



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

20 October 2023

Sample collection dates up to 13 October 2023  
(Epidemiological week 41)

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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 33-41

- From weeks 33-39, 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 33-41 increases and/or higher levels have been seen in Gauteng (Daspoort and Goudkoppies WWTW), Western Cape (Zandvleit and Borcheds Quarry WWTW ), and Free State (Sterktwater 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 33-39

- 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 eThekweni (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 Sterktwater WWTWs).

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

# 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

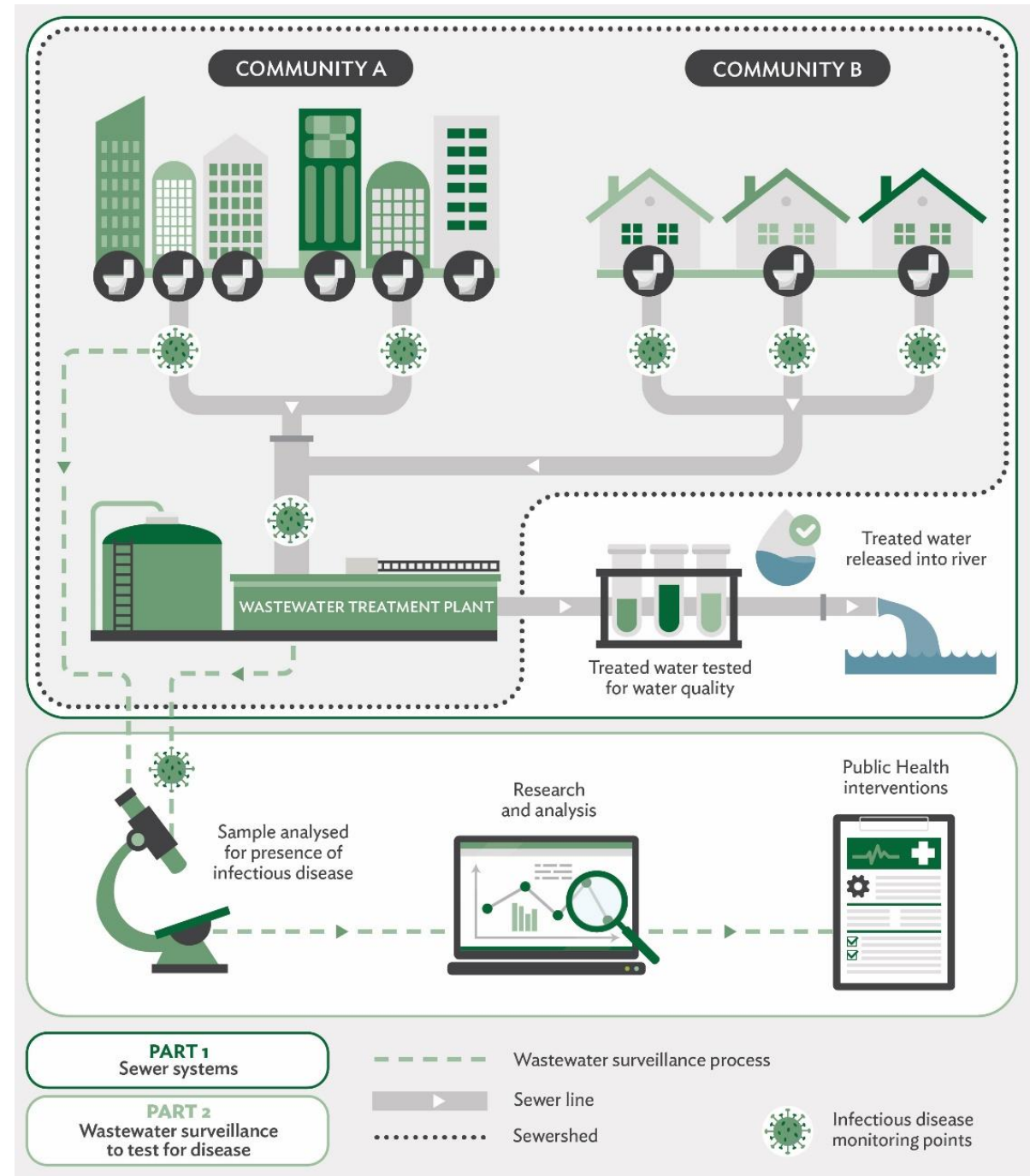
<https://www.gcro.ac.za/outputs/photo-essays/detail/photo-essay-sewersheds-what-can-wastewater-tell-us-about-community-health/>

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:

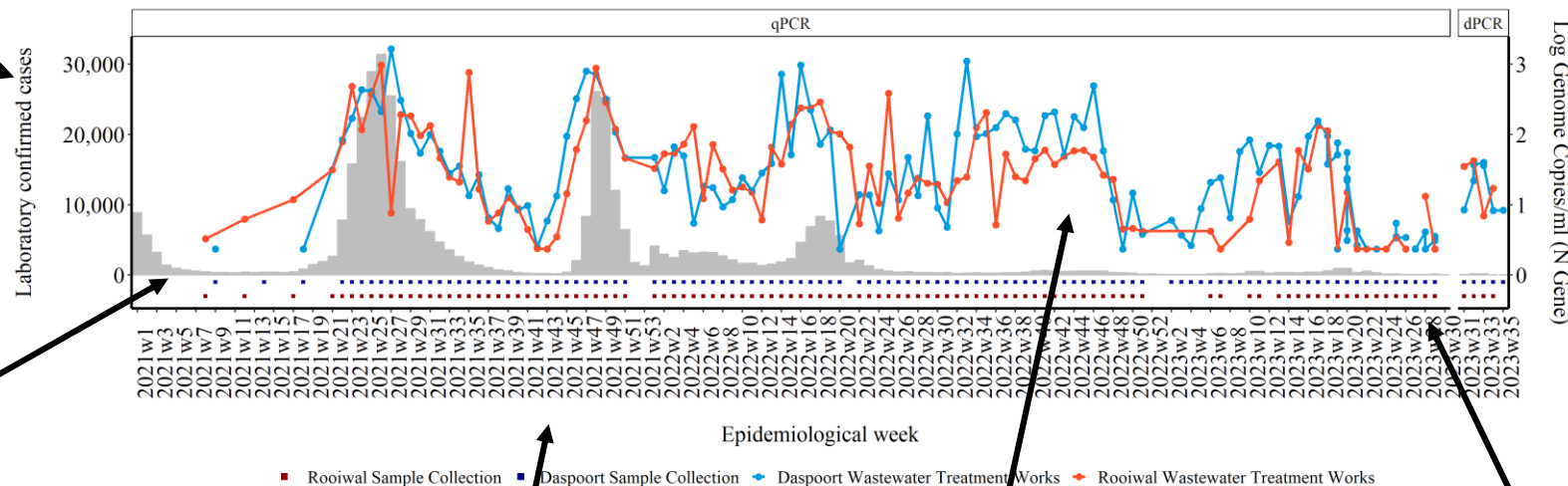
Epi weeks during which samples were collected

Facets:

Indicates the platform used to test samples

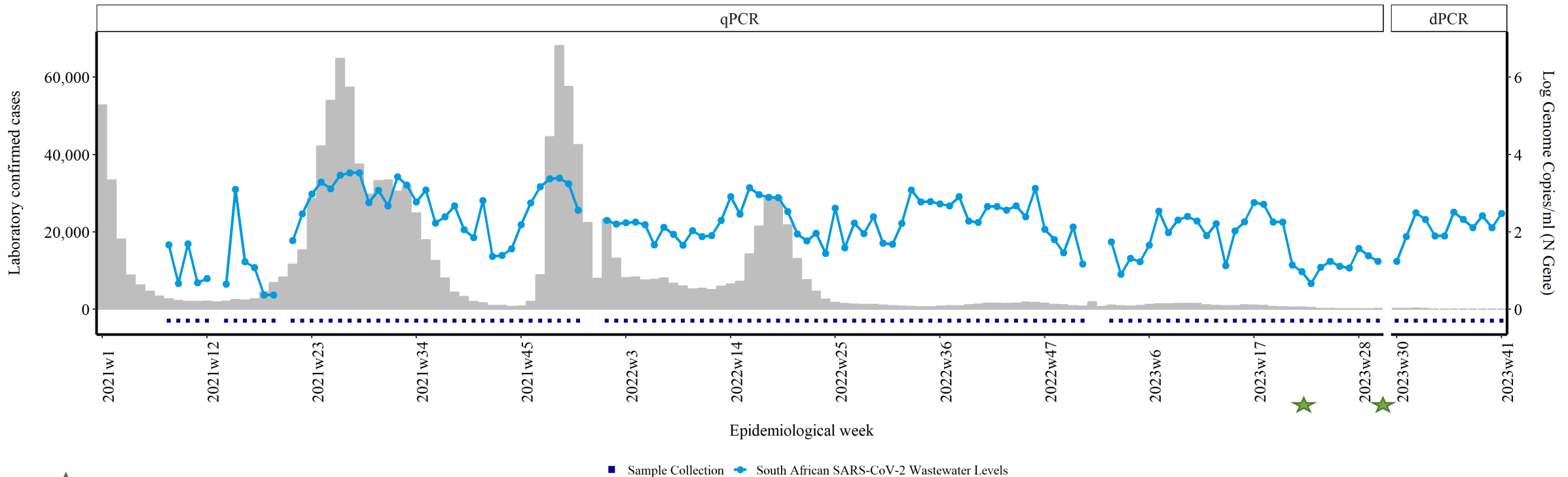
Right vertical axis:

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



★ Chloroform start and end date

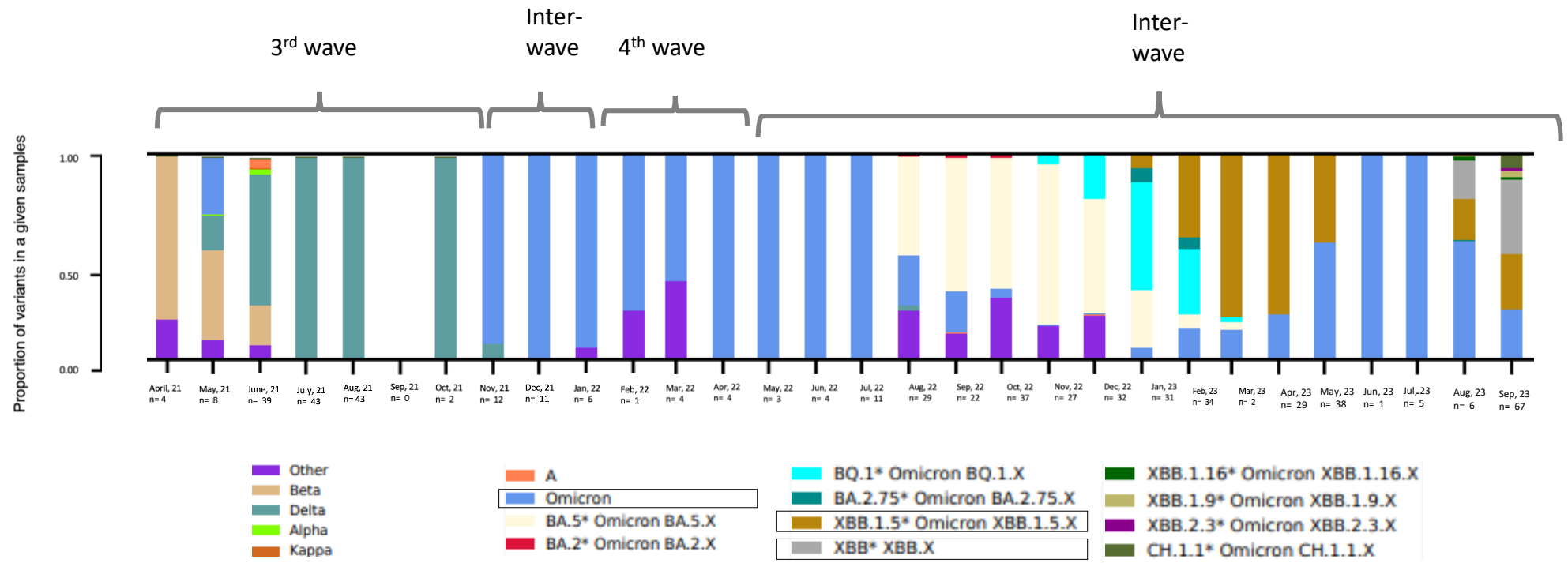
■ Sample Collection ● South African SARS-CoV-2 Wastewater Levels

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

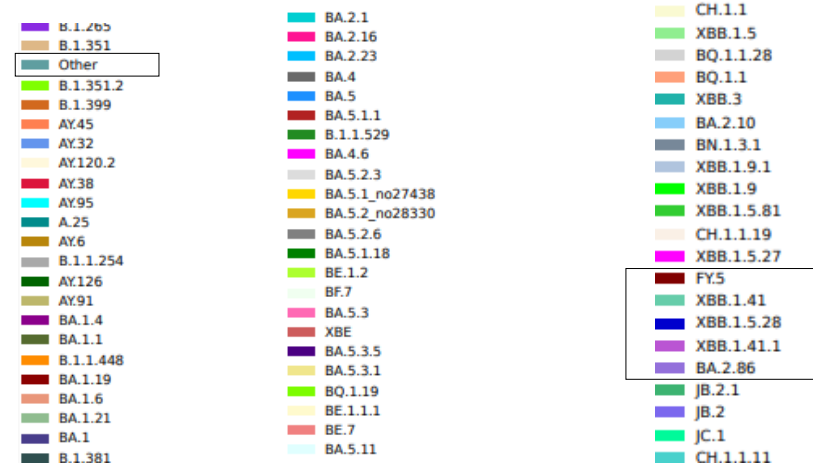
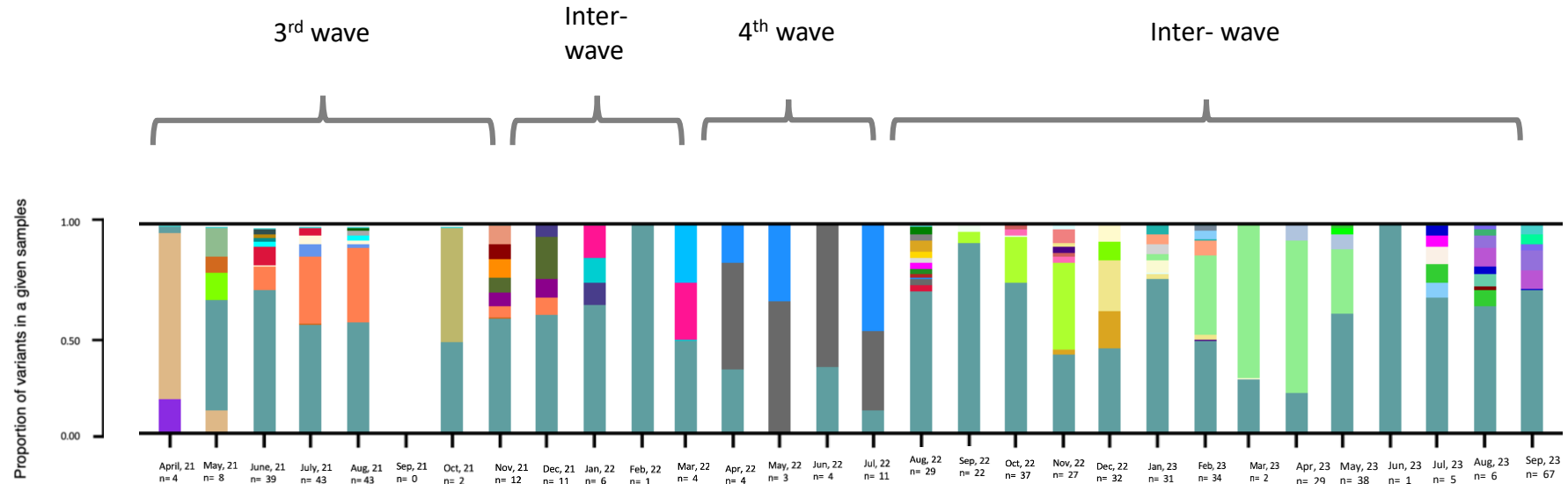
Inferred variants in wastewater samples from South African wastewater treatment plants by month, between April 2021- September 2023



# 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.



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

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

## Summary:

Summary of lineages associated with most common mutations associated

## Epi Week:

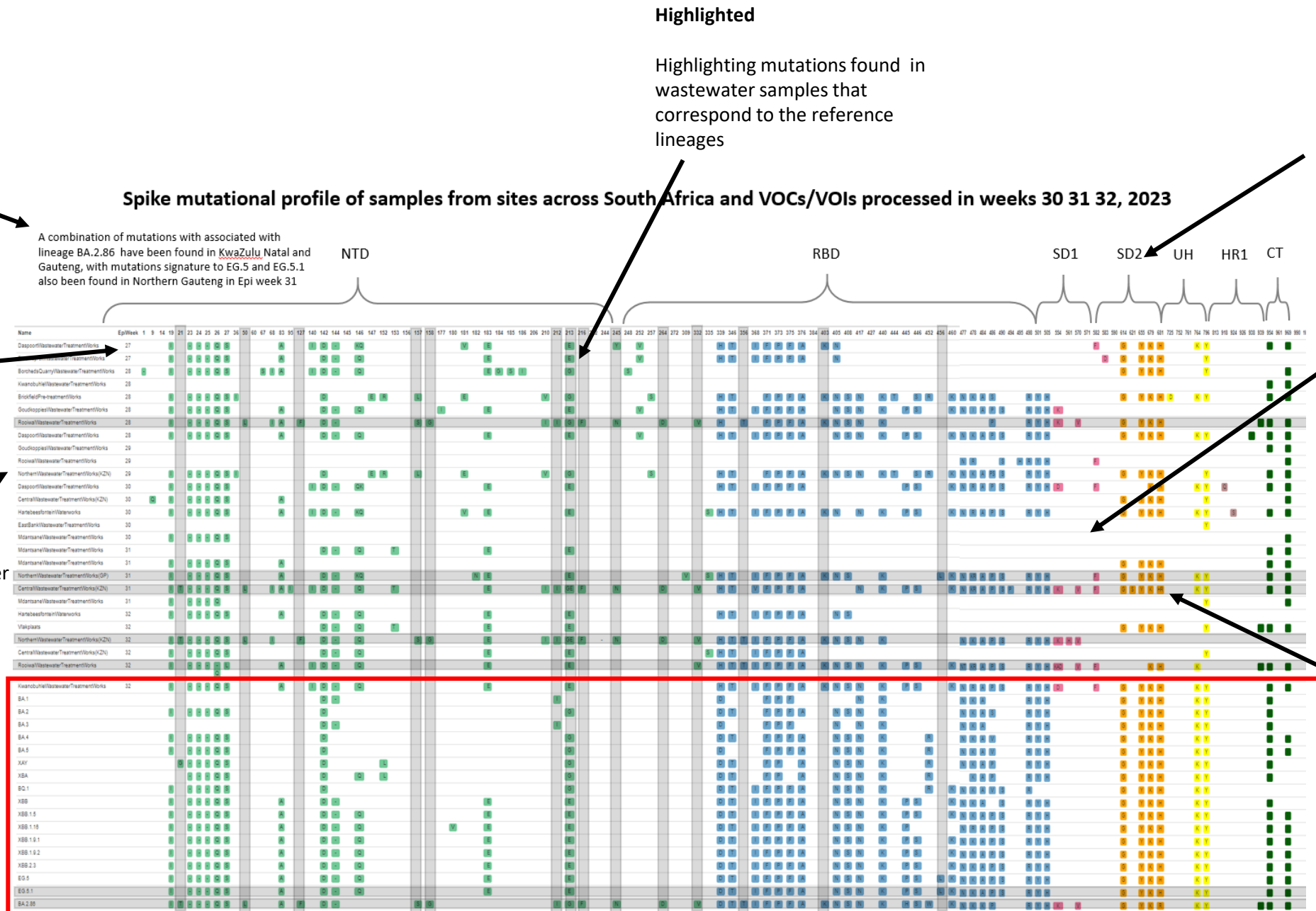
Epidemiological week during which samples were collected

## 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

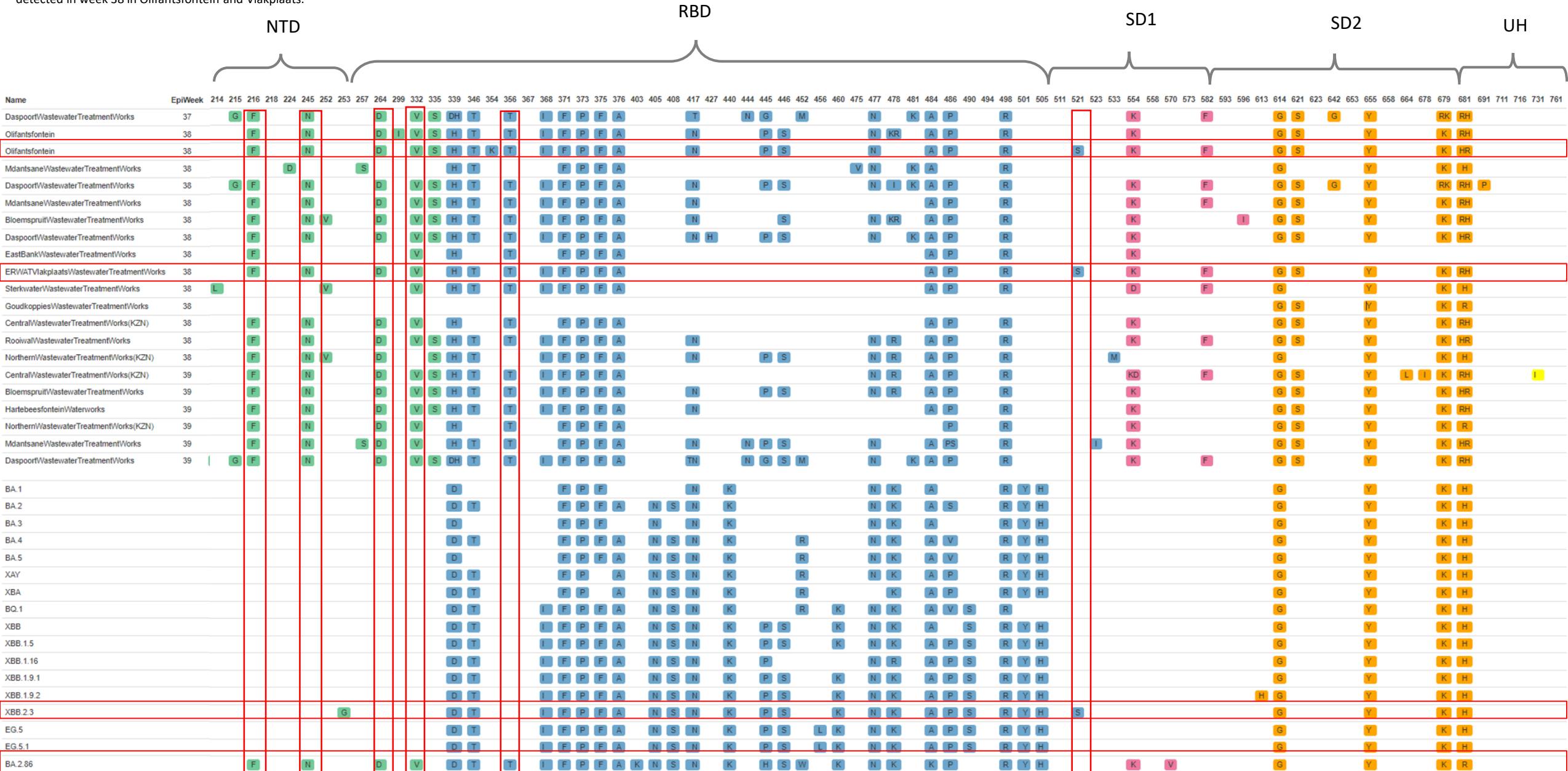






# Spike mutational profile of samples from sites across South Africa and VOCs/VOIs processed in weeks 33 - 39, 2023

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.





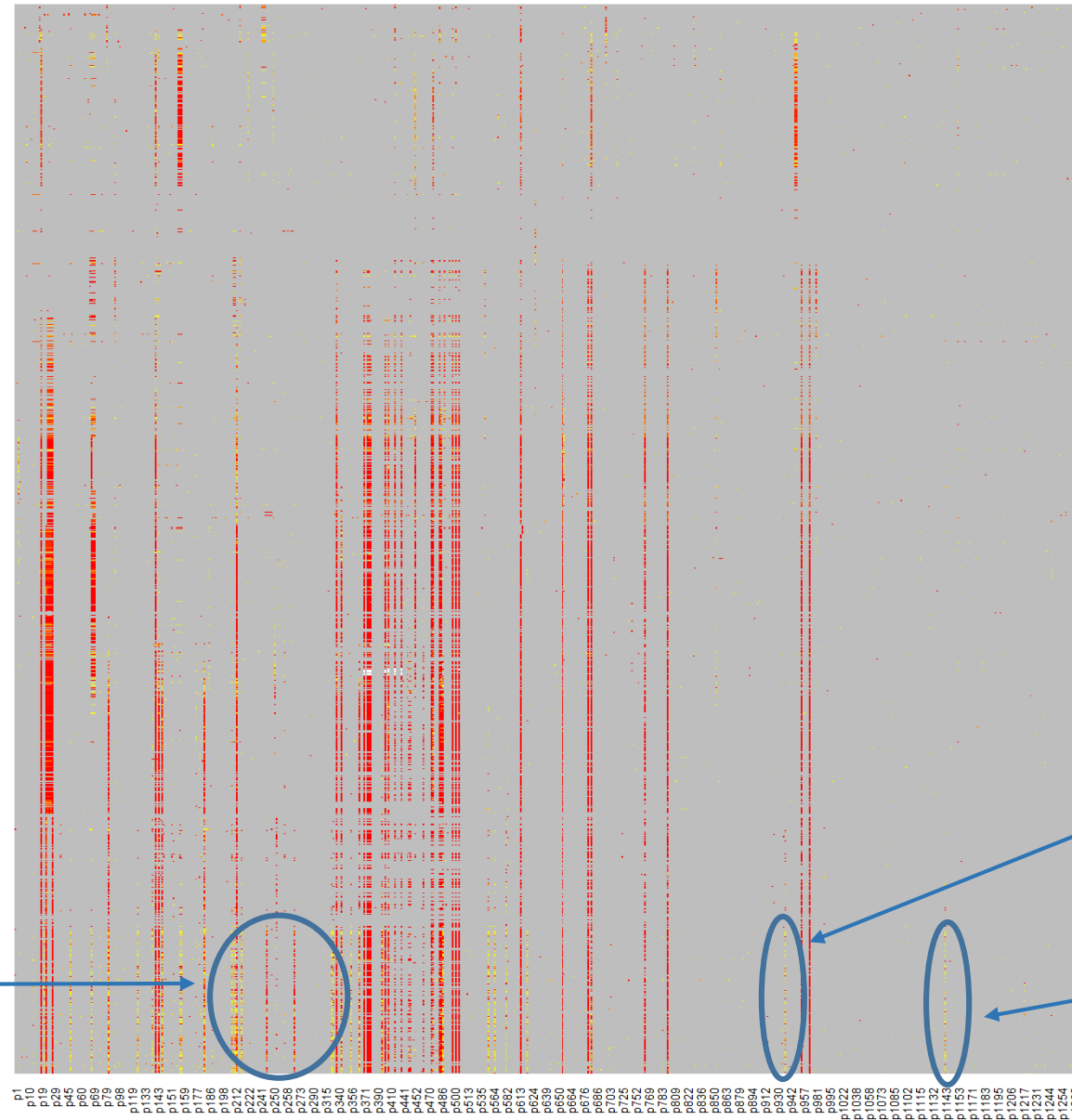
# Amino acid mutations and frequency – Spike protein

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\*

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



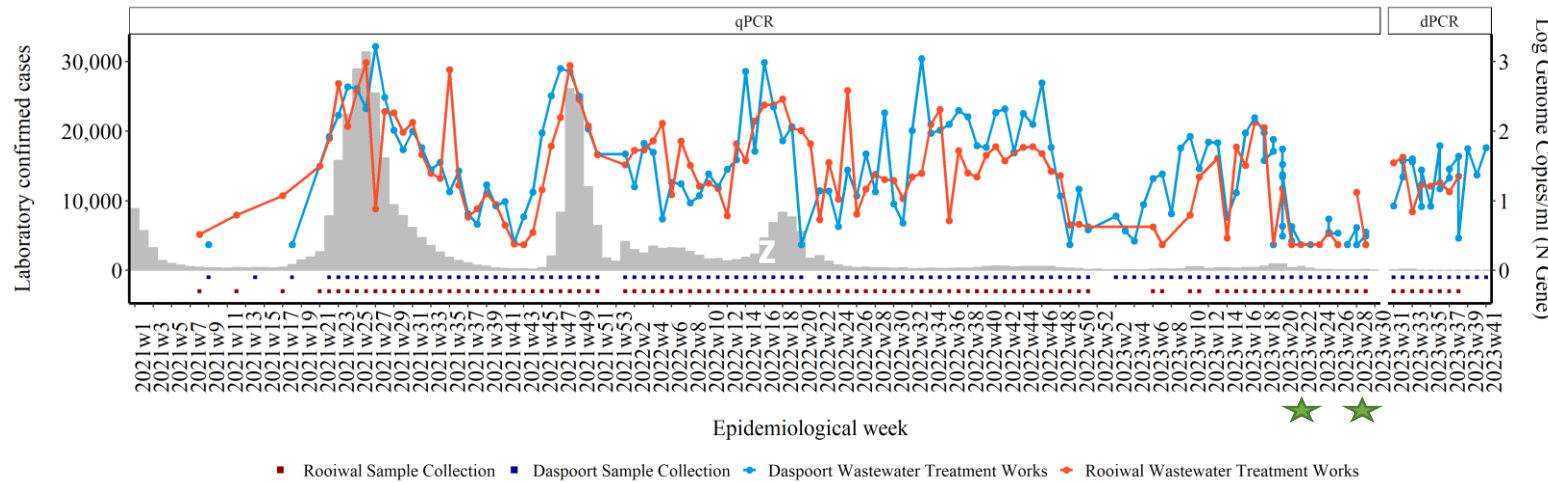
1149  
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1121  
1107  
1093  
1079  
1065  
1051  
1037  
1023  
1009  
995  
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911  
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253  
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71  
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15  
1

S939F  
 Spike protein mutation associated with the BA.2.86 lineage

P143L  
 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

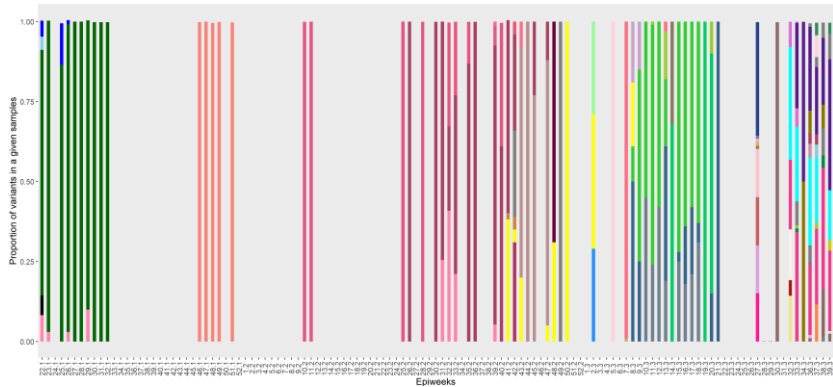
## SARS-CoV-2 levels and Genomic Results in Epi week 41:

- From Epi week 33, the SARS-CoV-2 levels in Daspoort WWTW is moderate.
- No new results for Epi week 41 in Rooiwal WWTW. However, in Epi week 41 SARS-CoV-2 levels increased slightly.

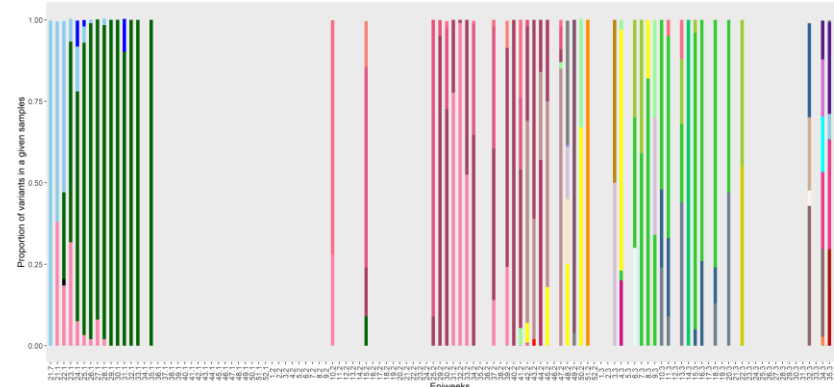
**\* Sequencing data ending in Epi week 38 in Rooiwal and 39 in Daspoort**

- 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.

## Daspoort



## Rooiwal

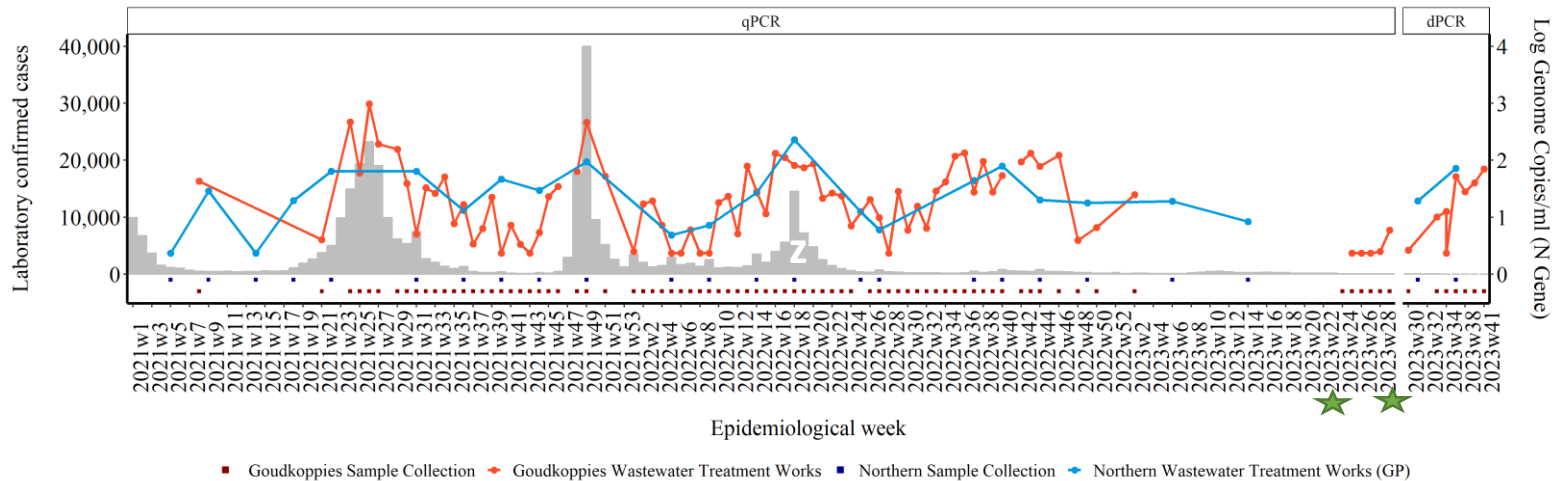


## SNP Analysis:

- 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 41:

- As of Epi week 41, SARS-CoV-2 levels in Goudkoppies WWTW have increased. Levels remain moderate.
- No new results for Northern WWTW in Epi week 41. However, the SARS-CoV-2 levels increased to moderate in Epi week 34.

**\* Sequencing data ending in Epi week 36 in Goudkoppies and 31 in Northern.**

- 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

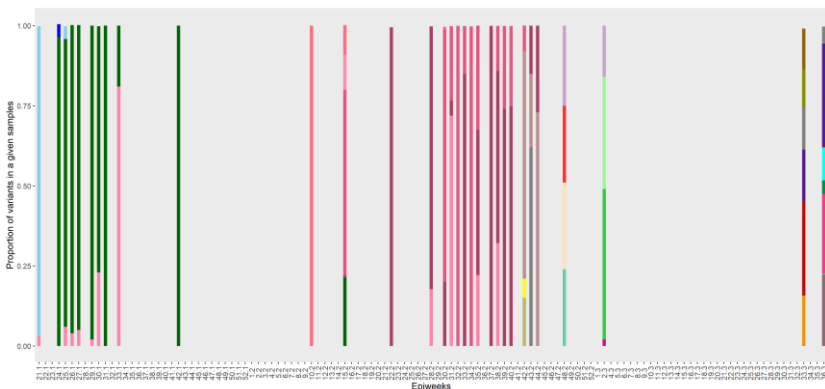
- Omicron lineages XBB.1.5.28, XBB.1.41.1, XBB.2.4, XBB.1.42.1 and XBB.2.9 were circulating during Epi week 31 in Northern Gauteng.

## SNP Analysis:

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

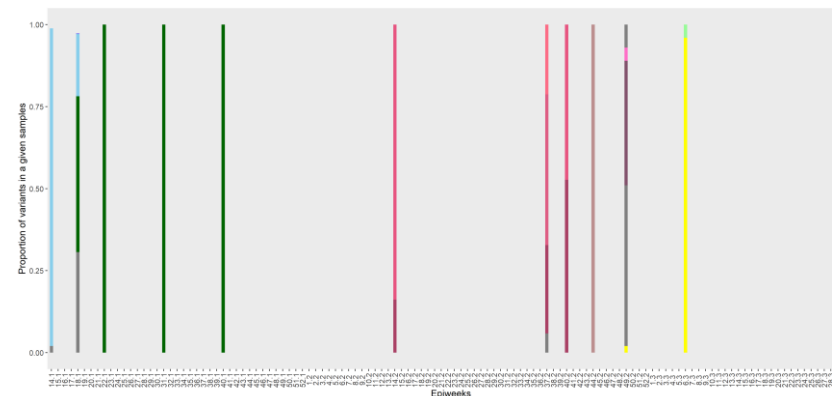
- SNP analysis could not be performed as the SARS-CoV-2 sequencing coverage in the Northern Johannesburg samples collected during Epi week 34 was too low for meaningful interpretation

## Goudkoppies



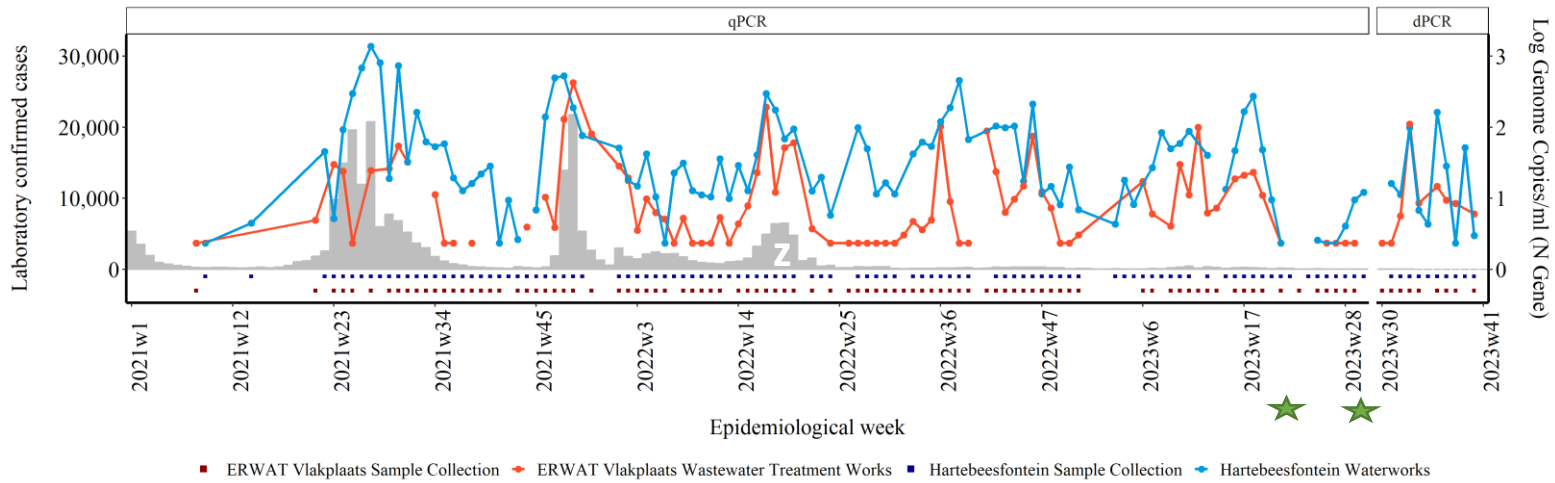
- |          |            |            |             |            |         |
|----------|------------|------------|-------------|------------|---------|
| Alpha    | AY.1       | AY.125     | BA.2        | BA.2.86    | BA.3    |
| BA.4     | BA.4.6     | BA.5       | BA.5.1      | BE.1       | BE.1.1  |
| BE.1.2   | BE.8       | Beta       | BQ.1        | Delta      | DR.1    |
| JB.2     | Other      | XAS        | XBB.1.16.17 | XBB.1.41.1 | XBB.1.5 |
| XBB.1.5* | XBB.1.5.40 | XBB.1.5.45 | XBB.1.5.81  | XBB.1.5.84 | XBB.3   |

## Northern



- |            |            |            |         |
|------------|------------|------------|---------|
| Alpha      | BA.2       | BA.4       | BA.4.6  |
| BA.4.8     | BA.5       | BA.5.2     | BE.1.1  |
| BE.1.2     | Beta       | BQ.1       | Delta   |
| XBB.1.41.1 | XBB.1.42.1 | XBB.1.5.28 | XBB.2.4 |
| XBB.2.9    |            |            |         |

# Gauteng - Ekurhuleni



★ Chloroform start and end date

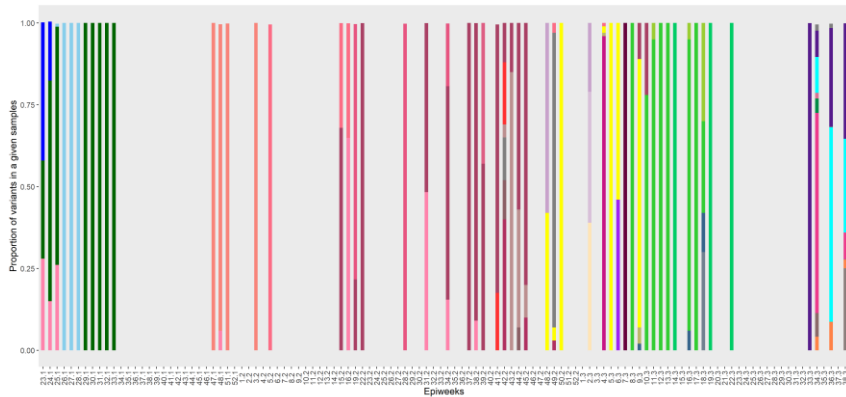
## SARS-CoV-2 levels and Genomic Results in Epi week 41:

- No new results were obtained in Epi week 41. The SARS-CoV-2 levels in Hartebeesfontein WWTW increased significantly from low levels in Epi week 33, to moderate levels in Epi week 39.
- No new result for Epi week 41, however, 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 38 in Vlakplaats and 39 in Hartebeesfontein.**

- 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.

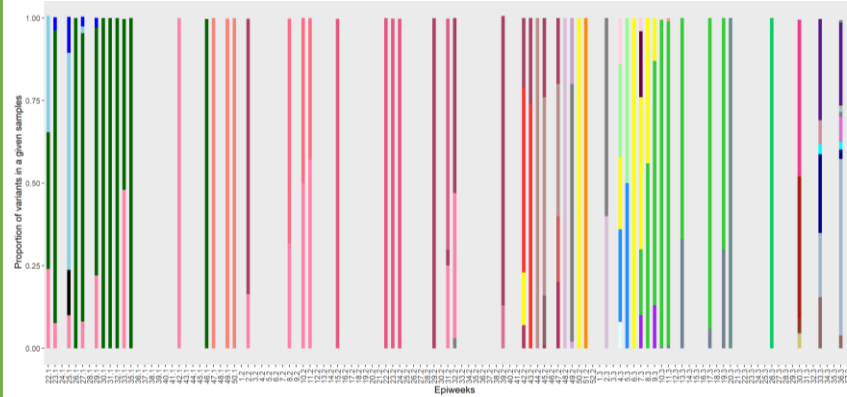
## ERWAT Vlakplaats



group

- Alpha
- BA.1
- BA.2
- BA.2.86
- BA.4
- BA.4.6
- BA.5
- BA.5.1
- BA.5.3
- BE.1
- BE.1.2
- BE.7
- BE.8
- Beta
- BQ.1
- CP.1
- Delta
- JB.2
- Other
- XAH
- XAS
- XBB
- XBB.1.16.17
- XBB.1.41.1
- XBB.1.5
- XBB.1.5\*
- XBB.1.5.81
- XBB.1.5.91
- XBB.1.9
- XBB.1.9.1
- XBB.2
- XBB.2.3.11
- XBB.3
- XBE

## Hartebeesfontein



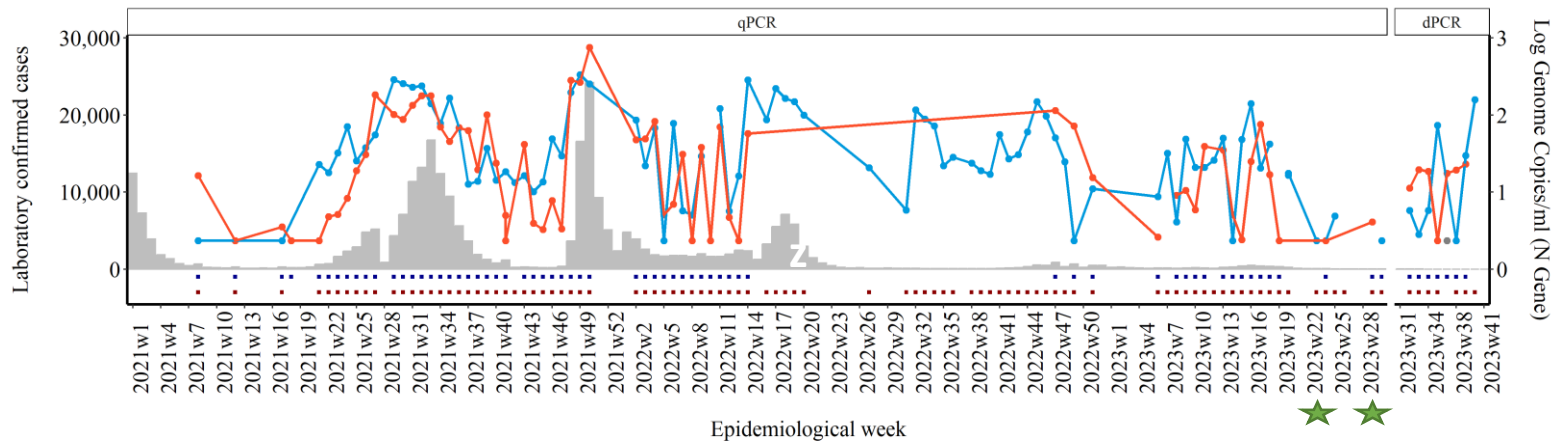
group

- Alpha
- BA.1
- BA.2
- BA.2.1
- BA.2.10
- BA.2.75
- BA.2.86
- BA.4
- BA.4.6
- BA.5
- BA.5.1
- BA.5.3
- BE.1
- BE.1.1
- BE.1.2
- BE.1.4
- BE.15
- BE.2
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- BE.97
- BE.98
- BE.99
- BE.100

## 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 - eThekweni

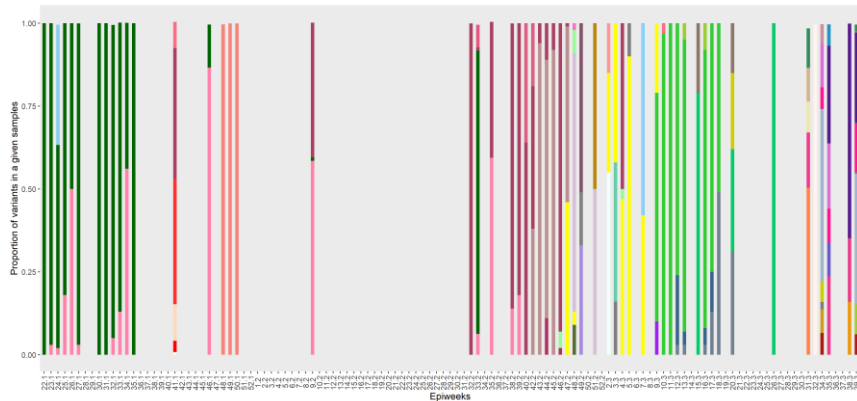


## SARS-CoV-2 levels and Genomic Results in Epi week 41:

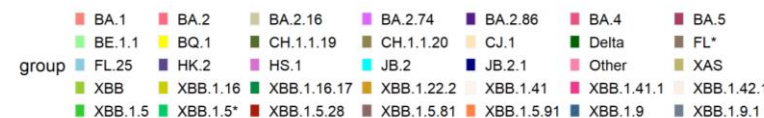
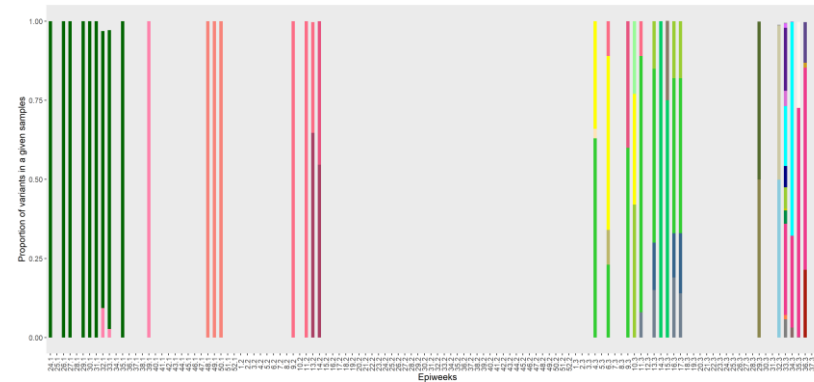
- No new results were obtained in Epi week 41 however, SARS-CoV-2 levels in Central WWTW in Epi week 39 showed a sharp increase from low levels in Epi week 38 (1 log copy/ml) to moderate levels (2 log copies/ml).
- No new results were obtained in Epi week 41 however, SARS-CoV-2 levels increased from low to moderate in week Epi 36 in Northern WWTW and remain low in Epi week 39.

**\* Sequencing data ending in Epi week 39 in Central eThekweni and in eThekweni North.**

## Central



## North

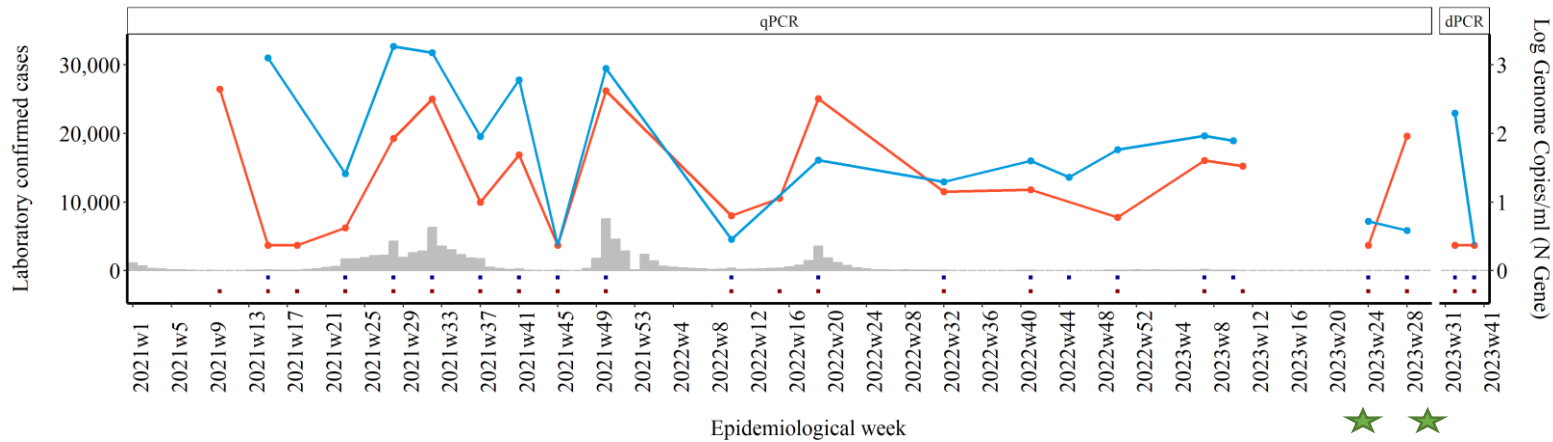


- XBB, XBB.1.5.28, JC.1, JB.2 were circulating in Epi week 39, with a BA.2.86 dominance at the Central eThekweni water treatment plant.
- In eThekweni North, XBB.1.42.1, JB.2, and BA.2.86 were circulating during Epi week 39

## SNP Analysis:

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

# Eastern Cape – Nelson Mandela

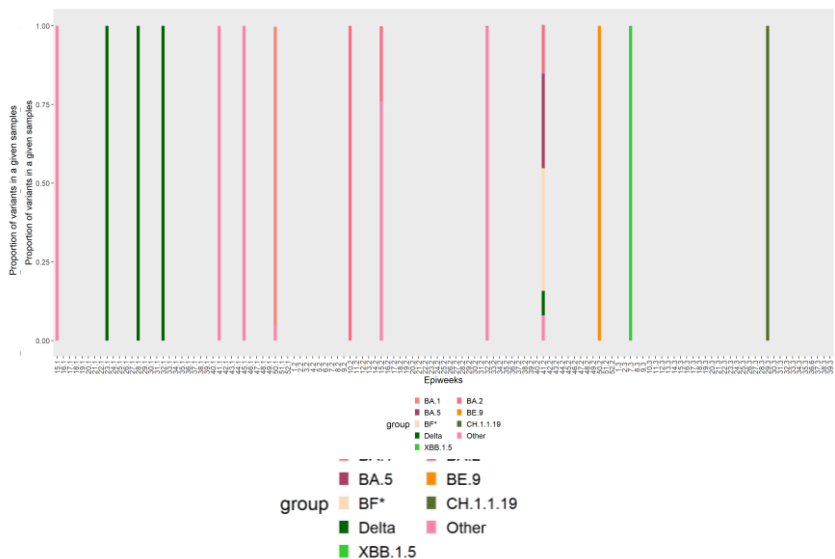


## SARS-CoV-2 levels and Genomic Results in Epi week 41:

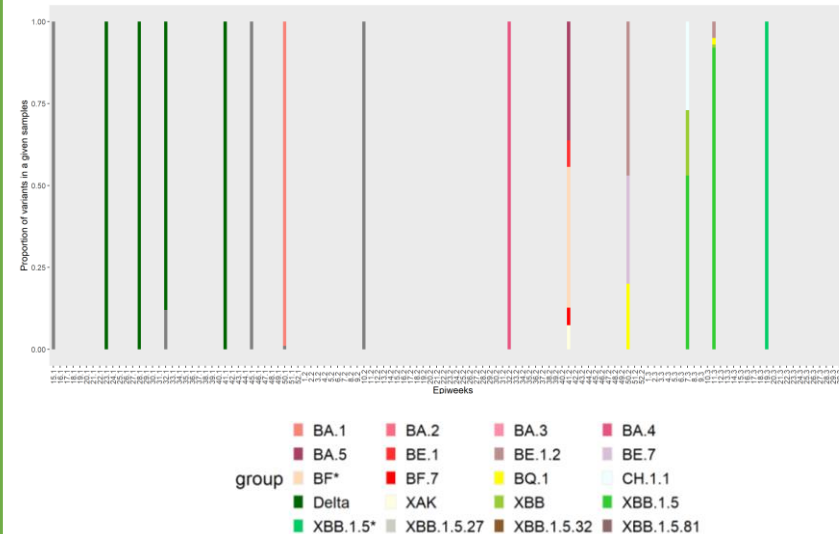
- No new results were obtained in Epi week 41, however, in Epi week 37, a sharp decline in SARS-CoV-2 levels were seen in Kwanobuhle WWTW.
- SARS-CoV-2 levels increased from low to intermediate from Epi week 24 to Epi week 31 in Brickfield Pre-treatment works. As of Epi week 37, SARS-CoV-2 levels remain low. No new results are available for Epi week 41.

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

## Brickfield



## Kwanobuhle

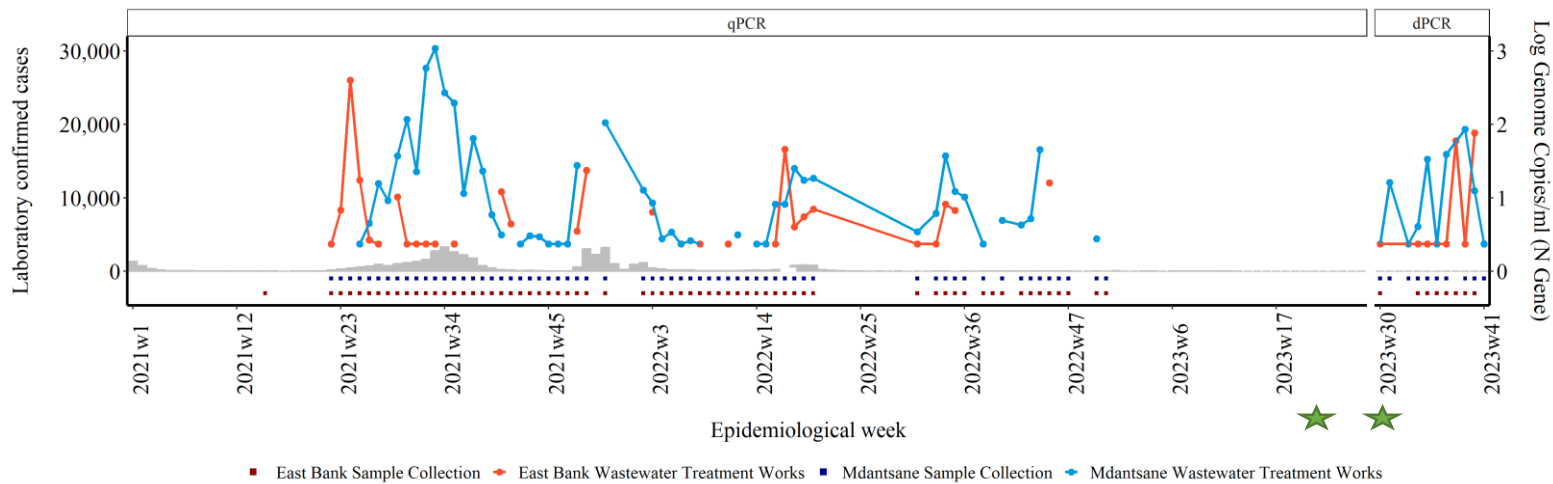


- SARS-CoV-2 sequencing coverage in the Brickfield samples collected during Epi weeks 30-32 are too low for meaningful interpretation
- Omicron lineages XBB.1.5.81, XBB.1.5.32 and XBB.1.5.27 were circulating in Kwanobuhle during epi week 32.

## 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.

# Eastern Cape – Buffalo City

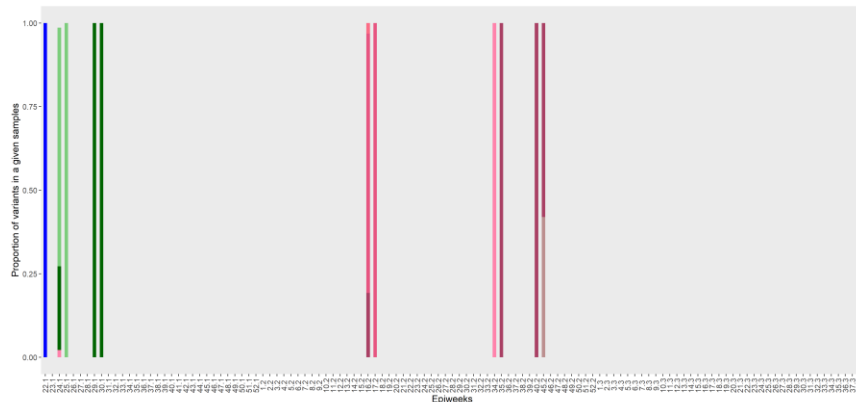


## SARS-CoV-2 levels and Genomic Results in Epi week 41:

- In Epi week 41, SARS-CoV-2 levels in Mdantsane WWTW decreased and remain low after an increase in SARS-CoV-2 levels was seen in Epi week 35.
- SARS-CoV-2 levels in East Bank WWTW in Epi week 39 remain moderate after an increase was seen in Epi week 38. No new results are available for Epi week 41.

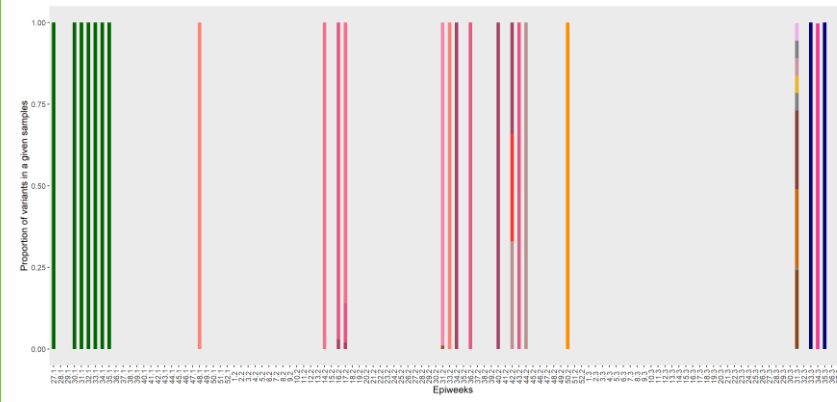
**\* Sequencing data ending in Epi week 38 in Eastbank and 39 in Mdantsane.**

### Eastbank



- |       |         |         |         |
|-------|---------|---------|---------|
| group | A       | Alpha   | BA.2    |
|       | BA.2.86 | BA.4    | BA.5    |
|       | BE.1.2  | Delta   | GG.1    |
|       | Other   | XBB.2.4 | XBB.2.9 |

### Mdantsane



- |       |            |            |            |            |            |            |
|-------|------------|------------|------------|------------|------------|------------|
| group | BA.1       | BA.2       | BA.2.86    | BA.2.86.1  | BA.3       | BA.4       |
|       | BA.4.6     | BA.5       | BE.1       | BE.1.2     | BE.9       | CH.1.1.11  |
|       | CJ.1       | Delta      | FY.3       | FY.5       | HS.1       | JB.2       |
|       | JB.2.1     | Other      | XAP        | XBB.1.22   | XBB.1.41.1 | XBB.1.5.32 |
|       | XBB.1.5.56 | XBB.1.5.81 | XBB.1.5.88 | XBB.1.5.91 |            |            |

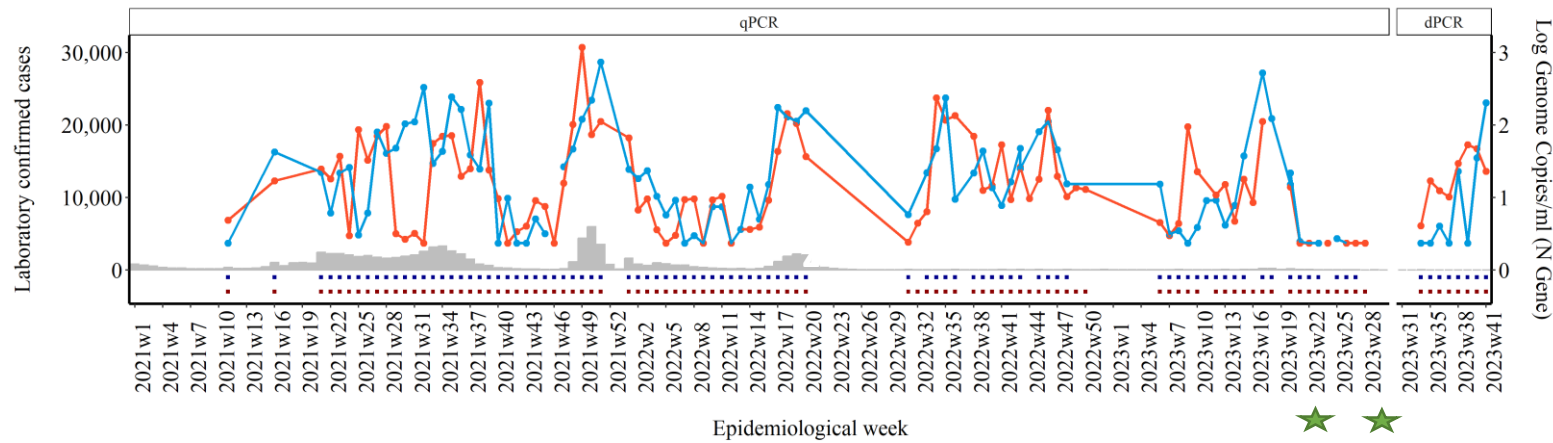
- Omicron lineage BA.2.86, XBB.2.9, XBB.2.9 and GG.1 were circulating in Eastbank during Epi week 38.
- Lineages CH.1.1.11, BA.2.86, XBB.1.5.81 and XBB.1.5.88 were circulating in Mdantsane during Epi week 39.

## 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.



# 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 41:

- In Bloemspruit WWTW, a decrease in SARS-CoV-2 levels were seen in Epi week 41. Levels remain moderate.
- A sharp increase in SARS-CoV-2 levels were seen in Sterkwater WWTW from Epi week 38, and levels remain moderate to high in Epi week 41.

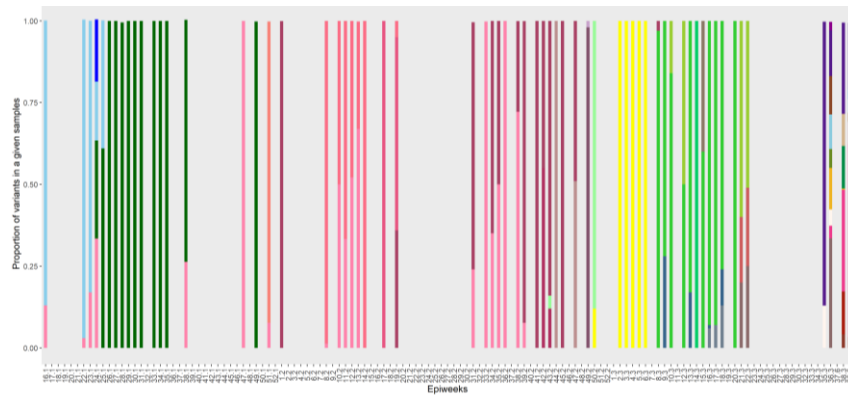
\* Sequencing data ending in Epi week 39 in Bloemspruit and 38 in Sterkwater.

- 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:

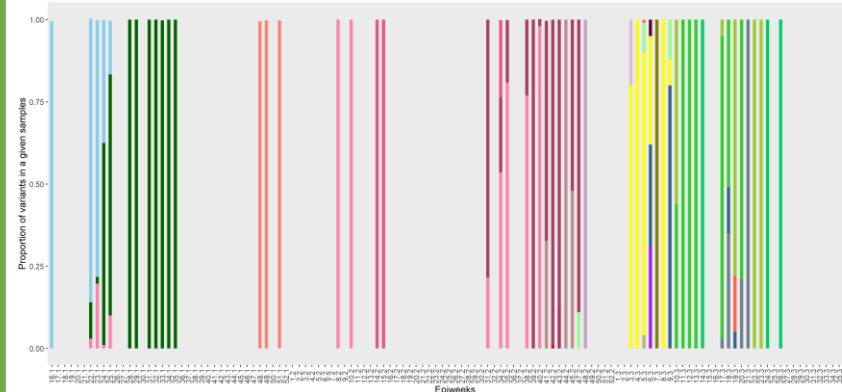
- A combination of mutations (V127F, L212I, V213G, L216F, H245N, A264D, I332V, K356T) associated with lineage BA.2.86 were found in both Bloemspruit and Sterkwater.

## Bloemspruit



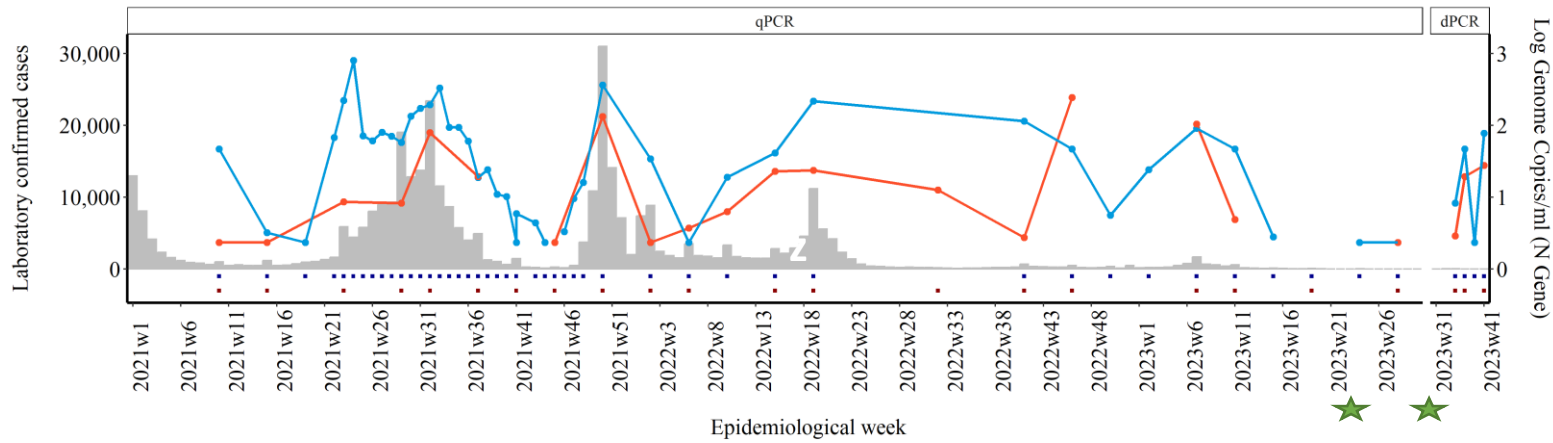
group ■ Alpha ■ BA.1 ■ BA.2 ■ BA.2.19 ■ BA.2.86 ■ BA.4 ■ BA.5 ■ BA.5.1 ■ BA.5.2 ■ BE.1.1 ■ BE.1.2 ■ Beta ■ BQ.1 ■ Delta ■ EG.2 ■ EG.5.1.1 ■ FL\* ■ FL.12 ■ FL.25 ■ FL.4.8 ■ FY.5 ■ HK.3.1 ■ JB.2 ■ Other ■ XBB ■ XBB.1.16.17 ■ XBB.1.22 ■ XBB.1.41 ■ XBB.1.41.1 ■ XBB.1.5 ■ XBB.1.5\* ■ XBB.1.5.17 ■ XBB.1.5.28 ■ XBB.1.5.81 ■ XBB.1.9 ■ XBB.1.9.1 ■ XBB.2.4 ■ XBE

## Sterkwater



group ■ BA.1 ■ BA.2 ■ BA.4 ■ BA.5 ■ BA.5.1 ■ BA.5.3 ■ BE.1.1 ■ BE.1.2 ■ BE.7 ■ Beta ■ BF.7 ■ BQ.1 ■ Delta ■ HS.1 ■ JB.2 ■ JB.2.1 ■ JC.1 ■ Other ■ XAS ■ XAV ■ XBB ■ XBB.1 ■ XBB.1.22 ■ XBB.1.41.1 ■ XBB.1.5 ■ XBB.1.5\* ■ XBB.1.5.17 ■ XBB.1.5.25 ■ XBB.1.5.81 ■ XBB.1.5.83 ■ XBB.1.9 ■ XBB.1.9.1 ■ XBB.2 ■ XBE

# Western Cape – City of Cape Town



★ Chloroform start and end date ■ Borcheds Quarry Sample Collection ◆ Borcheds Quarry Wastewater Treatment Works ■ Zandvleit Sample Collection ◆ Zandvleit Wastewater Treatment Works

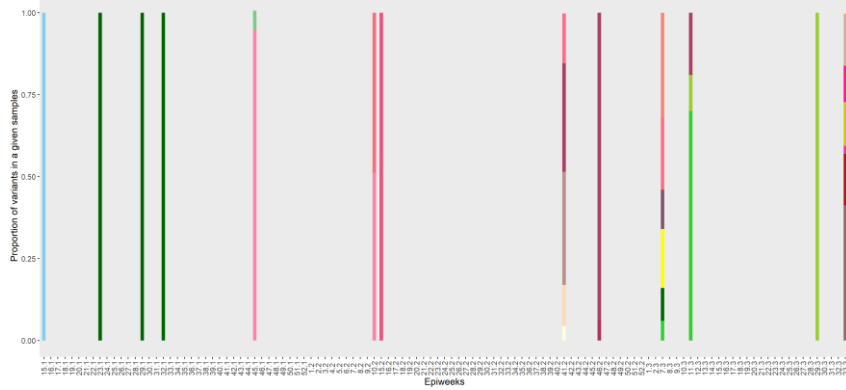
## SARS-CoV-2 levels and Genomic Results in Epi week 41:

- After a sharp increase in SARS-CoV-2 levels in Epi week 37, SARS-CoV-2 levels in Borcheds Quarry WWTW remain moderate.
- In Epi week 41, a 2-fold increase in SARS-CoV-2 levels were observed in Zandvleit WWTW. Levels are moderate.

*\* Sequencing data ending in Epi week 33 in Bloemspruit and in Sterkwater.*

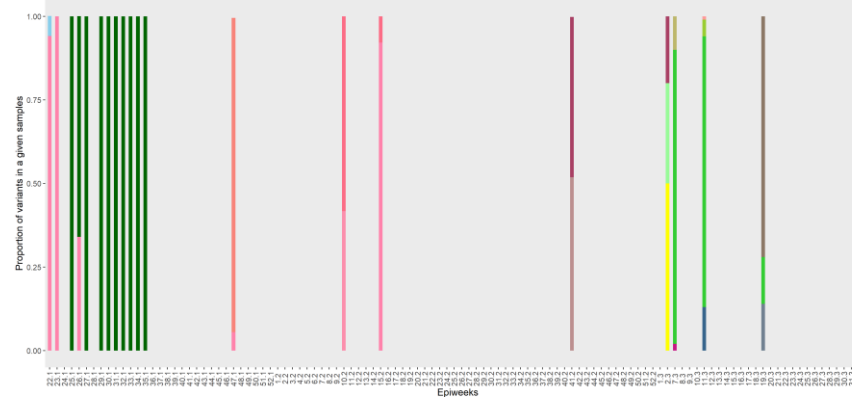
- During epiweek 33, lineages XBB.1.5.81, XBB.1.5.28, JB.2, and XBB.1.16 were detected in Borcheds Quarry.
- Omicron lineage XBB.1.5.28, XBB.1.5.1, XBB1.22, JB.2.1 and XBB.1.16.26 were circulating in Zandvleit in week 33.

## Borcheds Quarry



■ A	■ BA.1	■ BA.2	■ BA.4	■ BA.5
■ BA.5.2	■ BE.1.2	■ Beta	■ BF*	■ BQ.1
■ Delta	■ EG.2	■ JB.2	■ Other	■ XAK
■ XBB	■ XBB.1.16	■ XBB.1.41.1	■ XBB.1.5	■ XBB.1.5.28
■ XBB.1.5.81	■ XBE			

## Zandvleit



■ BA.1	■ BA.2	■ BA.2.75	■ BA.3	■ BA.4
■ BA.5	■ BE.1.1	■ BE.1.2	■ Beta	■ BQ.1
■ Delta	■ FL*	■ JB.2.1	■ Other	■ XAS
■ XBB	■ XBB.1.16.16	■ XBB.1.16.21	■ XBB.1.22	■ XBB.1.5
■ XBB.1.5.1	■ XBB.1.5.28	■ XBB.1.9	■ XBB.1.9.1	■ XBB.3

## 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 Zandvleit.

# COLLABORATORS



# FUNDERS



# TEAM

