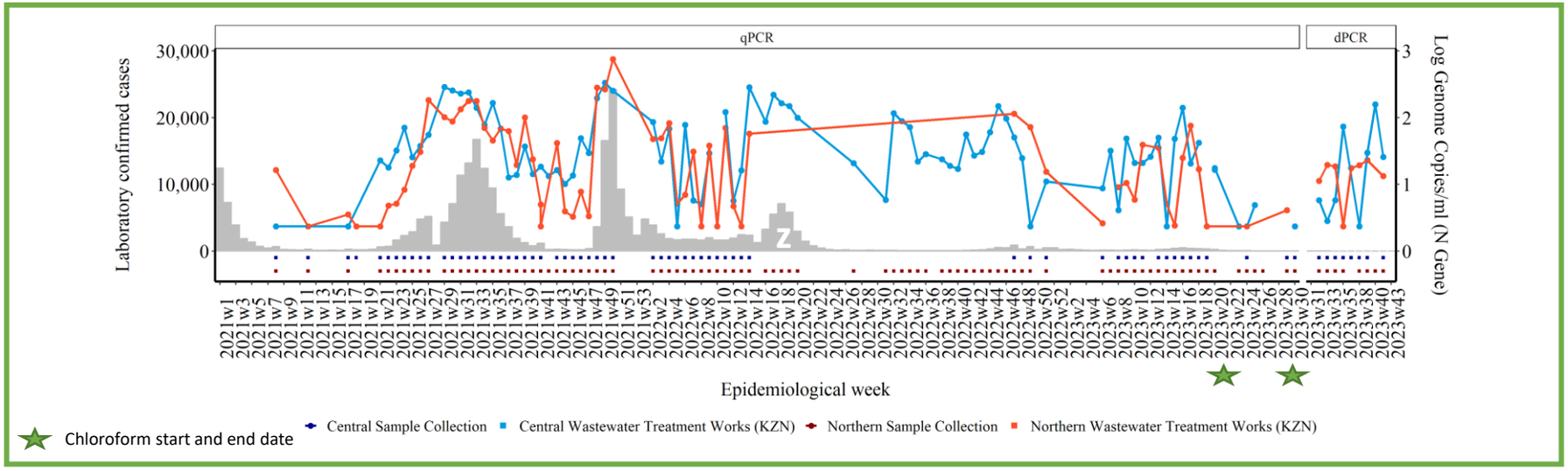
KwaZulu-Natal - eThekwini



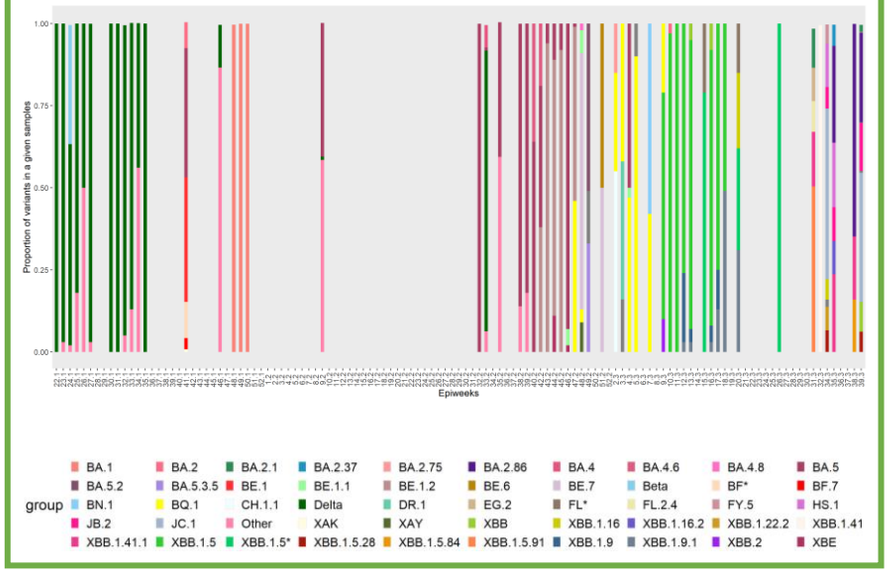
SARS-CoV-2 levels and Genomic Results in Epi week 43:

- No new results were obtained in Epi week 43 however, SARS-CoV-2 levels in Central WWTW in Epi week 39 showed a sharp increase from low levels in Epi week 38 (1 log copy/ml) to moderate levels (2 log copies/ml), followed by a decrease. Levels remain moderate.

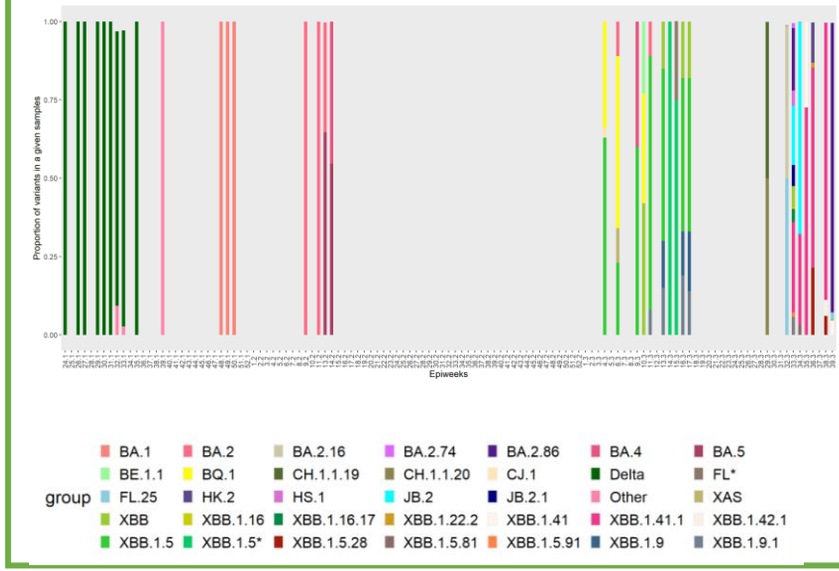
- No new results were obtained in Epi week 43 however, SARS-CoV-2 levels increased from low to moderate in week Epi 36 in Northern WWTW, after which there was a slight decrease. Levels remain moderate in Epi week 42.

**** Sequencing data ending in Epi week 39 in Central eThekwini and in eThekwini North. No new sequencing data available***

Central Wastewater Treatment Works



Northern Wastewater Treatment Works



- XBB, XBB.1.5.28, JC.1, JB.2 were circulating in Epi week 39, with a BA.2.86 dominance at the Central eThekwini water treatment plant.

- In eThekwini North, XBB.1.42.1, JB.2, and BA.2.86 were circulating during Epi week 39

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.

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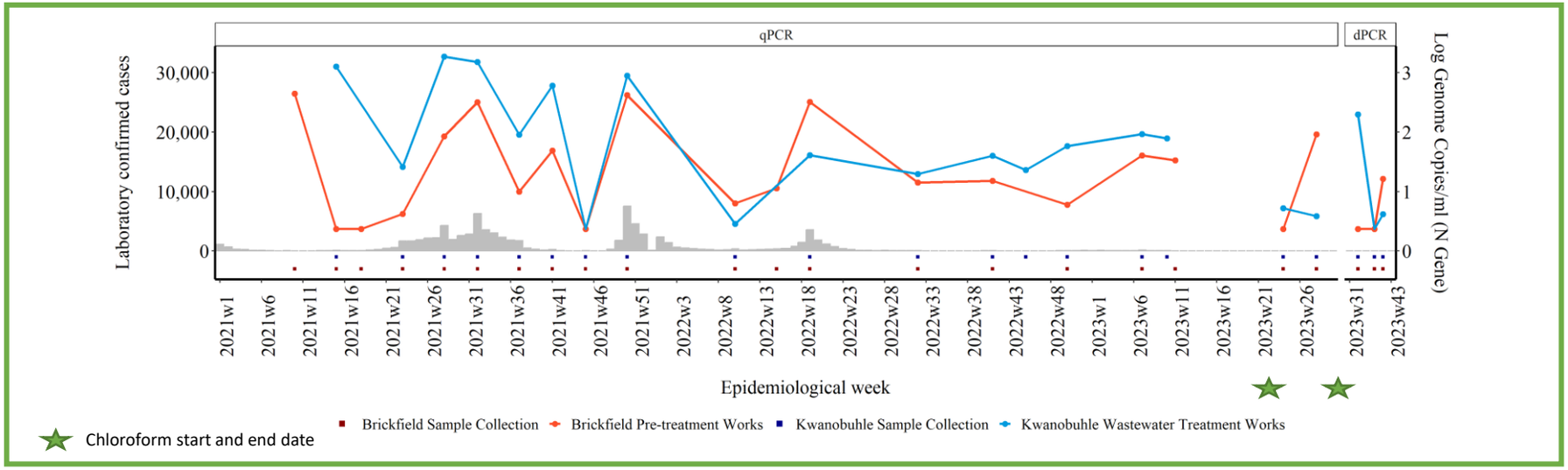
Eastern Cape – Nelson Mandela

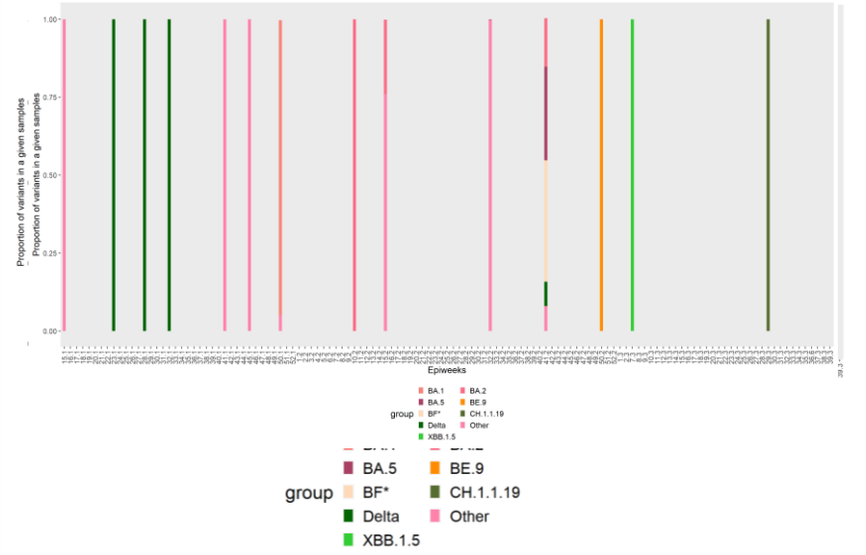


SARS-CoV-2 levels and Genomic Results in Epi week 43:

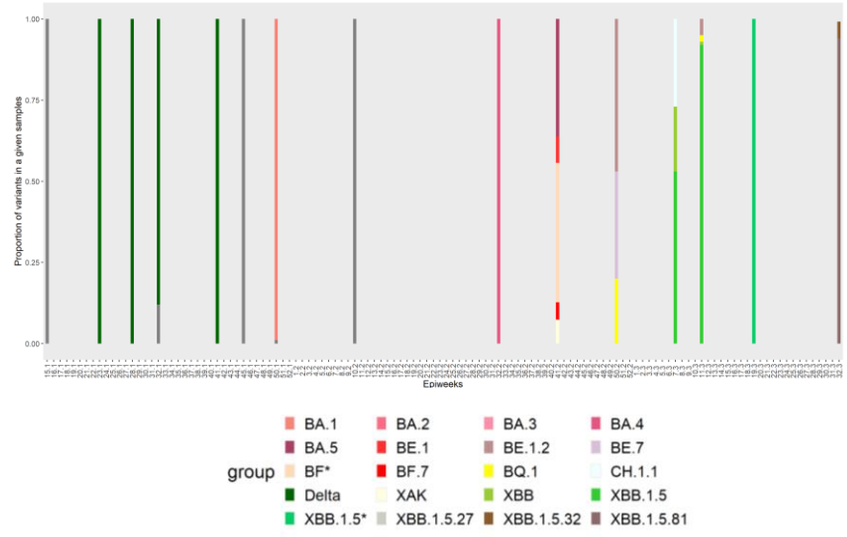
- No new results were obtained in Epi week 43, however, in Epi week 37, a sharp decrease in SARS-CoV-2 levels were seen in Kwanobuhle WWTW, followed by a slight increase. SARS-CoV-2 levels remain low.
- SARS-CoV-2 levels increased from low to moderate from Epi week 33 to Epi week 34 in Brickfield Pre-treatment works. No new results are available for Epi week 43.

**** Sequencing data ending in Epi week 29 in Brickfield and 32 in Kwanobuhle. No new sequencing data available***

Brickfield Pre-treatment works



Kwanobuhle Wastewater Treatment Works



- 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 and Kwanobuhle samples collected during Epi weeks 30-39 were too low for meaningful interpretation.

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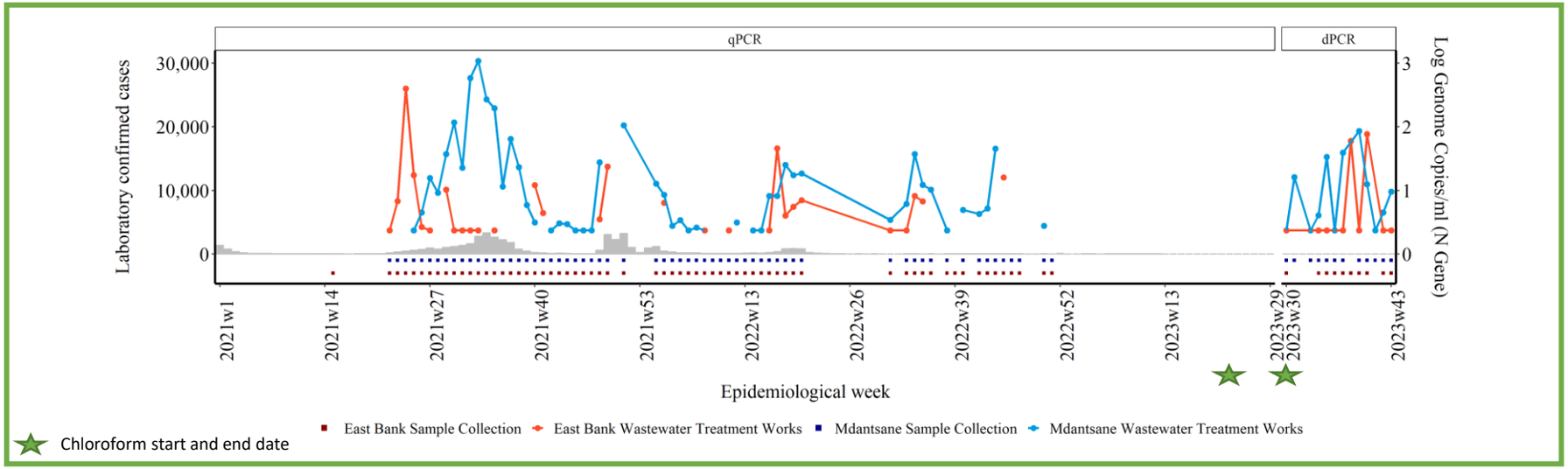
Eastern Cape – Buffalo City



SARS-CoV-2 levels and Genomic Results in Epi week 43:

- In Epi week 43, SARS-CoV-2 levels in Mdantsane WWTW increased but remain low.
- SARS-CoV-2 levels in East Bank WWTW in Epi week 43 were low after decrease was observed in Epi week 38.

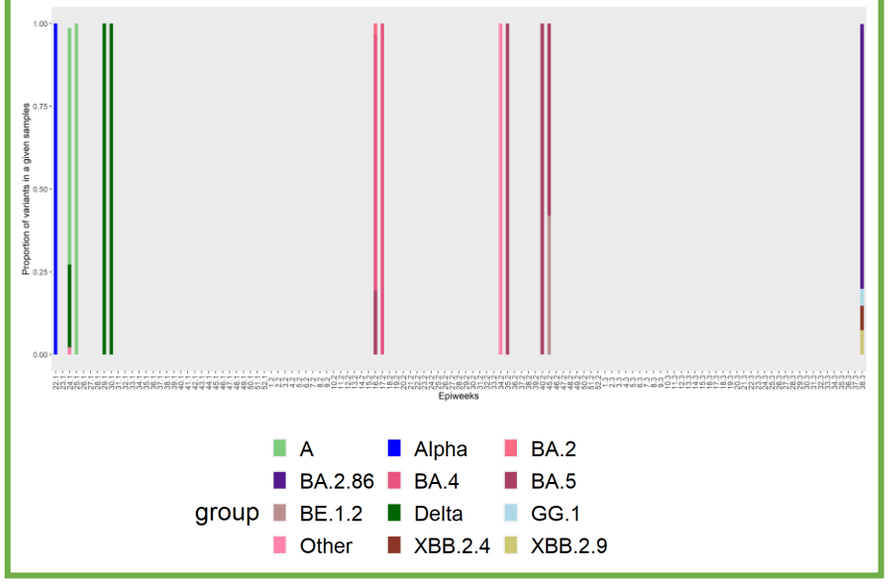
**** Sequencing data ending in Epi week 38 in Eastbank and 39 in Mdantsane. No new sequencing data available***

- Omicron lineage BA.2.86, XBB.2.9, XBB.2.9 and GG.1 were circulating in Eastbank during Epi week 38.
- Lineages CH.1.1.11, BA2.86, XBB.1.5.81 and XBB.1.5.88 were circulating in Mdantsane during Epi week 39.

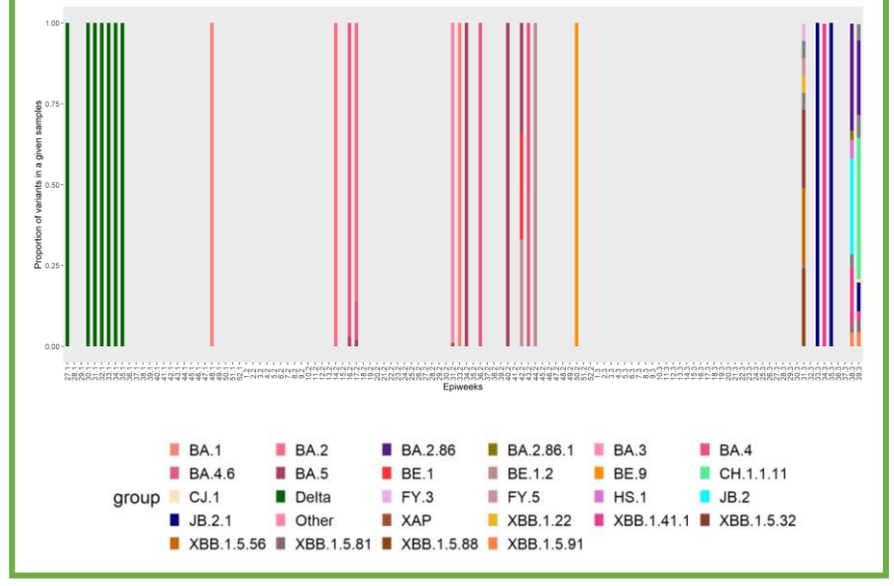
SNP Analysis:

- 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.

East Bank Wastewater Treatment Works



Mdantsane Wastewater Treatment Works



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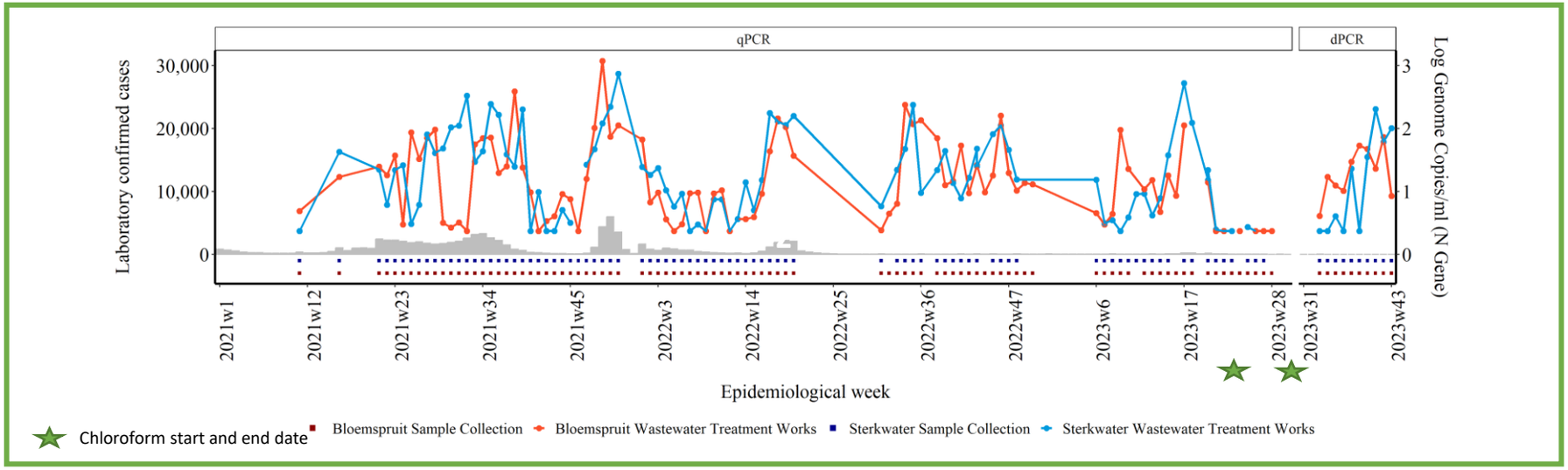


SARS-CoV-2 levels and Genomic Results in Epi week 43:

- In Bloemspruit WWTW, a decrease in SARS-CoV-2 levels were seen in Epi week 43. Levels are low.
- A sharp increase in SARS-CoV-2 levels were seen in Sterkwater WWTW from Epi week 38. In Epi week 42 levels decreased and increased again in Epi week 43 and remain moderate.

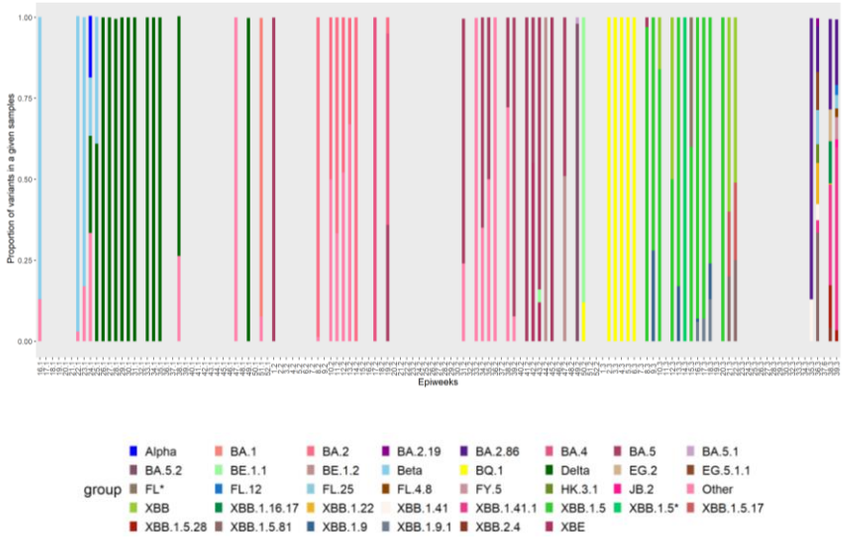
*** Sequencing data ending in Epi week 39 in Bloemspruit and 38 in Sterkwater. *No new sequencing data available***

- Lineage JB.2 was the dominant lineage circulating in Bloemspruit during week 39, with BA.2.86, FL.25, FY.5 and XBB.2.4 also circulating in the area.
- XBB.1.41.1 was the dominant lineage circulating in Sterkwater during week 38. HS.1, JB.2, XBB.1.22 and JB.2.1 were also circulating during this time.

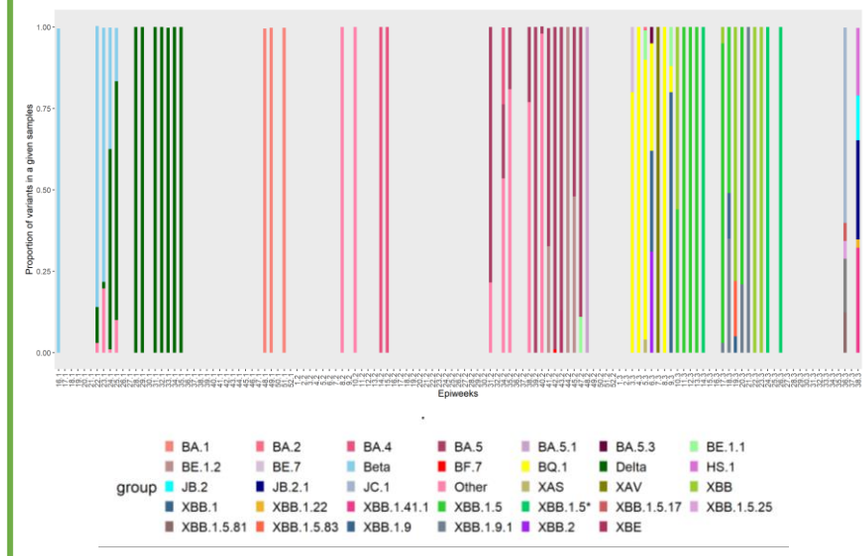
SNP Analysis:

- 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.

Bloemspruit Wastewater Treatment Works



Sterkwater Wastewater Treatment Works



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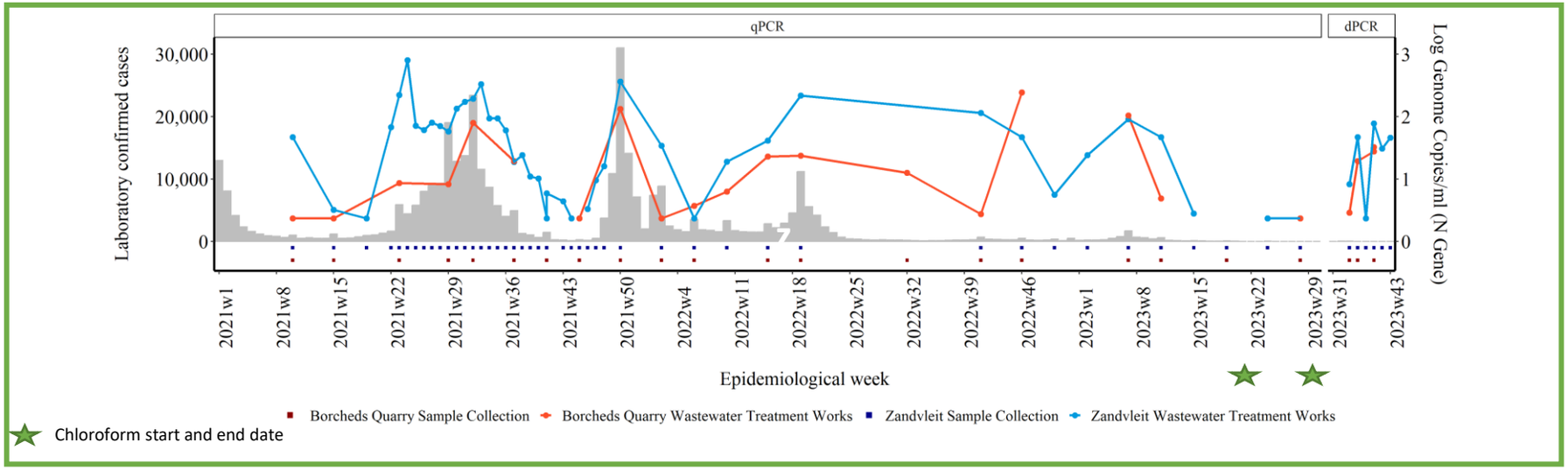
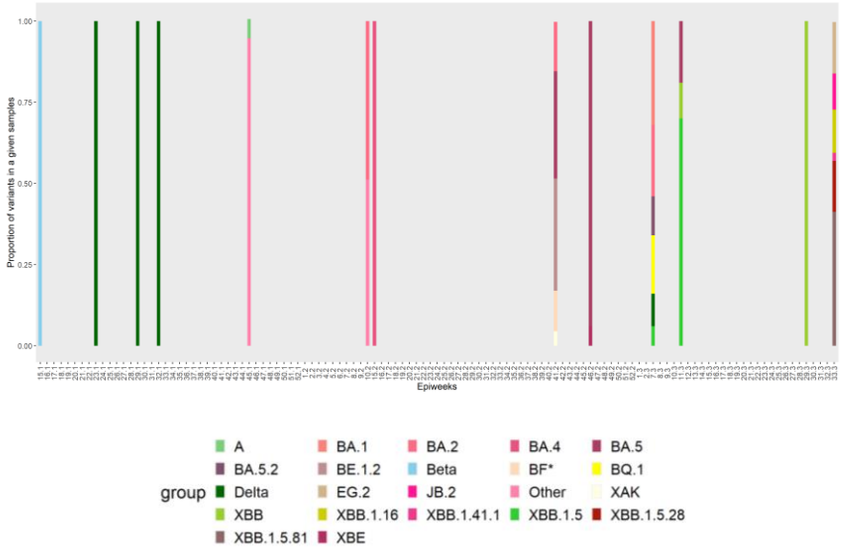


SARS-CoV-2 levels and Genomic Results in Epi week 43

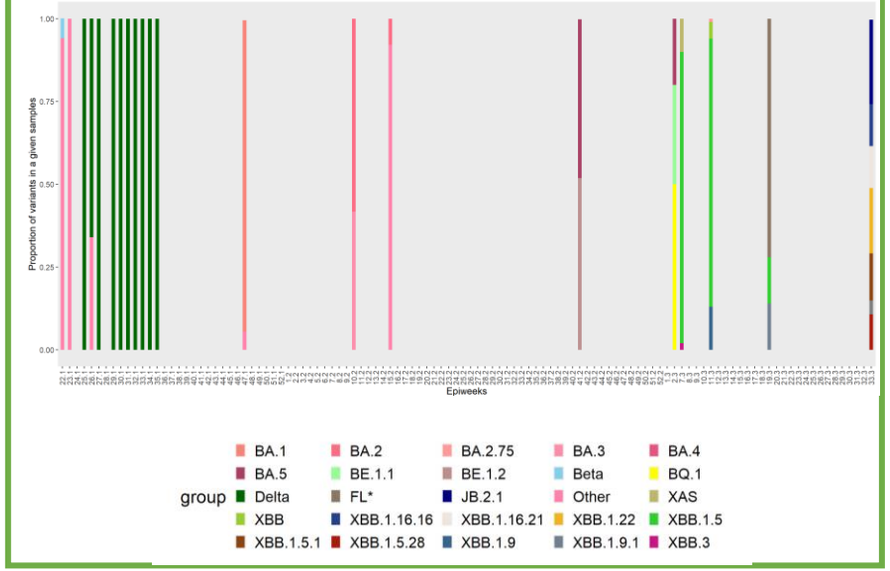
- After a sharp increase in SARS-CoV-2 levels in Epi week 37, SARS-CoV-2 levels in Borcheds Quarry WWTW remain moderate. No new results are available in Epi week 43.
- In Epi week 41, a 2-fold increase in SARS-CoV-2 levels were observed in Zandvleit WWTW. In Epi week 43, SARS-CoV-2 levels remain moderate.

**** Sequencing data ending in Epi week 33 in Bloemspruit and in Sterkwater. No new sequencing data available***

Borcheds Quarry Wastewater Treatment Works



Zandvleit Wastewater Treatment Works



- During epiweek 33, lineages XBB.1.5.81, XBB.1.5.28, JB.2, and XBB.1.16 were detected in Borcheds Quarry.
- Omicron lineage XBB.1.5.28, XBB.1.5.1, XBB1.22, JB.2.1 and XBB.1.16.26 were circulating in Zandvleit in week 33.

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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