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

26 April 2024

Sample collection dates up to 19 April 2024
(Epidemiological week 16)

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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 48 (2023) –16 (2024)

- From weeks 48-13 the cumulative SARS-CoV-2 levels measured at wastewater treatment works (WWTW) **has remained around one log genome copies/ml of wastewater**. This has followed on from the increases observed in weeks 31-41, when the cumulative SARS-CoV-2 levels in wastewater at sentinel sites in South Africa showed increases to levels above 2 log (100) genome copies/ml of wastewater, up from below one log copy/ml in epidemiological week 22 (first week in June 2023). **From week 45, levels measured below 2 log genome copies/ml of wastewater.**

- **In weeks 1-6 lower levels were observed nationally, with an increase seen in week 7 and a subsequent decrease in week 8. Levels increase to moderate in Epi week 10. Levels increased to high (above 2 log (100) genome copies/ml) in Epi week 14 and remain high in Epi week 16.**

- 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 48 (2023) – 16 (2024)

- **Omicron lineage BA.2.86.X, and JN.1.X sub-lineages** were the dominant lineages circulating in wastewater samples between January 2024 – March 2024 (Epiweek 1-10)

- In clinical samples, **BA.2.86** was also the dominant lineage circulating throughout November 2023 and January 2024, followed by **XBB.1.5***, **XBB.1.19*** and **JN.1***.

- The **Omicron lineage BA.2.86, JN.1* and Recombinant lineages are** circulating in KwaZulu-Natal in eThekwini (in the catchments of Northern and Central WWTWs). 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) BA.2.86.X and JN.1.X are the dominantly circulating. 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), Free State, in Mangaung (catchments Bloemspruit and Sterkwater WWTWs) and North West (Boitekong). In Limpopo (Musina) and Mpumalanga (Kingstonvale) BA.2.86.X lineage was the main lineage detected.

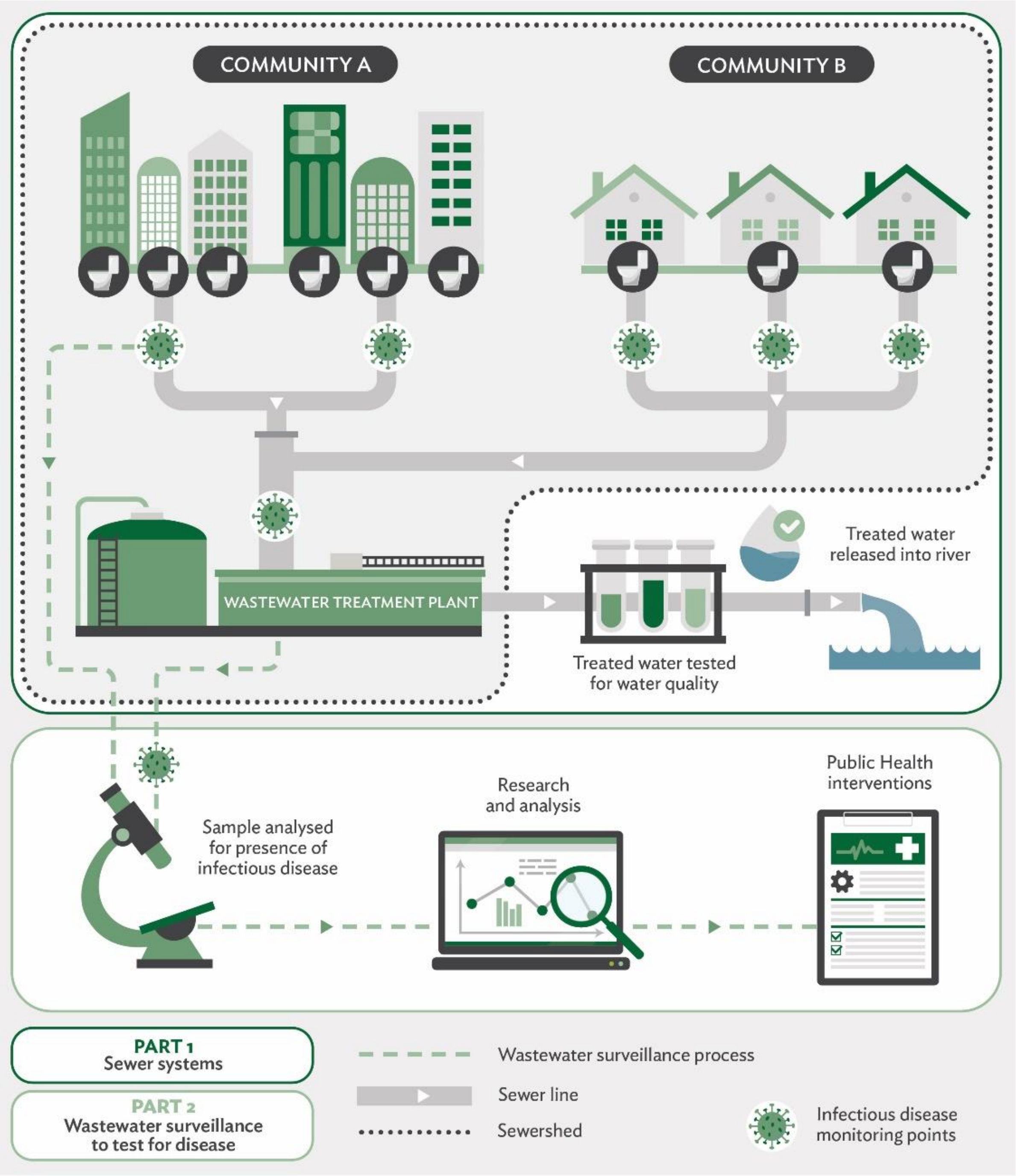
Interpretation: Ongoing transmission of SARS-CoV-2 due to Omicron lineages including the new BA.2.86 and JN.1* lineages.

Wastewater-based Epidemiology for COVID-19

How is wastewater tested for SARS-CoV-2?

For a full description of this process, see the photo essay developed in collaboration with the Gauteng City Region Observatory https://www.gcro.ac.za/outputs/photo-essays/detail/photo-essay-sewersheds-what-can-wastewater-tell-us-about-community-health/

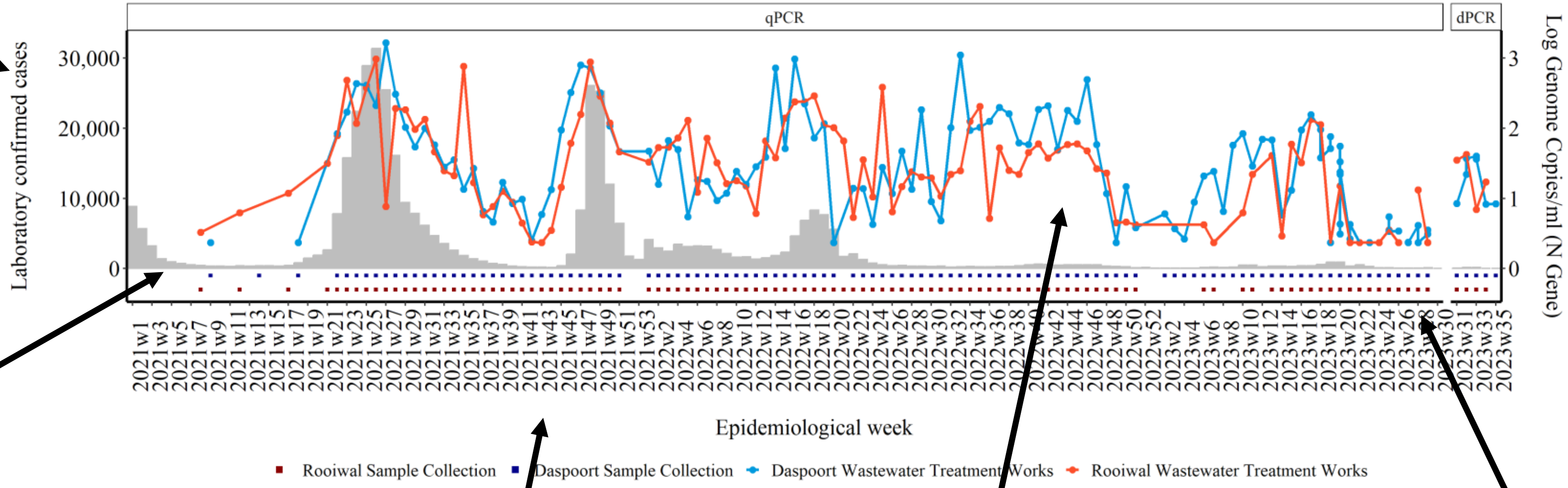
For a technical description and analysis of wastewater levels and results see
https://pubmed.ncbi.nlm.nih.gov/37506905/
https://www.medrxiv.org/content/10.1101/2022.12.15.22283506v1 (accepted by Nature Communications, publication pending)



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:

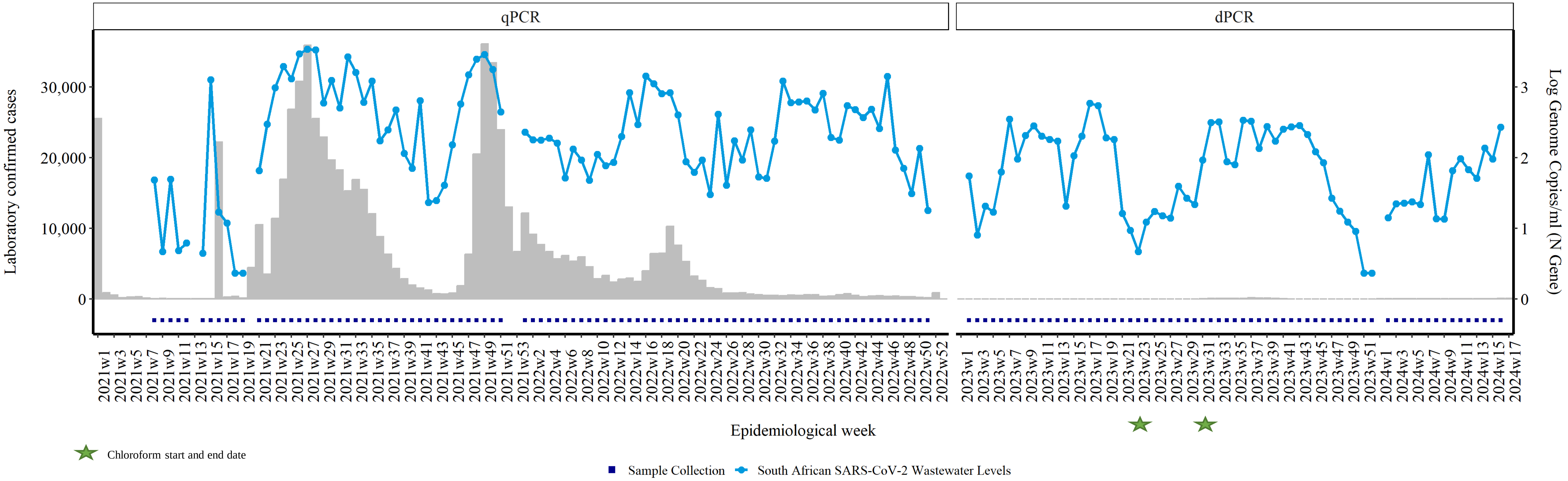
Changes in wastewater SARS-CoV-2 results over time for different treatment facilities

Coloured squares:

Epi weeks during which samples were collected

South Africa at a glance:

Summed total of clinical and genome copies

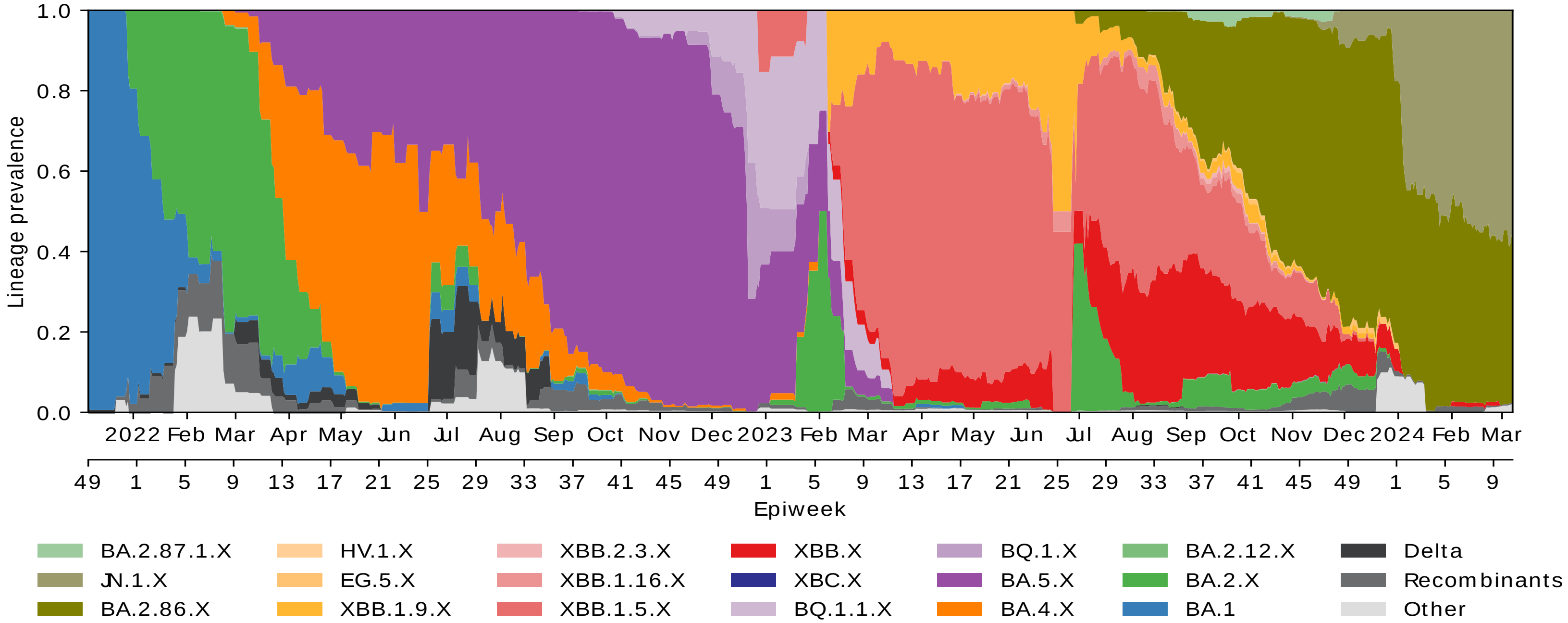


Changes in levels of SARS-Cov-2 in log-transformed genome copies/ml (right vertical axis, line graph) in in-flowing untreated wastewater from plants tested by NICD, compared with laboratory-confirmed cases from Tshwane, Johannesburg, Ekurhuleni, eThekwini, Mangaung, Nelson Mandela, Buffalo City, City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

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

Inferred variants in wastewater samples from South African wastewater treatment plants by month, between April 2021- March 2024

- 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 9 (March 2024)
  - Omicron lineages BA.2.86.X and JN.1.X were circulating from December to March, with Recombinants and XBB.X sub-lineages circulating at low proportion.



April, 21	May, 21	June, 21	July, 21	Aug, 21	Sep, 21	Oct, 21	Nov, 21	Dec, 21	Jan, 22	Feb, 22	Mar, 22	Apr, 22	May, 22	Jun, 22	Jul, 22	Aug, 22	Sep, 22	Oct, 22	Nov, 22	Dec, 22	Jan, 23	Apr, 23	May, 23	Jun, 23	Jul, 23	Aug, 23	Sep, 23	Oct, 23	Nov, 23	Dec, 23	Jan, 24.	Feb,24	Mar, 24
n= 4	n= 8	n= 39	n= 43	n= 43	n= 0	n= 2	n= 12	n= 11	n= 6	n= 1	n= 4	n= 4	n= 3	n= 4	n= 11	n= 29	n= 22	n= 37	n= 27	n= 32	n= 31	n= 29	n= 44	n= 9	n= 38	n= 59	n= 33	n= 45	n= 18	n= 14	n= 18.	n=15.	n=20

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

- Results from sequencing data ending in epi week 9 (March 2024)
  - Omicron lineages BA.2.86 and JN.1 were circulating from January to March with BA.2.86 dominance.
  - Omicron XBB sub-lineages were in circulation throughout.
  - The predominant lineage circulating in clinical samples in the recent week is BA.2.86 and JN.1 sublineages.

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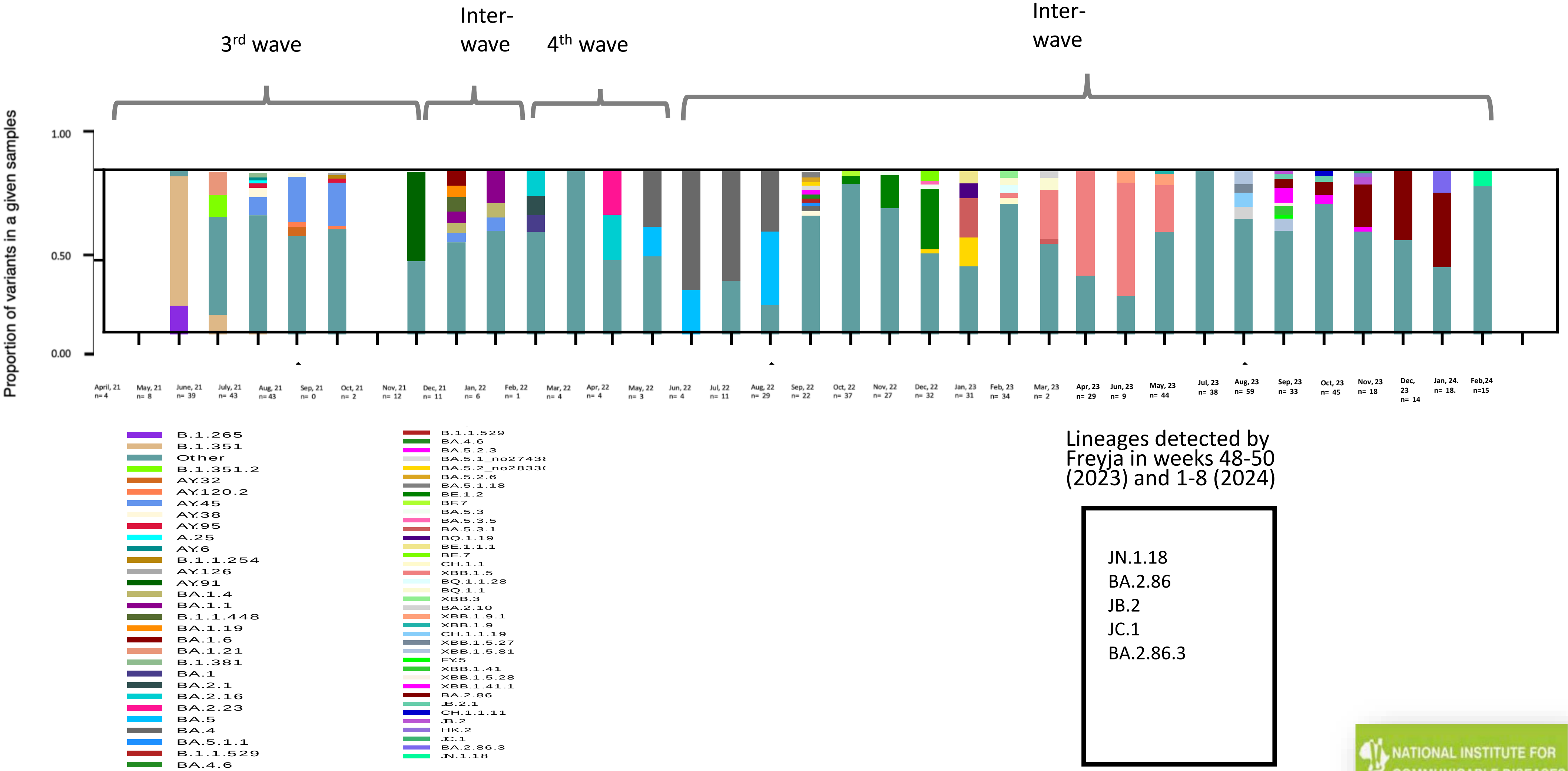


Lineages detected by Freyja in weeks 48-50 (2023) and 1-8 (2024)

JN.1.18
BA.2.86
JB.2
JC.1
BA.2.86.3

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

Summary:

Summary of lineages associated with most common mutations associated

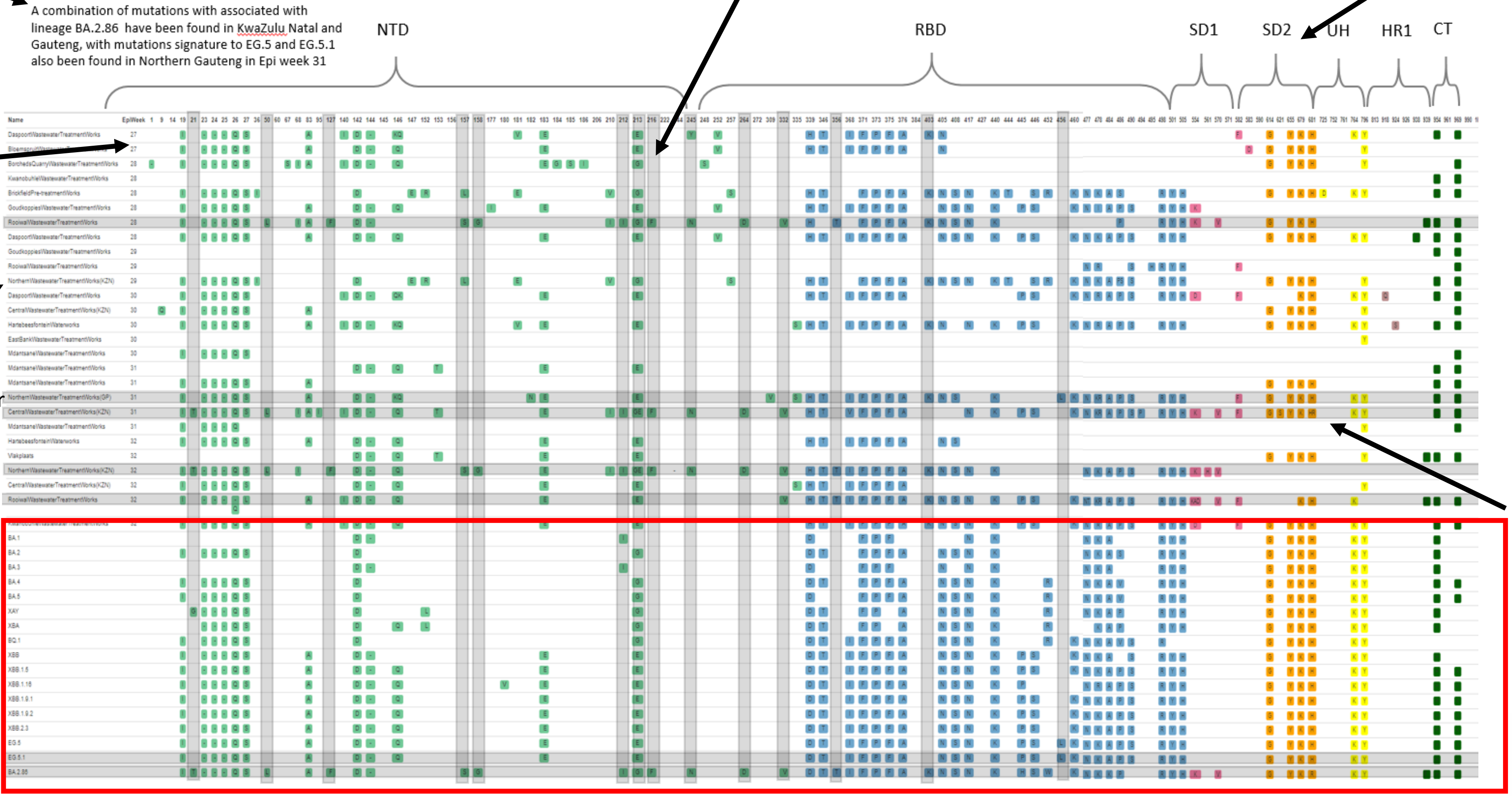
Highlighted

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

Top x-axis:

Position of the spike protein

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

Epi Week:

Epidemiological week during which samples were collected

Site Names:

Sites from which wastewater samples were collected

Coloured blocks

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

Reference lineages:

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

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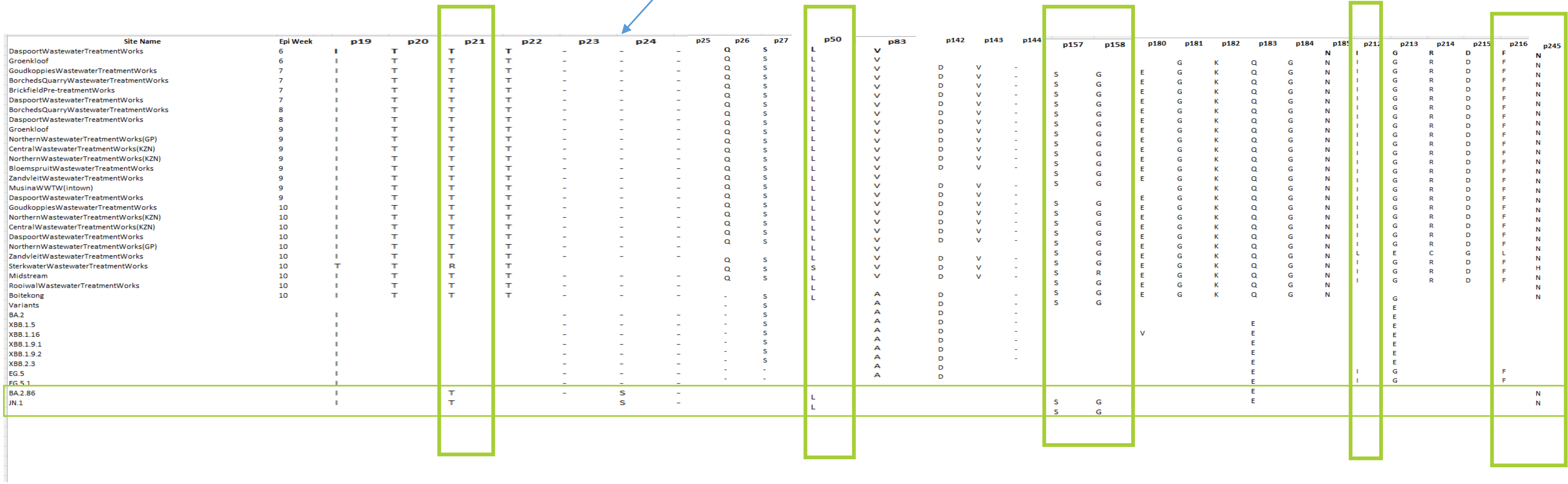
Spike mutational profile of samples from sites across South Africa and VOCs/VOIs processed in weeks 6 - 10, 2024

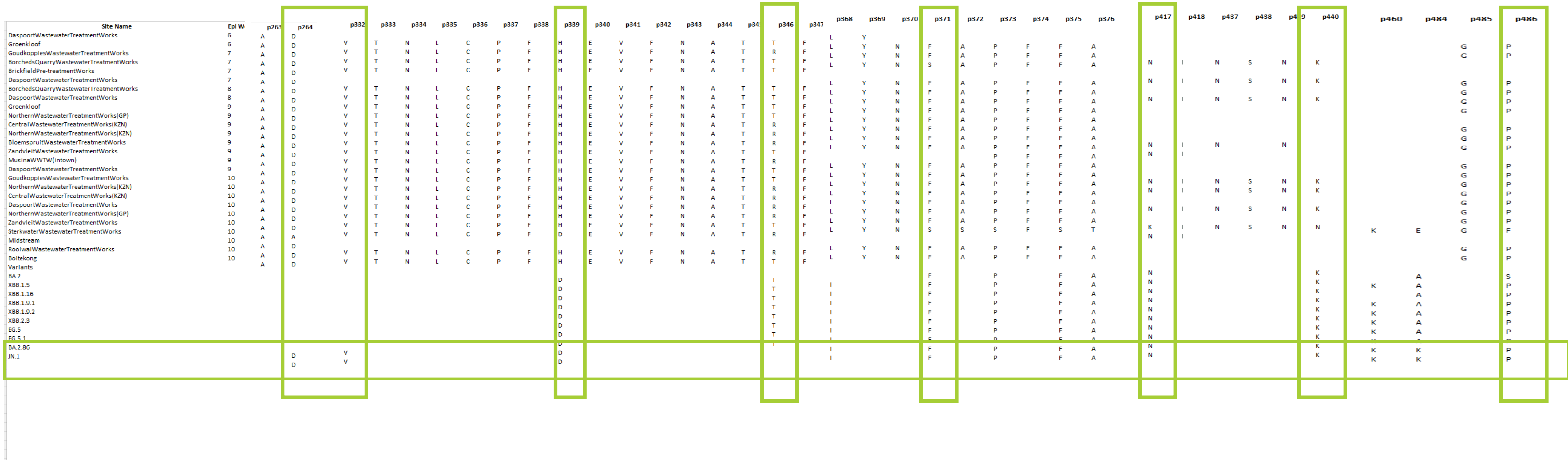


Deletion (-)

Site Name	Epi Week	p19	p20	p21	p22	p23	p24	p25	p26	p27	p50	p83	p142	p143	p144	p157	p158	p180	p181	p182	p183	p184	p185	p212	p213	p214	p215	p216	p245
DaspoortWastewaterTreatmentWorks	6	I	T	T	T	-	-	-	Q	S	L	V											N	I	G	R	D	F	N
Groenkloof	6	I	T	T	T	-	-	-	Q	S	L	V							G	K	Q	G	N	I	G	R	D	F	N
GoudkoppiesWastewaterTreatmentWorks	7	I	T	T	T	-	-	-	Q	S	L	V	D	V	-	S	G	E	G	K	Q	G	N	I	G	R	D	F	N
BorchedsQuarryWastewaterTreatmentWorks	7	I	T	T	T	-	-	-	Q	S	L	V	D	V	-	S	G	E	G	K	Q	G	N	I	G	R	D	F	N
BrickfieldPre-treatmentWorks	7	I	T	T	T	-	-	-	Q	S	L	V	D	V	-	S	G	E	G	K	Q	G	N	I	G	R	D	F	N
DaspoortWastewaterTreatmentWorks	7	I	T	T	T	-	-	-	Q	S	L	V	D	V	-	S	G	E	G	K	Q	G	N	I	G	R	D	F	N
BorchedsQuarryWastewaterTreatmentWorks	8	I	T	T	T	-	-	-	Q	S	L	V	D	V	-	S	G	E	G	K	Q	G	N	I	G	R	D	F	N
DaspoortWastewaterTreatmentWorks	8	I	T	T	T	-	-	-	Q	S	L	V	D	V	-	S	G	E	G	K	Q	G	N	I	G	R	D	F	N
Groenkloof	9	I	T	T	T	-	-	-	Q	S	L	V	D	V	-	S	G	E	G	K	Q	G	N	I	G	R	D	F	N
NorthernWastewaterTreatmentWorks(GP)	9	I	T	T	T	-	-	-	Q	S	L	V	D	V	-	S	G	E	G	K	Q	G	N	I	G	R	D	F	N
CentralWastewaterTreatmentWorks(KZN)	9	I	T	T	T	-	-	-	Q	S	L	V	D	V	-	S	G	E	G	K	Q	G	N	I	G	R	D	F	N
NorthernWastewaterTreatmentWorks(KZN)	9	I	T	T	T	-	-	-	Q	S	L	V	D	V	-	S	G	E	G	K	Q	G	N	I	G	R	D	F	N
BloemspruitWastewaterTreatmentWorks	9	I	T	T	T	-	-	-	Q	S	L	V	D	V	-	S	G	E	G	K	Q	G	N	I	G	R	D	F	N
ZandvleitWastewaterTreatmentWorks	9	I	T	T	T	-	-	-	Q	S	L	V	D	V	-	S	G	E	G	K	Q	G	N	I	G	R	D	F	N
MusinaWWTW(intown)	9	I	T	T	T	-	-	-	Q	S	L	V							G	K	Q	G	N	I	G	R	D	F	N
DaspoortWastewaterTreatmentWorks	9	I	T	T	T	-	-	-	Q	S	L	V	D	V	-	S	G	E	G	K	Q	G	N	I	G	R	D	F	N
GoudkoppiesWastewaterTreatmentWorks	10	I	T	T	T	-	-	-	Q	S	L	V	D	V	-	S	G	E	G	K	Q	G	N	I	G	R	D	F	N
NorthernWastewaterTreatmentWorks(KZN)	10	I	T	T	T	-	-	-	Q	S	L	V	D	V	-	S	G	E	G	K	Q	G	N	I	G	R	D	F	N
CentralWastewaterTreatmentWorks(KZN)	10	I	T	T	T	-	-	-	Q	S	L	V	D	V	-	S	G	E	G	K	Q	G	N	I	G	R	D	F	N
DaspoortWastewaterTreatmentWorks	10	I	T	T	T	-	-	-	Q	S	L	V	D	V	-	S	G	E	G	K	Q	G	N	I	G	R	D	F	N
NorthernWastewaterTreatmentWorks(GP)	10	I	T	T	T	-	-	-	Q	S	L	V	D	V	-	S	G	E	G	K	Q	G	N	I	G	R	D	F	N
ZandvleitWastewaterTreatmentWorks	10	I	T	T	T	-	-	-	Q	S	L	V	D	V	-	S	G	E	G	K	Q	G	N	I	G	R	D	F	N
SterkwaterWastewaterTreatmentWorks	10	T	T	R	T						S	V				S	R	E	G	K	Q	G	N	L	E	C	G	L	H
Midstream	10	I	T	T	T	-	-	-	Q	S	L	V	D	V	-	S	G	E	G	K	Q	G	N	I	G	R	D	F	N
RooiwalWastewaterTreatmentWorks	10	I	T	T	T	-	-	-	Q	S	L	V	D	V	-	S	G	E	G	K	Q	G	N	I	G	R	D	F	N
Boitekong	10	I	T	T	T	-	-	-	Q	S	L	V	D	V	-	S	G	E	G	K	Q	G	N	I	G	R	D	F	N
Variants																													
BA.2		I				-	-	-	-	S		A	D		-										G				
XBB.1.5		I				-	-	-	-	S		A	D		-						E				E				
XBB.1.16		I				-	-	-	-	S		A	D		-			V			E				E				
XBB.1.9.1		I				-	-	-	-	S		A	D		-						E				E				
XBB.1.9.2		I				-	-	-	-	S		A	D		-						E				E				
XBB.2.3		I				-	-	-	-	S		A	D		-						E				E				
EG.5		I				-	-	-	-	S		A	D		-						E				E				
EG.5.1		I				-	-	-	-	S		A	D		-						E				E				
BA.2.86		I		T		-	S	-	-	-	L	A	D			S	G				E			I	G			F	N
JN.1		I		T			S	-	-	-	L	A	D			S	G				E			I	G			F	N

A. combination of mutations associated with lineage BA.2.86, JN.1 and XBB sublineages have been found in sites across all South African provinces during Epi weeks 6-10

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



Site Name	Epi W	p263	p264	p332	p333	p334	p335	p336	p337	p338	p339	p340	p341	p342	p343	p344	p345	p346	p347	p368	p369	p370	p371	p372	p373	p374	p375	p376	p417	p418	p437	p438	p4 9	p440	p460	p484	p485	p486
DaspoortWastewaterTreatmentWorks	6	A	D																																			
Groenkloof	6	A	D	V	T	N	L	C	P	F	H	E	V	F	N	A	T	T	F	L	Y																	
GoudkoppiesWastewaterTreatmentWorks	7	A	D	V	T	N	L	C	P	F	H	E	V	F	N	A	T	R	F	L	Y	N	F	A	P	F	F	A									G	P
BorchedsQuarryWastewaterTreatmentWorks	7	A	D	V	T	N	L	C	P	F	H	E	V	F	N	A	T	T	F	L	Y	N	F	A	P	F	F	A									G	P
BrickfieldPre-treatmentWorks	7	A	D	V	T	N	L	C	P	F	H	E	V	F	N	A	T	T	F	L	Y	N	S	A	P	F	F	A	N	I	N	S	N	K				
DaspoortWastewaterTreatmentWorks	7	A	D																																			
BorchedsQuarryWastewaterTreatmentWorks	8	A	D	V	T	N	L	C	P	F	H	E	V	F	N	A	T	T	F	L	Y	N	F	A	P	F	F	A	N	I	N	S	N	K			G	P
DaspoortWastewaterTreatmentWorks	8	A	D	V	T	N	L	C	P	F	H	E	V	F	N	A	T	T	F	L	Y	N	F	A	P	F	F	A									G	P
Groenkloof	9	A	D	V	T	N	L	C	P	F	H	E	V	F	N	A	T	T	F	L	Y	N	F	A	P	F	F	A	N	I	N	S	N	K			G	P
NorthernWastewaterTreatmentWorks(GP)	9	A	D	V	T	N	L	C	P	F	H	E	V	F	N	A	T	T	F	L	Y	N	F	A	P	F	F	A									G	P
CentralWastewaterTreatmentWorks(KZN)	9	A	D	V	T	N	L	C	P	F	H	E	V	F	N	A	T	R	F	L	Y	N	F	A	P	F	F	A										
NorthernWastewaterTreatmentWorks(KZN)	9	A	D	V	T	N	L	C	P	F	H	E	V	F	N	A	T	R	F	L	Y	N	F	A	P	F	F	A									G	P
BloemspruitWastewaterTreatmentWorks	9	A	D	V	T	N	L	C	P	F	H	E	V	F	N	A	T	R	F	L	Y	N	F	A	P	F	F	A									G	P
ZandvleitWastewaterTreatmentWorks	9	A	D	V	T	N	L	C	P	F	H	E	V	F	N	A	T	T	F	L	Y	N	F	A	P	F	F	A	N	I	N		N				G	P
MusinaWWTW(intown)	9	A	D	V	T	N	L	C	P	F	H	E	V	F	N	A	T	R	F						P	F	F	A	N	I								
DaspoortWastewaterTreatmentWorks	9	A	D	V	T	N	L	C	P	F	H	E	V	F	N	A	T	T	F	L	Y	N	F	A	P	F	F	A									G	P
GoudkoppiesWastewaterTreatmentWorks	10	A	D	V	T	N	L	C	P	F	H	E	V	F	N	A	T	T	F	L	Y	N	F	A	P	F	F	A									G	P
NorthernWastewaterTreatmentWorks(KZN)	10	A	D	V	T	N	L	C	P	F	H	E	V	F	N	A	T	R	F	L	Y	N	F	A	P	F	F	A	N	I	N	S	N	K			G	P
CentralWastewaterTreatmentWorks(KZN)	10	A	D	V	T	N	L	C	P	F	H	E	V	F	N	A	T	R	F	L	Y	N	F	A	P	F	F	A	N	I	N	S	N	K			G	P
DaspoortWastewaterTreatmentWorks	10	A	D	V	T	N	L	C	P	F	H	E	V	F	N	A	T	R	F	L	Y	N	F	A	P	F	F	A									G	P
NorthernWastewaterTreatmentWorks(GP)	10	A	D	V	T	N	L	C	P	F	H	E	V	F	N	A	T	R	F	L	Y	N	F	A	P	F	F	A	N	I	N	S	N	K			G	P
ZandvleitWastewaterTreatmentWorks	10	A	D	V	T	N	L	C	P	F	H	E	V	F	N	A	T	T	F	L	Y	N	F	A	P	F	F	A									G	P
SterkwaterWastewaterTreatmentWorks	10	A	A	V	T	N	L	C	P	F	D	E	V	F	N	A	T	R	F	L	Y	N	S	S	S	F	S	T	K	I	N	S	N	N	K	E	G	F
Midstream	10	A	D																										N	I								
RooiwalWastewaterTreatmentWorks	10	A	D	V	T	N	L	C	P	F	H	E	V	F	N	A	T	R	F	L	Y	N	F	A	P	F	F	A									G	P
Boitekong	10	A	D	V	T	N	L	C	P	F	H	E	V	F	N	A	T	T	F	L	Y	N	F	A	P	F	F	A									G	P
Variants																																						
BA.2											D							T					F		P		F	A	N					K		A		S
XBB.1.5											D							T		I			F		P		F	A	N					K	K	A		P
XBB.1.16											D							T		I			F		P		F	A	N					K		A		P
XBB.1.9.1											D							T		I			F		P		F	A	N					K	K	A		P
XBB.1.9.2											D							T		I			F		P		F	A	N					K	K	A		P
XBB.2.3											D							T		I			F		P		F	A	N					K	K	A		P
EG.5											D							T		I			F		P		F	A	N					K	K	A		P
EG.5.1											D							T		I			F		P		F	A	N					K	K	A		P
BA.2.86			D	V							D									I			F		P		F	A	N					K	K	K		P
JN.1			D	V							D									I			F		P		F	A	N					K	K	K		P

A. combination of mutations associated with lineage BA.2.86, JN.1 and XBB sublineages have been found in sites across all South African provinces during Epi weeks 6-10

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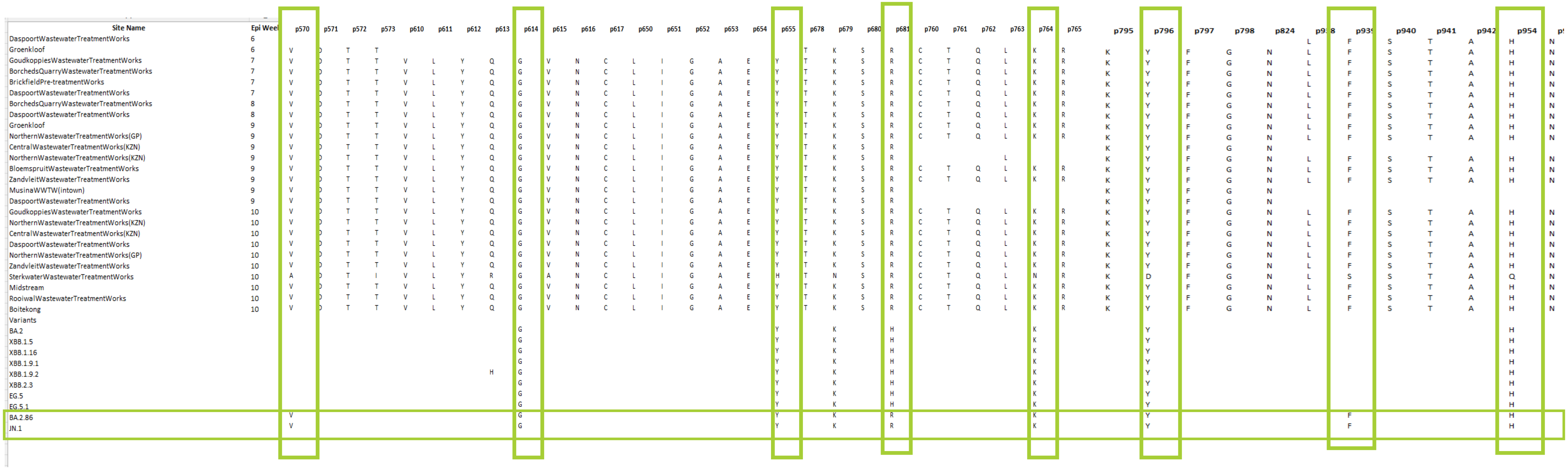
Spike mutational profile of samples from sites across South Africa and VOCs/VOIs processed in weeks 42 - 46, 2023



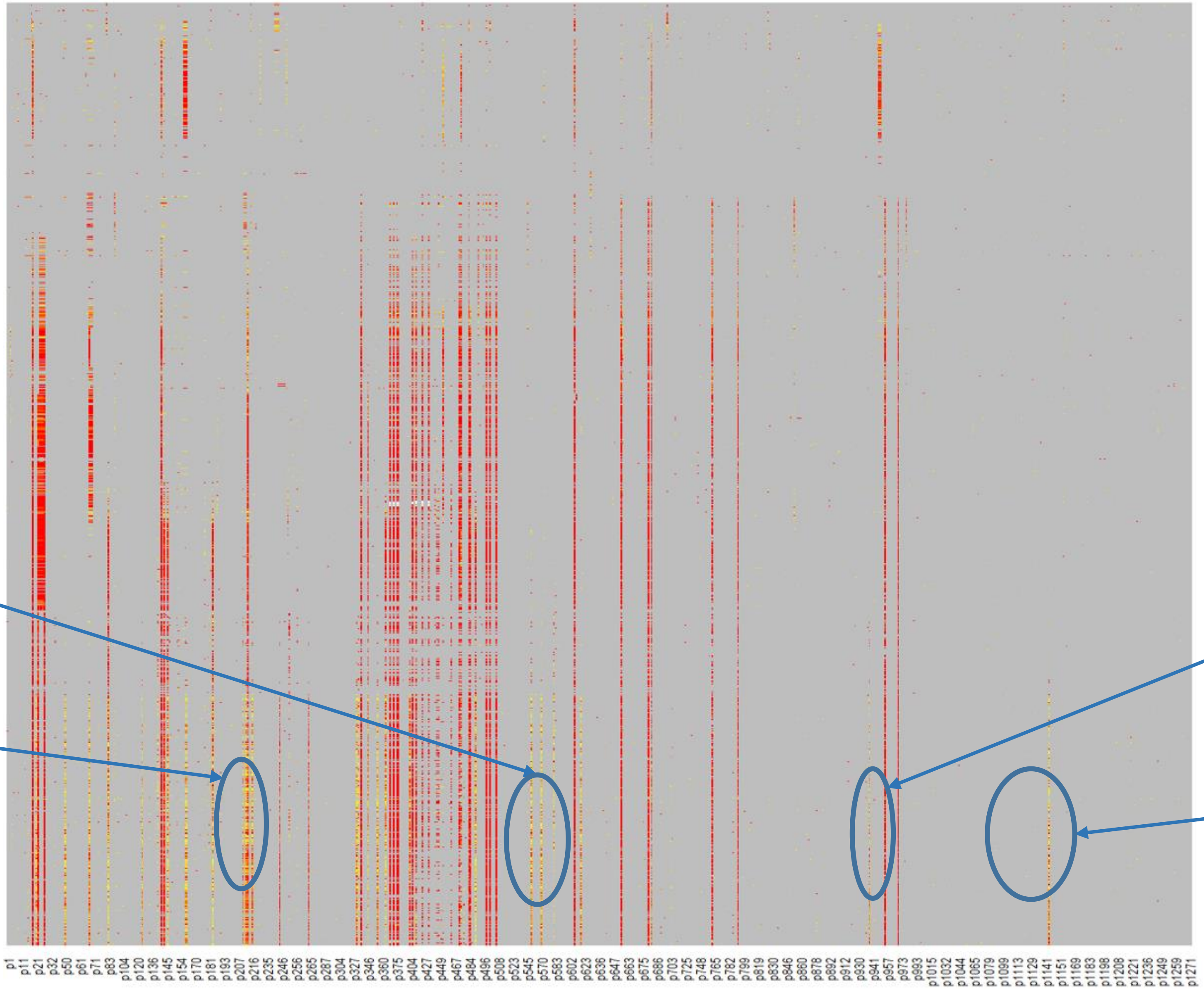
Site Name	Epi Week	p570	p571	p572	p573	p610	p611	p612	p613	p614	p615	p616	p617	p650	p651	p652	p653	p654	p655	p678	p679	p680	p681	p760	p761	p762	p763	p764	p765	p795	p796	p797	p798	p824	p938	p939	p940	p941	p942	p954	p9
DaspoortWastewaterTreatmentWorks	6																																		L	F	S	T	A	H	N
Groenkloof	6	V	D	T	T															T	K	S	R	C	T	Q	L	K	R	K	Y	F	G	N	L	F	S	T	A	H	N
GoudkoppiesWastewaterTreatmentWorks	7	V	D	T	T	V	L	Y	Q	G	V	N	C	L	I	G	A	E	Y	T	K	S	R	C	T	Q	L	K	R	K	Y	F	G	N	L	F	S	T	A	H	N
BorchedsQuarryWastewaterTreatmentWorks	7	V	D	T	T	V	L	Y	Q	G	V	N	C	L	I	G	A	E	Y	T	K	S	R	C	T	Q	L	K	R	K	Y	F	G	N	L	F	S	T	A	H	N
BrickfieldPre-treatmentWorks	7	V	D	T	T	V	L	Y	Q	G	V	N	C	L	I	G	A	E	Y	T	K	S	R	C	T	Q	L	K	R	K	Y	F	G	N	L	F	S	T	A	H	N
DaspoortWastewaterTreatmentWorks	7	V	D	T	T	V	L	Y	Q	G	V	N	C	L	I	G	A	E	Y	T	K	S	R	C	T	Q	L	K	R	K	Y	F	G	N	L	F	S	T	A	H	N
BorchedsQuarryWastewaterTreatmentWorks	8	V	D	T	T	V	L	Y	Q	G	V	N	C	L	I	G	A	E	Y	T	K	S	R	C	T	Q	L	K	R	K	Y	F	G	N	L	F	S	T	A	H	N
DaspoortWastewaterTreatmentWorks	8	V	D	T	T	V	L	Y	Q	G	V	N	C	L	I	G	A	E	Y	T	K	S	R	C	T	Q	L	K	R	K	Y	F	G	N	L	F	S	T	A	H	N
Groenkloof	9	V	D	T	T	V	L	Y	Q	G	V	N	C	L	I	G	A	E	Y	T	K	S	R	C	T	Q	L	K	R	K	Y	F	G	N	L	F	S	T	A	H	N
NorthernWastewaterTreatmentWorks(GP)	9	V	D	T	T	V	L	Y	Q	G	V	N	C	L	I	G	A	E	Y	T	K	S	R	C	T	Q	L	K	R	K	Y	F	G	N	L	F	S	T	A	H	N
CentralWastewaterTreatmentWorks(KZN)	9	V	D	T	T	V	L	Y	Q	G	V	N	C	L	I	G	A	E	Y	T	K	S	R							K	Y	F	G	N							
NorthernWastewaterTreatmentWorks(KZN)	9	V	D	T	T	V	L	Y	Q	G	V	N	C	L	I	G	A	E	Y	T	K	S	R				L			K	Y	F	G	N	L	F	S	T	A	H	N
BloemspruitWastewaterTreatmentWorks	9	V	D	T	T	V	L	Y	Q	G	V	N	C	L	I	G	A	E	Y	T	K	S	R	C	T	Q	L	K	R	K	Y	F	G	N	L	F	S	T	A	H	N
ZandvleitWastewaterTreatmentWorks	9	V	D	T	T	V	L	Y	Q	G	V	N	C	L	I	G	A	E	Y	T	K	S	R	C	T	Q	L	K	R	K	Y	F	G	N	L	F	S	T	A	H	N
MusinaWWTW(intown)	9	V	D	T	T	V	L	Y	Q	G	V	N	C	L	I	G	A	E	Y	T	K	S	R							K	Y	F	G	N							
DaspoortWastewaterTreatmentWorks	9	V	D	T	T	V	L	Y	Q	G	V	N	C	L	I	G	A	E	Y	T	K	S	R							K	Y	F	G	N							
GoudkoppiesWastewaterTreatmentWorks	10	V	D	T	T	V	L	Y	Q	G	V	N	C	L	I	G	A	E	Y	T	K	S	R	C	T	Q	L	K	R	K	Y	F	G	N	L	F	S	T	A	H	N
NorthernWastewaterTreatmentWorks(KZN)	10	V	D	T	T	V	L	Y	Q	G	V	N	C	L	I	G	A	E	Y	T	K	S	R	C	T	Q	L	K	R	K	Y	F	G	N	L	F	S	T	A	H	N
CentralWastewaterTreatmentWorks(KZN)	10	V	D	T	T	V	L	Y	Q	G	V	N	C	L	I	G	A	E	Y	T	K	S	R	C	T	Q	L	K	R	K	Y	F	G	N	L	F	S	T	A	H	N
DaspoortWastewaterTreatmentWorks	10	V	D	T	T	V	L	Y	Q	G	V	N	C	L	I	G	A	E	Y	T	K	S	R	C	T	Q	L	K	R	K	Y	F	G	N	L	F	S	T	A	H	N
NorthernWastewaterTreatmentWorks(GP)	10	V	D	T	T	V	L	Y	Q	G	V	N	C	L	I	G	A	E	Y	T	K	S	R	C	T	Q	L	K	R	K	Y	F	G	N	L	F	S	T	A	H	N
ZandvleitWastewaterTreatmentWorks	10	V	D	T	T	V	L	Y	Q	G	V	N	C	L	I	G	A	E	Y	T	K	S	R	C	T	Q	L	K	R	K	Y	F	G	N	L	F	S	T	A	H	N
SterkwaterWastewaterTreatmentWorks	10	A	D	T	I	V	L	Y	R	G	A	N	C	L	I	G	A	E	H	T	N	S	R	C	T	Q	L	N	R	K	D	F	G	N	L	S	S	T	A	Q	N
Midstream	10	V	D	T	T	V	L	Y	Q	G	V	N	C	L	I	G	A	E	Y	T	K	S	R	C	T	Q	L	K	R	K	Y	F	G	N	L	F	S	T	A	H	N
RooiwalWastewaterTreatmentWorks	10	V	D	T	T	V	L	Y	Q	G	V	N	C	L	I	G	A	E	Y	T	K	S	R	C	T	Q	L	K	R	K	Y	F	G	N	L	F	S	T	A	H	N
Boitekong	10	V	D	T	T	V	L	Y	Q	G	V	N	C	L	I	G	A	E	Y	T	K	S	R	C	T	Q	L	K	R	K	Y	F	G	N	L	F	S	T	A	H	N
Variants																																									
BA.2										G									Y		K		H					K			Y									H	
XBB.1.5										G									Y		K		H					K			Y									H	
XBB.1.16										G									Y		K		H					K			Y									H	
XBB.1.9.1										G									Y		K		H					K			Y									H	
XBB.1.9.2									H	G									Y		K		H					K			Y									H	
XBB.2.3										G									Y		K		H					K			Y									H	
EG.5										G									Y		K		H					K			Y									H	
EG.5.1										G									Y		K		H					K			Y									H	
BA.2.86		V								G									Y		K		R					K			Y					F				H	
JN.1		V								G									Y		K		R					K			Y					F				H	

A. combination of mutations associated with lineage BA.2.86, JN.1 and XBB sublineages have been found in sites across all South African provinces during Epi weeks 6-10

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)



E554K
Mutations in spike protein associated with BA.2.86

V213E,
R346T

Mutations in spike protein associated with XBB* sub-lineages

S939F

Spike protein mutation associated with the BA.2.86 lineage

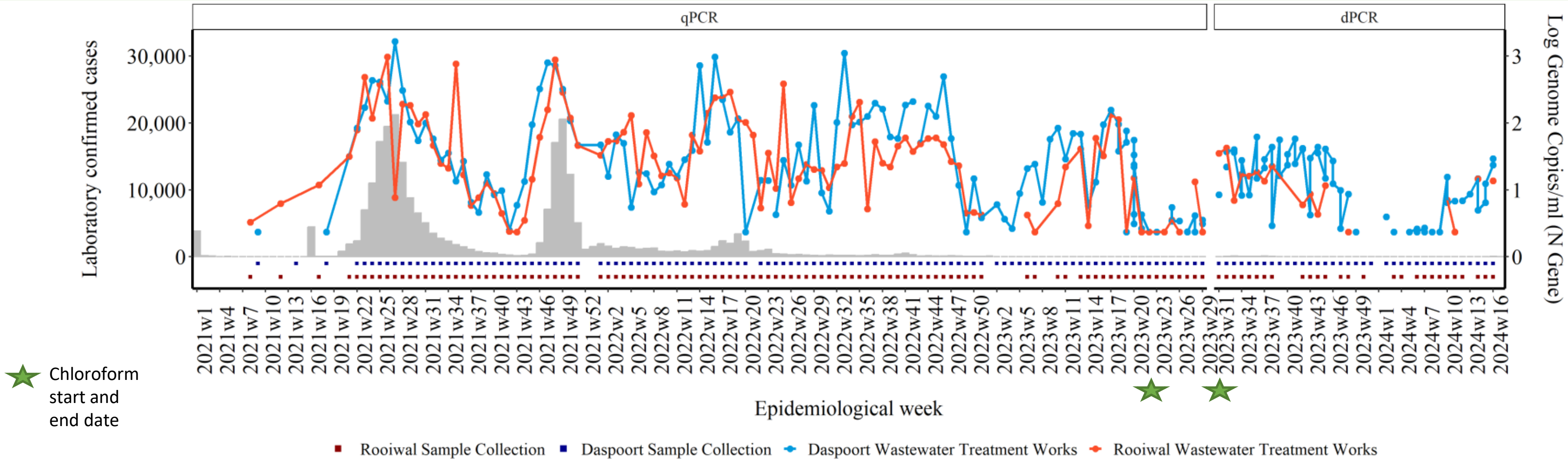
P1143L

Spike protein mutation associated with the BA.2.86 lineage

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*

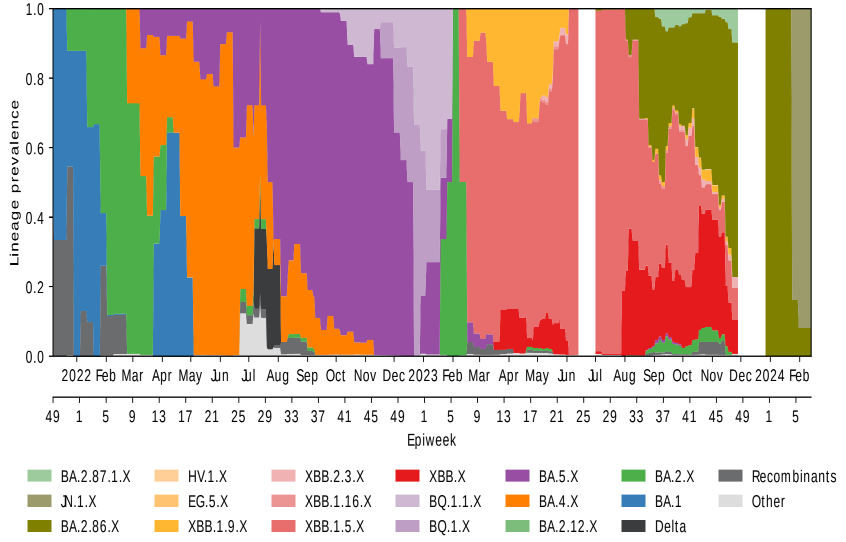
Heatmap showing patterns of emerging mutations in the spike region of SARS-CoV-2, collected from April, 2021 - November, 2023. Mutations appearing in yellow have a low read frequency, those appearing in orange have a medium read frequency and those appearing in red have a high read frequency. Mutations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

Gauteng - Tshwane

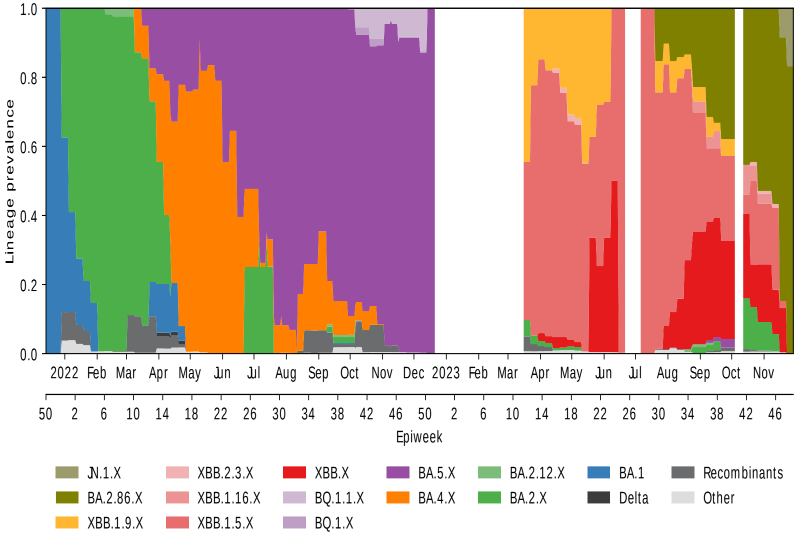


Wastewater levels of SARS-CoV-2 in log-transformed genome copies/ml (right vertical axis, line graph) and laboratory confirmed cases of SARS-CoV-2 for Tshwane (left vertical axis, grey bars) by epidemiological week. Coloured dots below the horizontal axis signify if a sample was collected. SARS-CoV-2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

Daspoort Wastewater Treatment Works



Rooiwal Wastewater Treatment Works



SARS-CoV-2 levels and Genomic Results in Epi week 16:

- SARS-CoV-2 levels in Daspoort WWTW increase to moderate in Epi week 14 and decrease to low again in Epi week 15. Levels increase to moderate again in Epi week 16.
- SARS-CoV-2 levels in Rooiwal WWTW have decreased in Epi week 47. Levels remain low in Epi week 11 and increase to moderate in Epi week 14. Levels remain moderate in Epi week 16.

**** Sequencing data ending in Epi week 5 in Daspoort and 46 in Rooiwal.***

- Omicron lineages JN.1.X and BA.2.86.X, XBB.1.19.X and JN.1.X were circulating in Daspoort during Epi week 5.

- BA.2.86.X, XBB.1.5.X, XBB.2.3.X and JN.1.X was also the dominant lineage detected in Rooiwal during Epi week 42-46

SNP Analysis:

- A combination of mutations (V127F, L212I, V213G, L216F, H245N, A264D, I332V, K356T) associated with lineage BA.2.86 and JN.1 were found in both Daspoort and Rooiwal.

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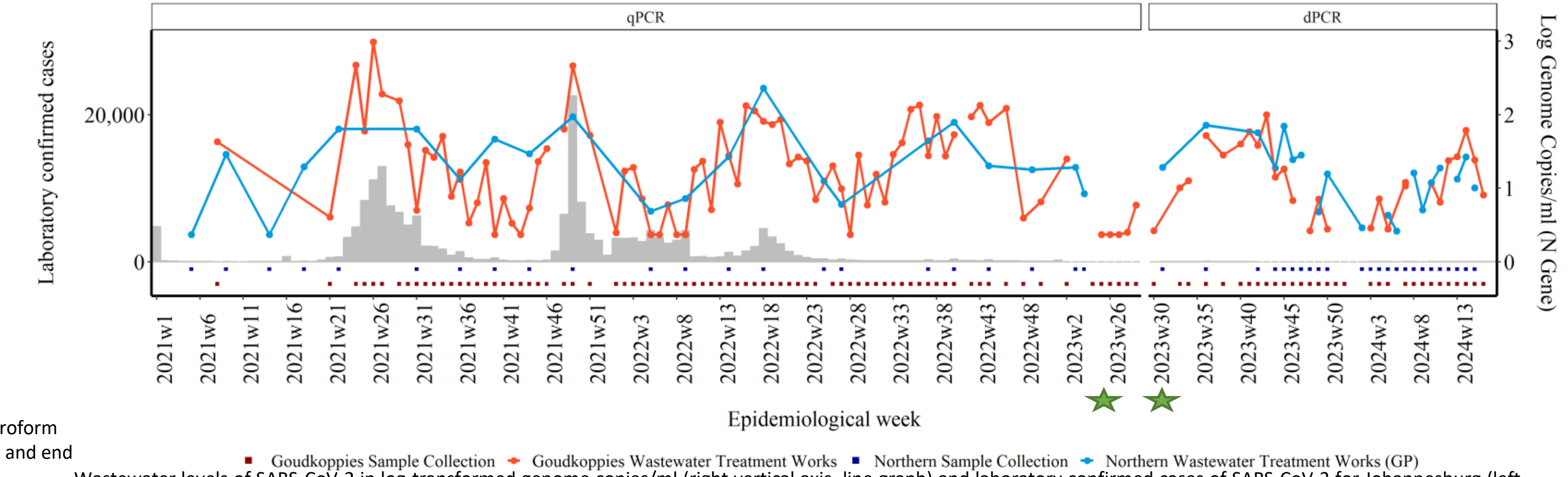
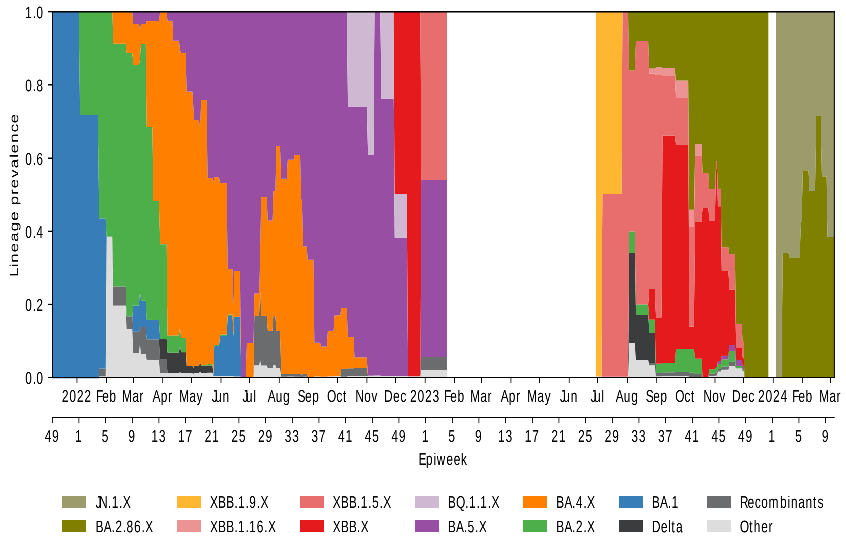
Gauteng - Johannesburg



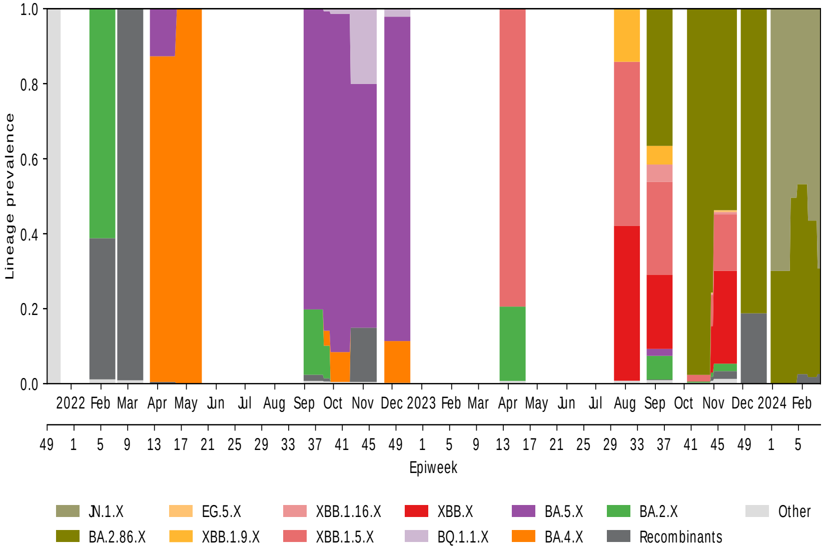
★ Chloroform start and end date

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

Goudkoppies Wastewater Treatment Works



Northern Wastewater Treatment Works



SARS-CoV-2 levels and Genomic Results in Epi week 16:

- SARS-CoV-2 levels in Goudkoppies WWTW increased from Epi week 5 to Epi week 7, but levels remain low. Levels increase to moderate in Epi week 12. Levels decreased in Epi week 15 but remain moderate. Levels decreased further in Epi week 16 to low.
- In Northern WWTW, SARS-CoV-2 levels increased to moderate in week 8 from low levels seen in week 6. Levels decreased to low in Epi week 9, followed by an increase to moderate in Epi week 10. Levels remain moderate in Epi week 14. Levels decrease to low in Epi week 15. No new results for Epi week 16 are available.

**** Sequencing data ending in Epi week 9 in Goudkoppies and 5 in Northern Wastewater Treatment Works.***

- JN.1.X and BA.2.8.X lineages were dominating during epiweek 1 - 9 in Goudkoppies.
- During Epi week 1 – 5 BA.2.86.X, JN.1.X lineages were circulating in Northern (GP). In Epi week 5, Recombinants were also observed.

SNP Analysis:

- A combination of mutations (V127F, L212I, V213G, L216F, H245N, A264D, I332V, K356T) associated with lineage BA.2.86 were found in Goudkoppies.
- A combination of mutations (V127F, L212I, V213G, L216F, H245N, A264D, I332V, K356T) associated with lineage BA.2.86 and JN.1were found in Northern Johannesburg.

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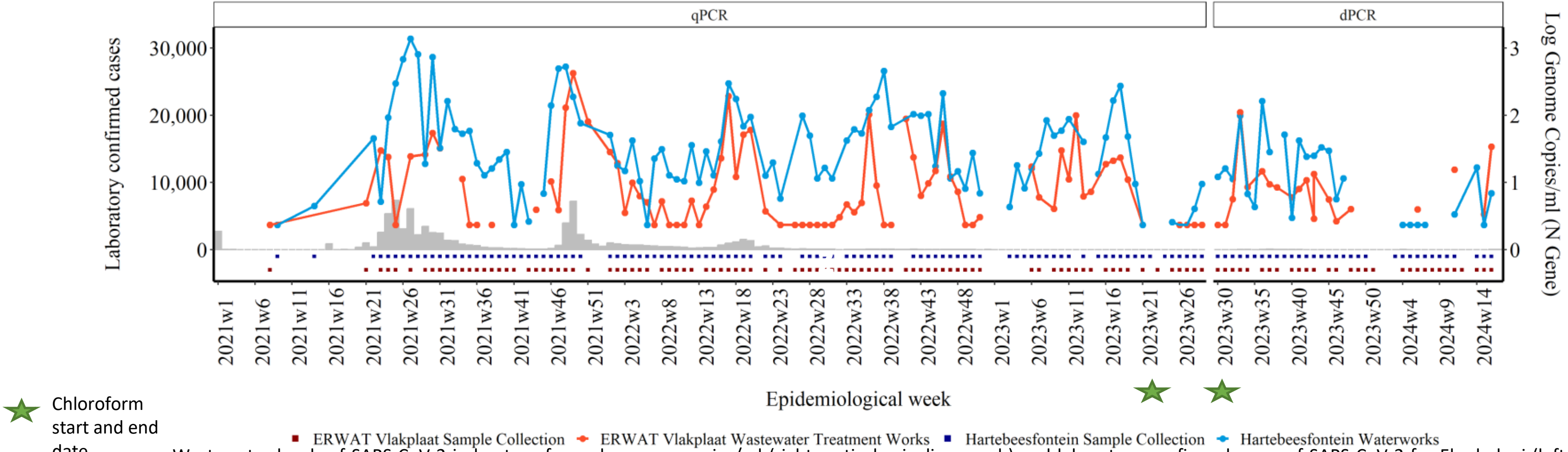
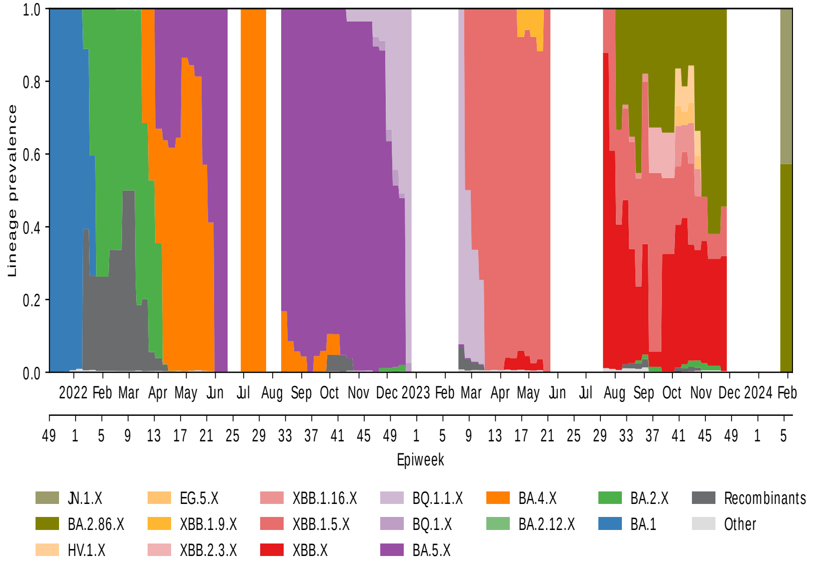
Gauteng - Ekurhuleni

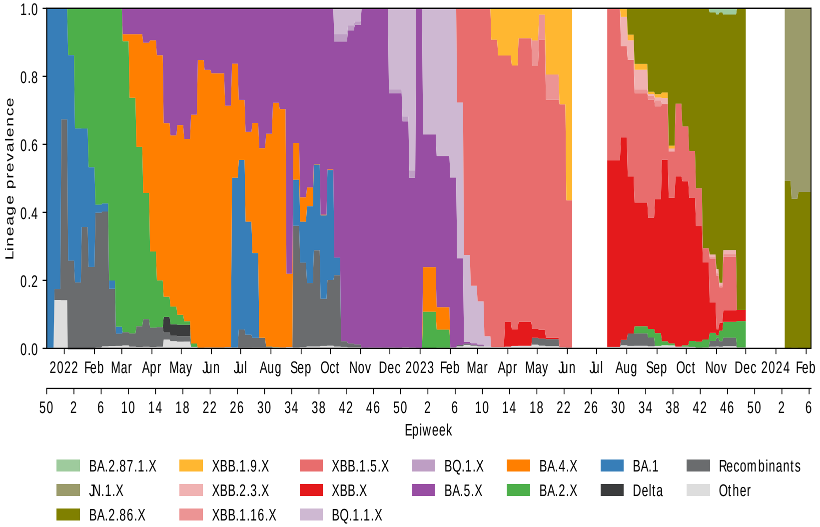


Wastewater levels of SARS-CoV-2 in log-transformed genome copies/ml (right vertical axis, line graph) and laboratory confirmed cases of SARS-CoV-2 for Ekurhuleni (left vertical axis, grey bars) by epidemiological week. Coloured dots below the horizontal axis signify if a sample was collected. SARS-CoV-2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

ERWAT Vlakplaats Wastewater Treatment Works



Hartebeesfontein Wastewater Treatment Works



SARS-CoV-2 levels and Genomic Results in Epi week 16:

- The SARS-CoV-2 levels in Hartebeesfontein WWTW decreased in Epi weeks 4 and remain low in Epi week 7. Levels remain low in Epi week 11. Levels increase to moderate in Epi week 14. Levels decrease to low in Epi week 15 and remain low in Epi week 16.
- As of Epi week 43, there was a slight increase in SARS-CoV-2 levels in Vlakplaats WWTW, after a decrease from Epi week 35. Levels remain low in Epi week 6. Levels rise to moderate in Epi week 11. Levels decrease to low in Epi week 15 and increased to moderate in Epi week 16.

**** Sequencing data ending in Epi week 5 in Vlakplaats and 6 in Hartebeesfontein.***

- Omicron lineages BA.2.86.X and JN.1.X were circulating in Vlakplaats during Epi week 5.
- Lineages BA.2.86 and JN.1.X were predominantly circulating during Epi week 6 at the Hartebeesfontein water treatment plant.

SNP Analysis:

- A combination of mutations (V127F, L212I, V213G, L216F, H245N, A264D, I332V, K356T) associated with lineage BA.2.86 and JN.1were found in both Ekurhuleni treatment plants.

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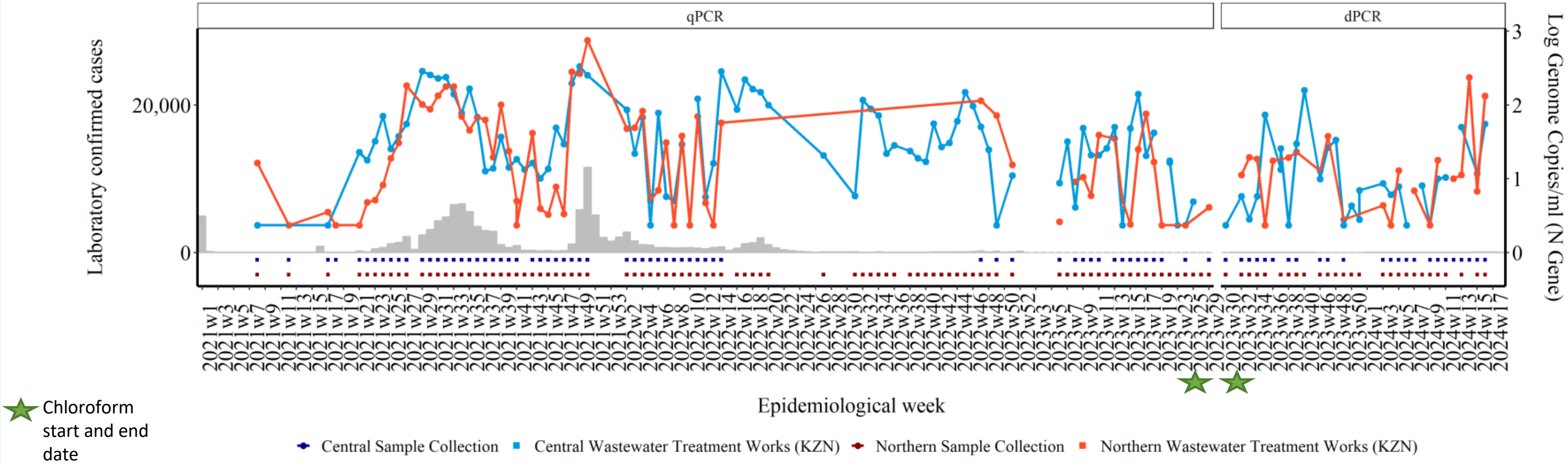
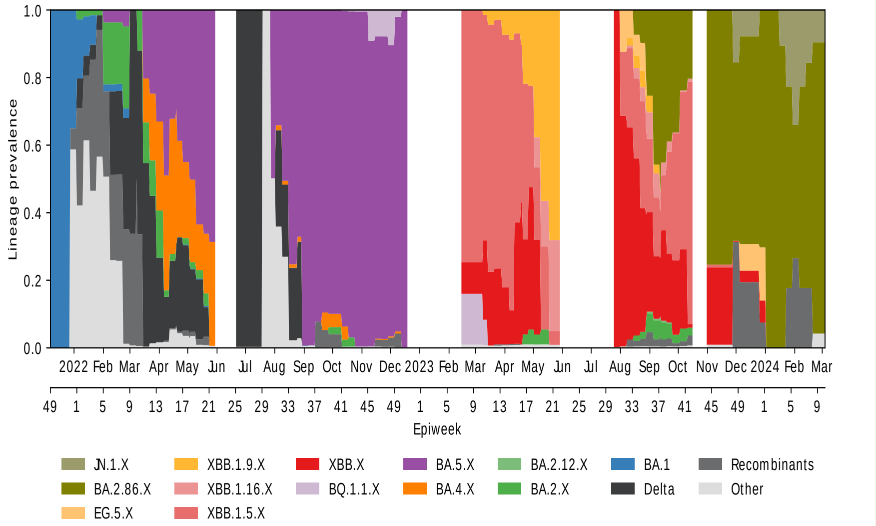
KwaZulu-Natal - eThekwini

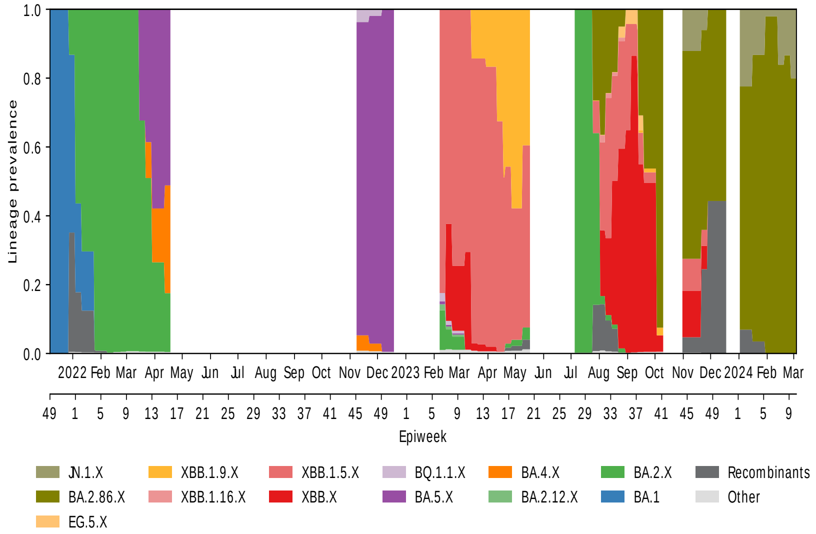


Wastewater levels of SARS-CoV-2 in log-transformed genome copies/ml (right vertical axis, line graph) and laboratory confirmed cases of SARS-CoV-2 for eThekwini (left vertical axis, grey bars) by epidemiological week. Coloured dots below the horizontal axis signify if a sample was collected. SARS-CoV-2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

Central Wastewater Treatment Works



Northern Wastewater Treatment Works



SARS-CoV-2 levels and Genomic Results in Epi week 16:

- SARS-CoV-2 levels in Central WWTW increase in Epi week 10 but remain low. Levels increase to moderate in Epi week 13. Levels decreased to low in Epi week 15 and increased to moderate in Epi week 16.
- SARS-CoV-2 levels increased in Epi week 5 in Northern WWTW. Levels increase to moderate in Epi week 10 and remain moderate in Epi week 13. Levels increase to high in Epi week 14. Levels decrease to low in Epi week 15 and increase to high in Epi week 16.

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

- During Epiweek 1 - 9 Lineage BA.2.86.X, JN.1.X were dominantly circulating in eThekwini Central. XBB.X, XBB.1.9.X and Recombinants were also circulating in the recent weeks.
- In eThekwini North, BA.2.86.X lineages were dominating. In circulation there were also lineages JN.1.X and Recombinant lineages during Epi week 1 - 9.

SNP Analysis:

- A combination of mutations (V127F, L212I, V213G, L216F, H245N, A264D, I332V, K356T) associated with lineage BA.2.86 and JN.1were found in both eThekwini wastewater treatment plants.

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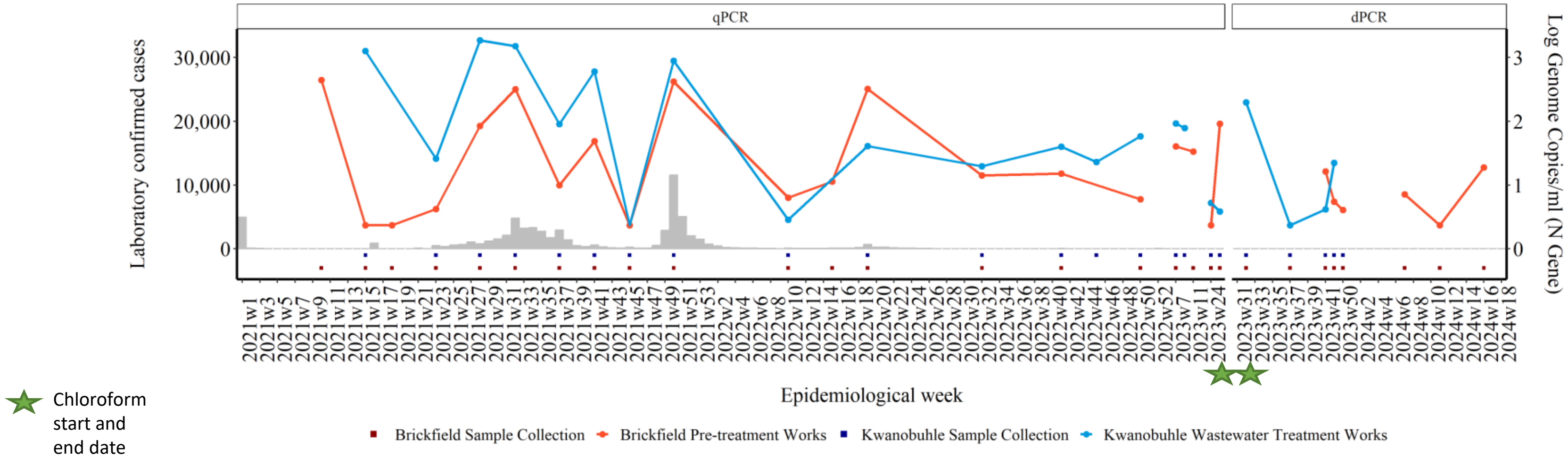
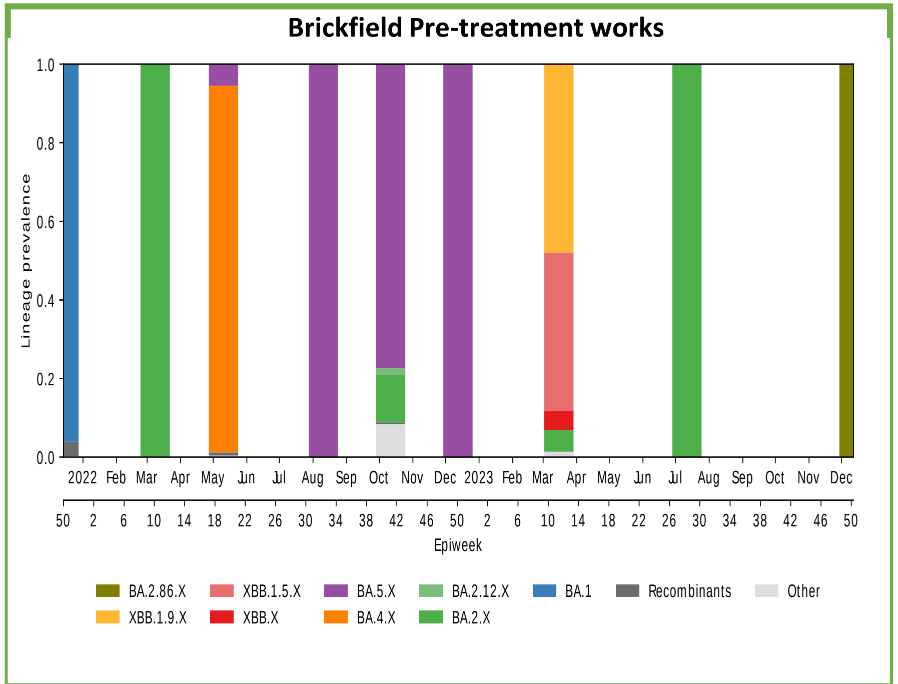
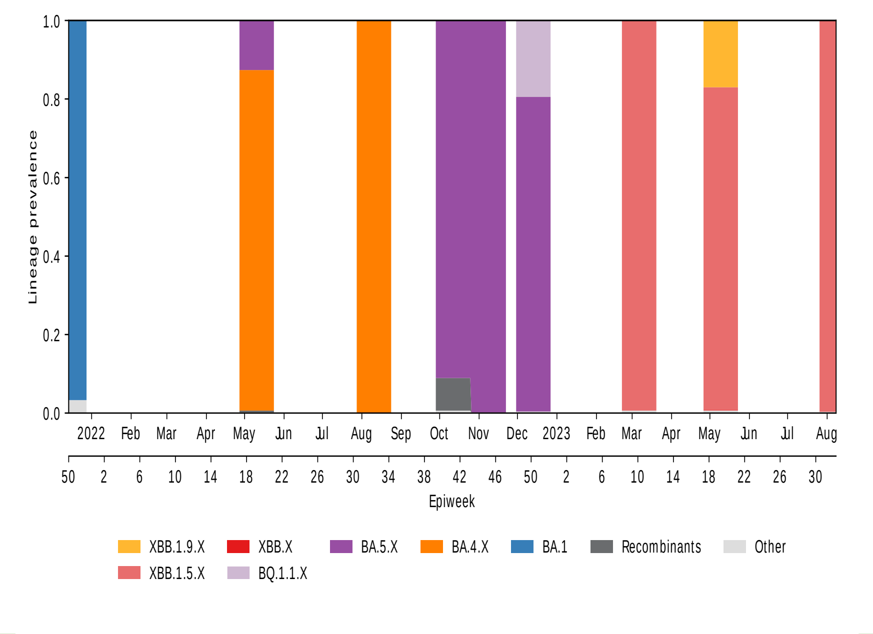
Eastern Cape – Nelson Mandela



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





SARS-CoV-2 levels and Genomic Results in Epi week 16:

- As of Epi week 46, SARS-CoV-2 levels are moderate after an increase was observed after Epi week 36 in the Kwanobuhle WWTW. No new results are available for Epi week 16.
- SARS-CoV-2 levels decreased from moderate to low from Epi week 43 to Epi week 46 in Brickfield Pre-treatment works. There was a slight increase in level in Epi week 7, but levels remain low. Levels decrease and remain low in Epi week 11. Levels increase to moderate in Epi week 16.

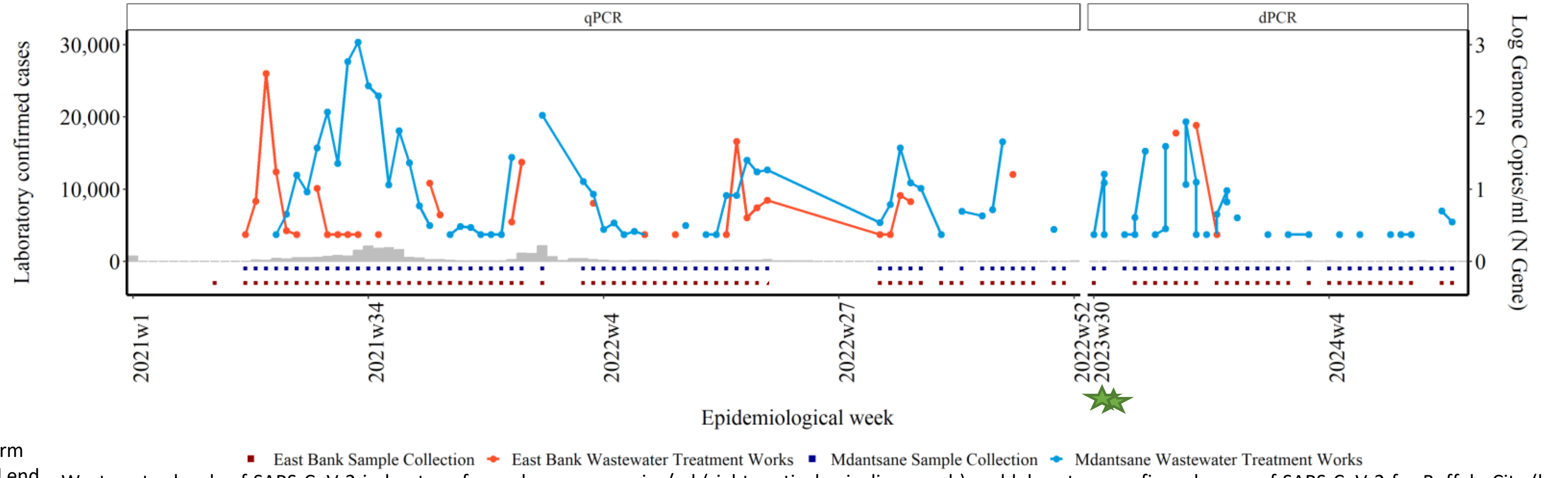
**** Sequencing data ending in Epi week 50 in Brickfield and 30 in Kwanobuhle.***

- BA.2.86.X lineage was dominating during Epi week 50 in Mdantsane
- Omicron lineages XBB.1.5.X sub-lineages were circulating in Kwanobuhle during Epi week 30.

SNP Analysis:

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

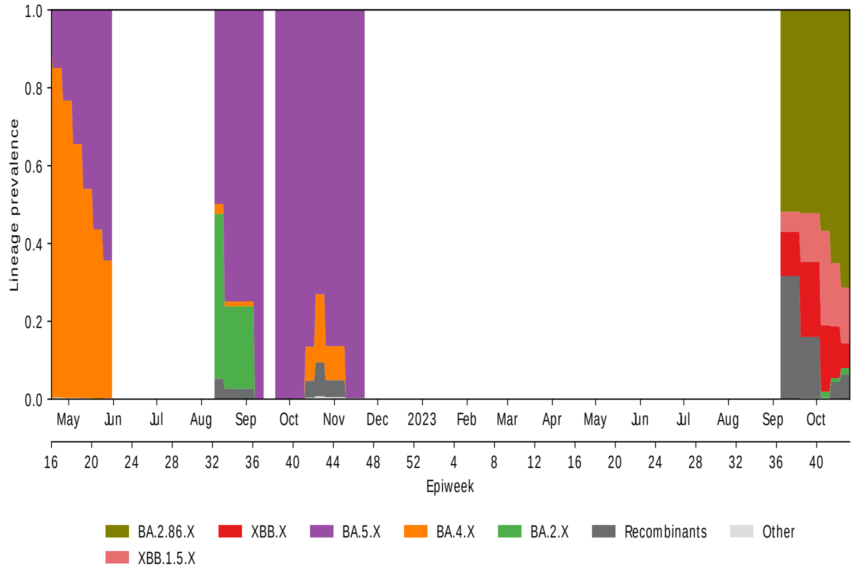
Eastern Cape – Buffalo City



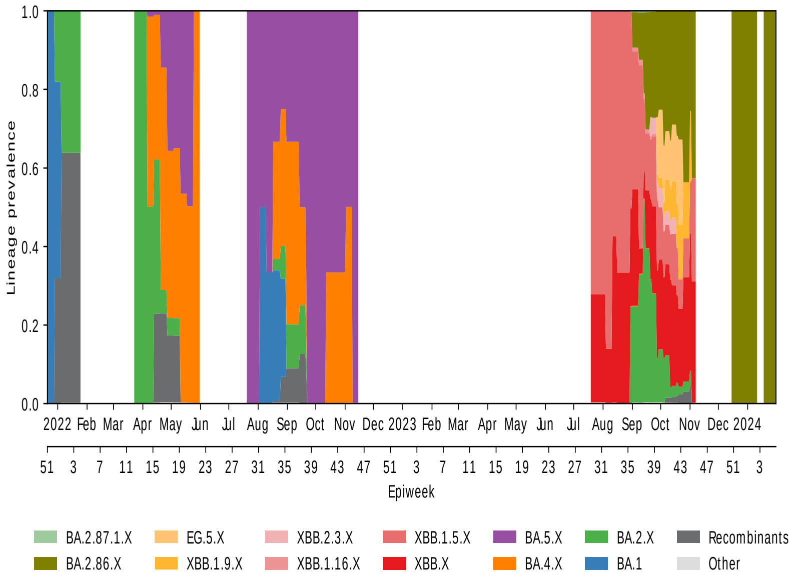
Chloroform start and end date

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

East Bank Wastewater Treatment Works



Mdantsane Wastewater Treatment Works



SARS-CoV-2 levels and Genomic Results in Epi week 16:

- In Epi week 46, SARS-CoV-2 levels in Mdantsane WWTW decreased and levels are low. Levels in Epi week 12 remain low. Levels increase slightly in Epi week 15 but remain low. Levels remain low in Epi week 16.
- SARS-CoV-2 levels in East Bank WWTW in Epi week 44 remain low after decrease was observed from Epi week 38. No new results for Epi week 16 are available.

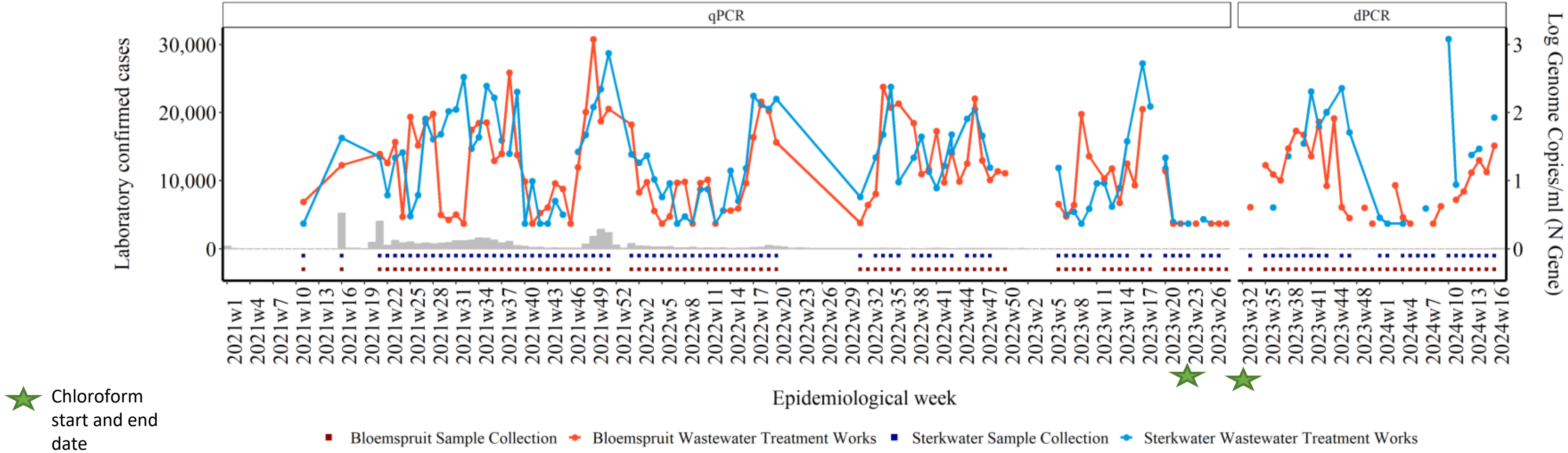
**** Sequencing data ending in Epi week 40 in Eastbank and Epiweek 3 in Mdantsane.***

- Omicron lineages BA.2.86.X, XBB.1.5.X, XBB.X and Recombinantswere circulating in Eastbank during Epi week 40.
- Lineage BA.2.86.X was dominating in Mdantsane during Epi week 3.

SNP Analysis:

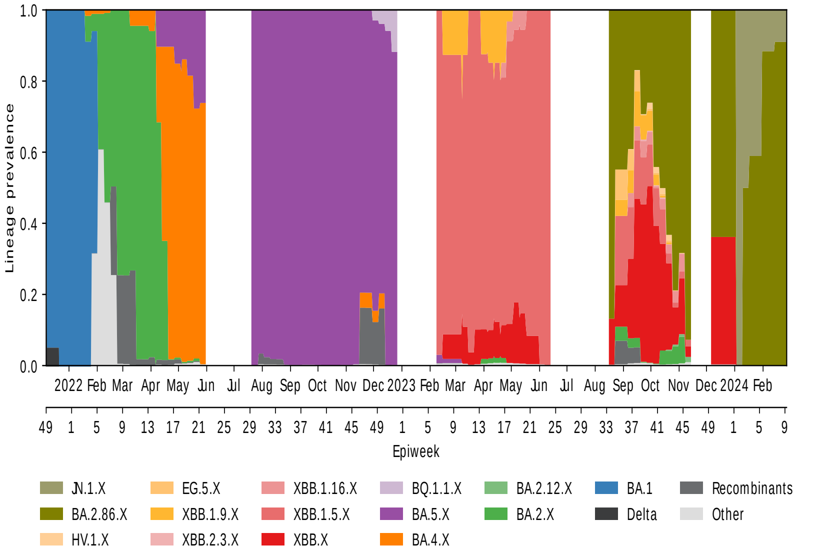
- A combination of mutations (V127F, L212I, V213G, L216F, H245N, A264D, I332V, K356T) associated with lineage BA.2.86 were found in both Eastbank and Mdantsane.

Free State – Mangaung

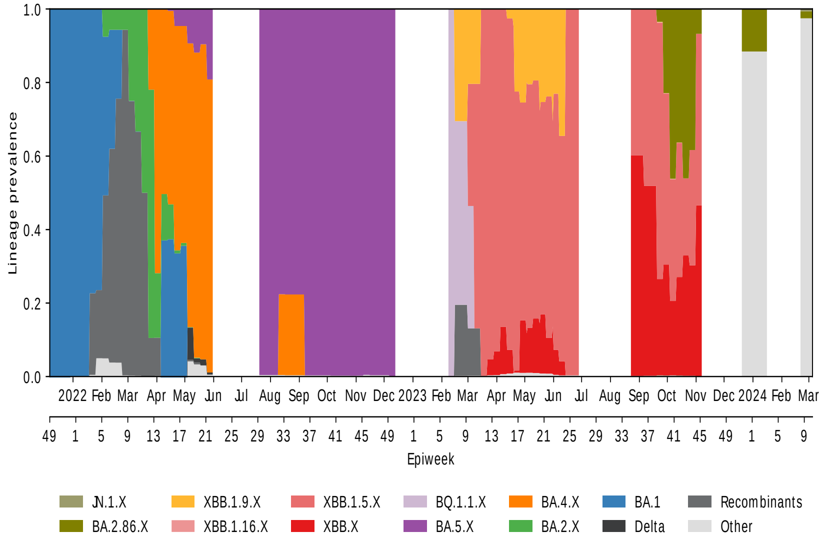


Wastewater levels of SARS-CoV-2 in log-transformed genome copies/ml (right vertical axis, line graph) and laboratory confirmed cases of SARS-CoV-2 for Manguang (left vertical axis, grey bars) by epidemiological week. Coloured dots below the horizontal axis signify if a sample was collected. SARS-CoV-2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

Bloemspruit Wastewater Treatment Works



Sterkwater Wastewater Treatment Works



SARS-CoV-2 levels and Genomic Results in Epi week 16:

- In Bloemspruit WWTW, a 2-fold increase in SARS-CoV-2 levels were seen in Epi week 44. In Epi week 4 levels decreased and remain low in Epi week 9. Levels increase to moderate in Epi week 13 and remain moderate in Epi week 16.
- Sterkwater SARS-CoV-2 levels in Epi week 10 increase to high (above 3 genome copies/ml). Levels drop to low in Epi week 11 and increase to moderate in Epi week 13 and remain moderate in Epi week 14. Levels increase further in Epi week 16 and remain moderate.

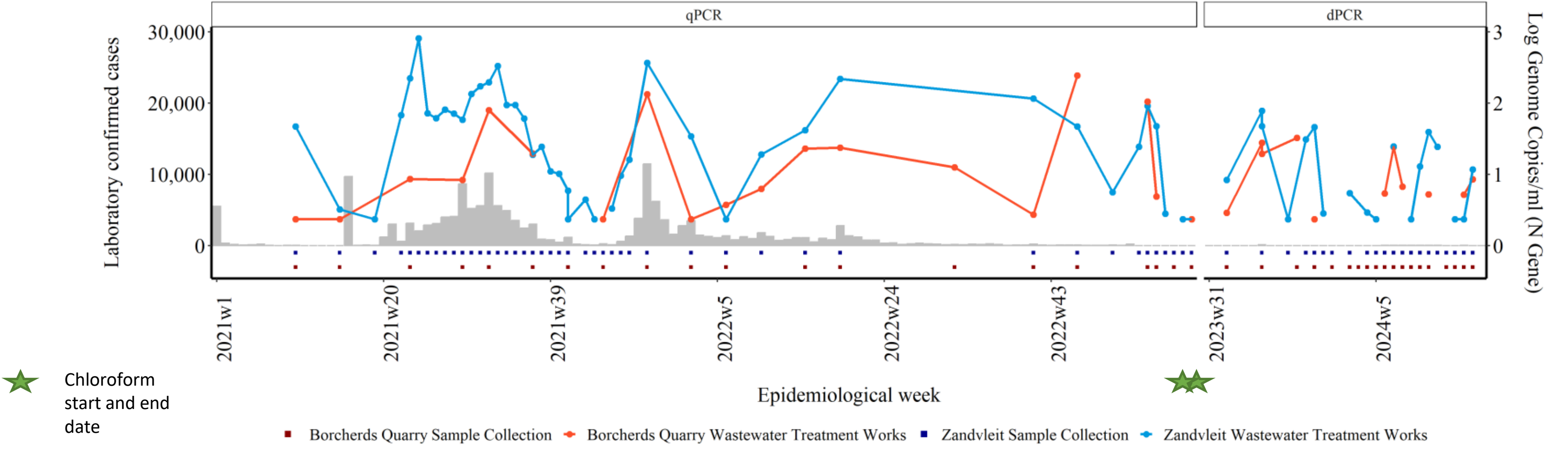
*** Sequencing data ending in Epi week 9 in Bloemspruit and Sterkwater Treatment Works.**

- BA.2.86.X, JN..1.X and XBB.X sub-lineages were the dominant lineage circulating in Bloemspruit during Epi week 1 to 9.
- JN.1.X and BA.2.86.X lineages were predominantly circulating in Epi week 9 in Bloemspruit
- BA.2.86.X (at low proportions) and Other Omicron lineages were circulating in Sterkwater in epiweek 9.

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.

Western Cape – City of Cape Town



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

SARS-CoV-2 levels and Genomic Results in Epi week 16:

- After a sharp increase in SARS-CoV-2 levels was seen in Epi week 37, a subsequent decrease in SARS-CoV-2 levels in Borcherds Quarry WWTW was seen. Levels are low in Epi week 6 and increase to moderate in Epi week 7. Levels decrease in Epi week 8 to low and remain low in Epi week 16.
- In Epi week 4, SARS-CoV-2 levels were low at Zandvleit WWTW. In Epi week 7 levels increase to moderate. Levels drop to low in Epi week 9 and increase to moderate in Epi week 11. Levels remain moderate in Epi week 12 and decrease to low in Epi week 14 and remain low in Epi week 15. Levels increase to moderate in Epi week 16.

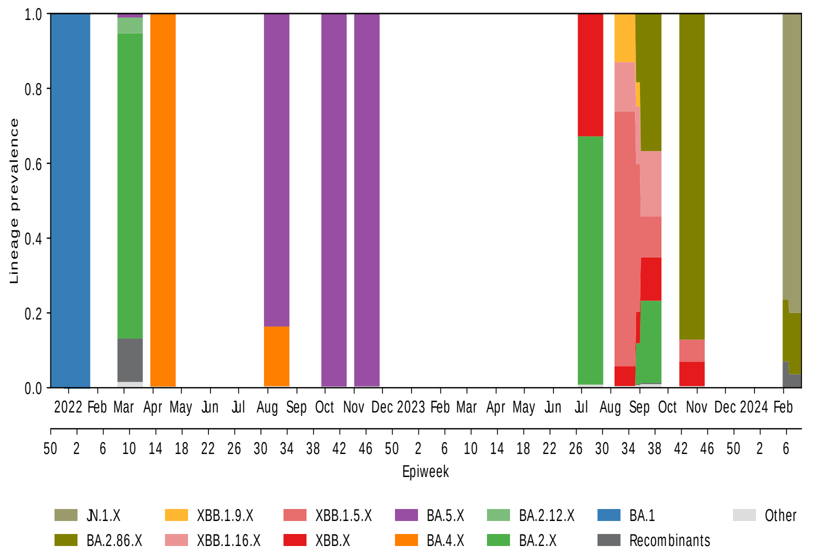
**** Sequencing data ending in Epi week 6 in Borcheds Quarry and Epi week 10 in Zandvleit.***

- During Epi week 6, JN.1.X sub-lineages were dominantly circulating in Borcheds. BA.2.86.X and Recombinants were also detected.
- During Epi week 2 - 6, lineages BA.2.86.X and JN.1.X were circulating in Zandvlet, with JN.1.X sub-lineages being dominant.
- In epi week 10 BA.2.86,X, JN.1.X and Recombinants lineages were circulating in Zandvlet, with JN.1.X lineages at highest proportion

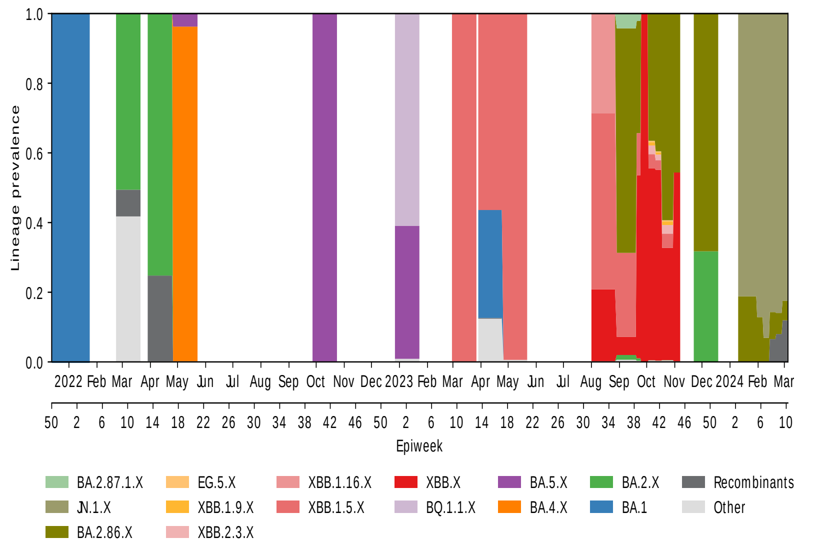
SNP Analysis:

- A combination of mutations (V127F, L212I, V213G, L216F, H245N, A264D, I332V, K356T) associated with lineage BA.2.86 and JN.1 were found in both Borcheds Quarry and Zandevleit.

Borcheds Quarry Wastewater Treatment Works



Zandvleit Wastewater Treatment Works



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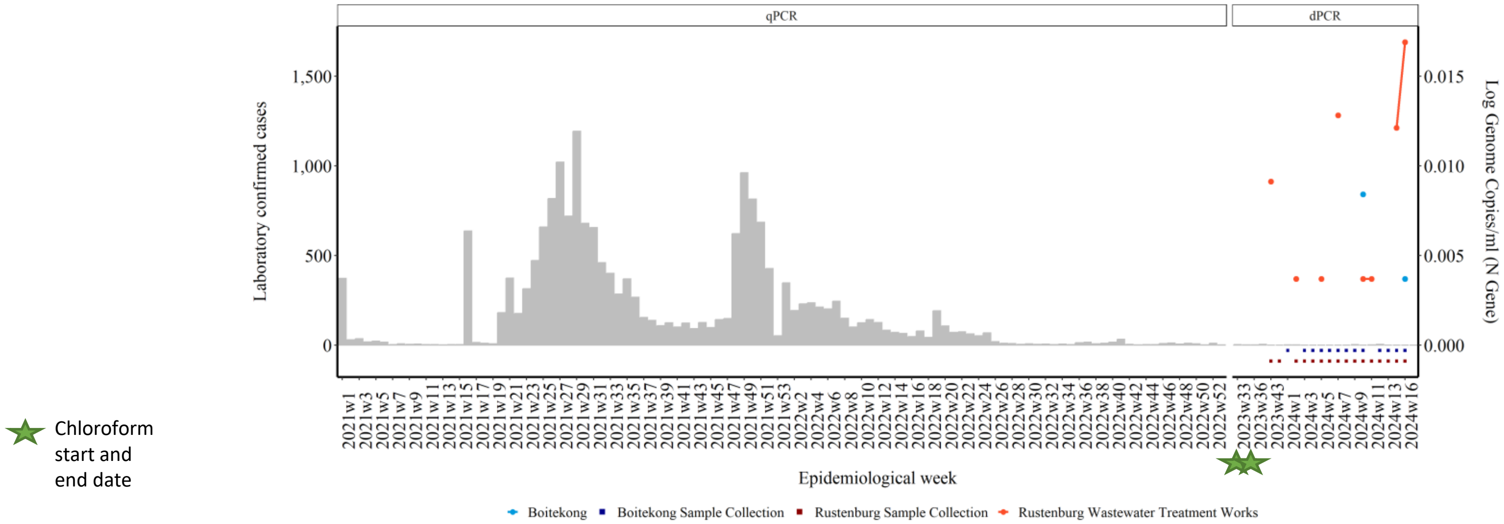
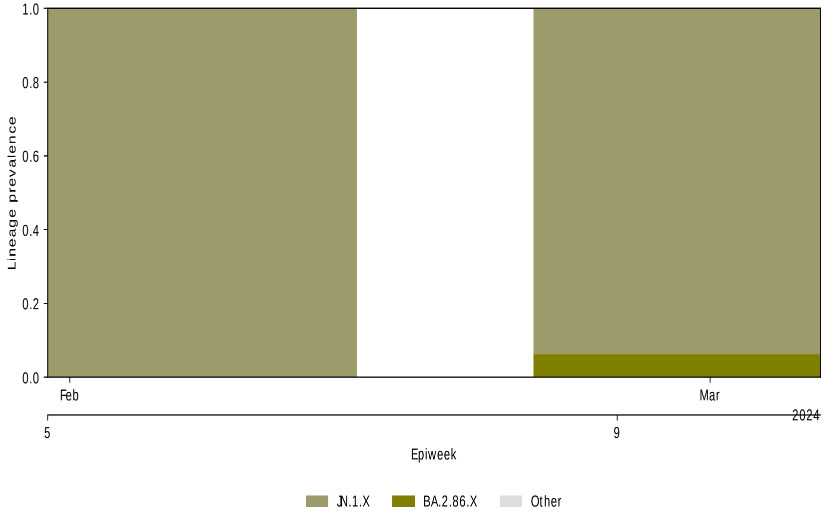
North West – Bojanala Platinum



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

Boitekong



Rustenburg

****No sequencing data currently available***

*** SARS-CoV-2 wastewater sample collection and testing at Rustenburg WWTW began in Epi week 43, 2023.**

*** SARS-CoV-2 wastewater sample collection and testing at Boitekong began in Epi week 1, 2024.**

SARS-CoV-2 levels and Genomic Results in Epi week 16:

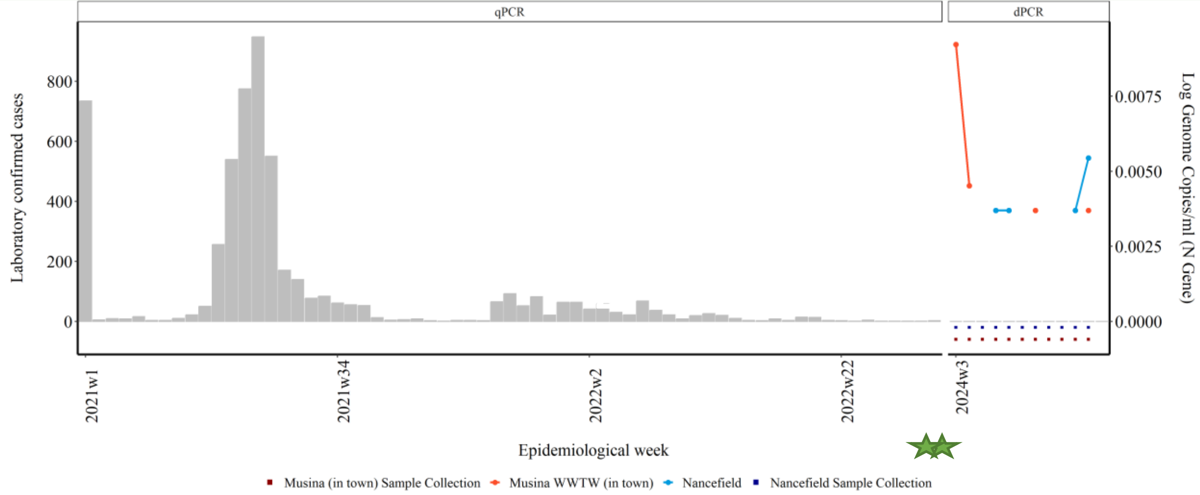
- At Rustenburg WWTW levels from Epi week 43 remain below 0.015 log genome copies/ml and remain low up until Epi week 16.
- At Boitekong wastewater results for SARS-CoV-2 were low in Epi week 10, 2024. Levels remain low in Epi week 16.

**** Sequencing data ending in Epi week 9 in Boitekong***

SARS-CoV-2 levels and Genomic Results in Epi week 13:

- At Boitekong, JN.1.X sub-lineages were dominantly circulating in Epi week 5 and 9. In Epi week 9, BA.2.86.X was also circulating at low proportions.

Limpopo – Vhembe



★ Chloroform start and end date

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

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

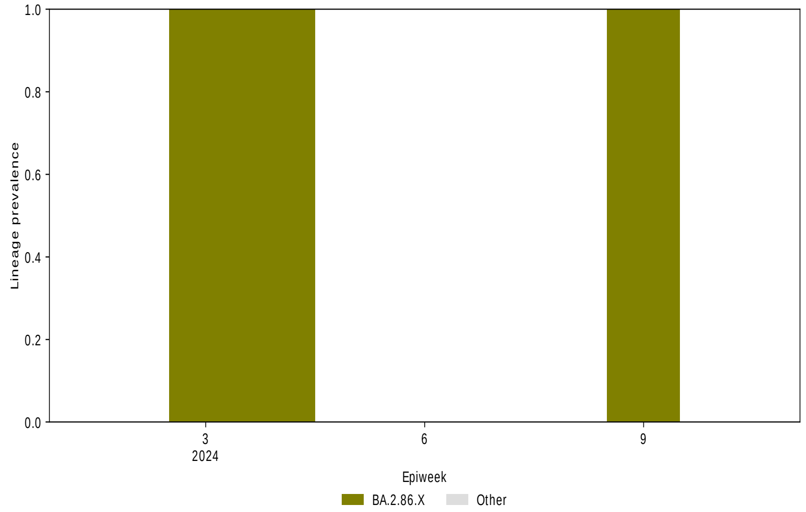
*** SARS-CoV-2 wastewater sample collection and testing at Nancefield began in Epi week 3, 2024.**

SARS-CoV-2 levels and Genomic Results in Epi week 16:

- At Musina WWTW levels from Epi week 3 remain below 0.01 log genome copies/ml and remain low up until Epi week 4, 2024. Levels remain low in Epi week 9. No new results for Epi week 16 are available.
- At Nancefield levels were low in Epi weeks 6 and 7, below 0.005 log genome copies/ml. They remain low in Epi week 12. No new results for Epi week 16 are available.

**** Sequencing data ending in Epiweek 13 in Musina***

Musina



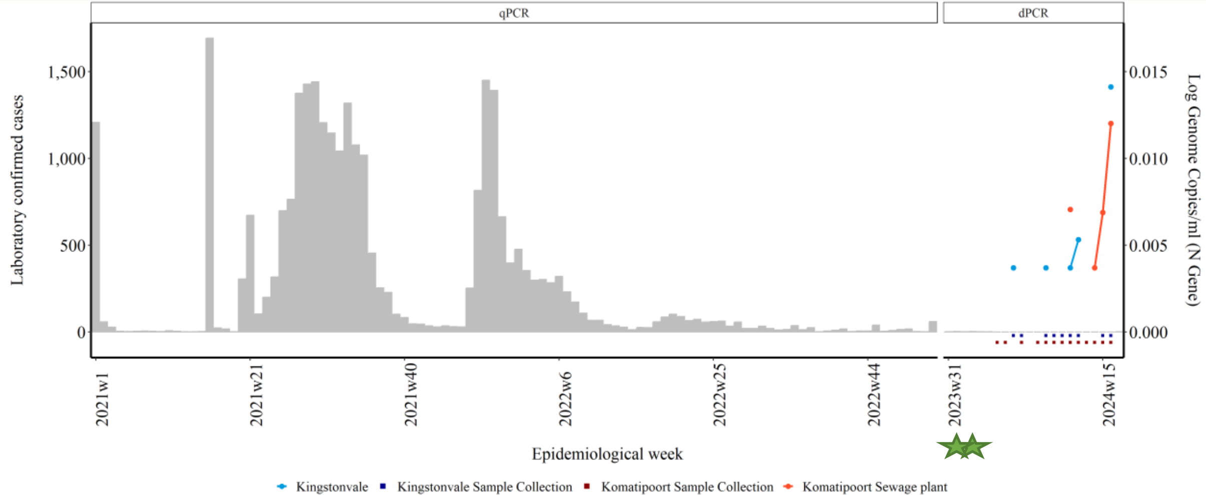
Nancefield

****No sequencing data currently available***

SARS-CoV-2 levels and Genomic Results in Epi week 13:

- Sequencing in Musina began in Epiweek 3, and BA.2.86.X sub-lineages were found to be the main circulating lineages. In Epi week 9 BA.2.86.X sub-lineages were still the dominant lineages detected in Musina WWTW

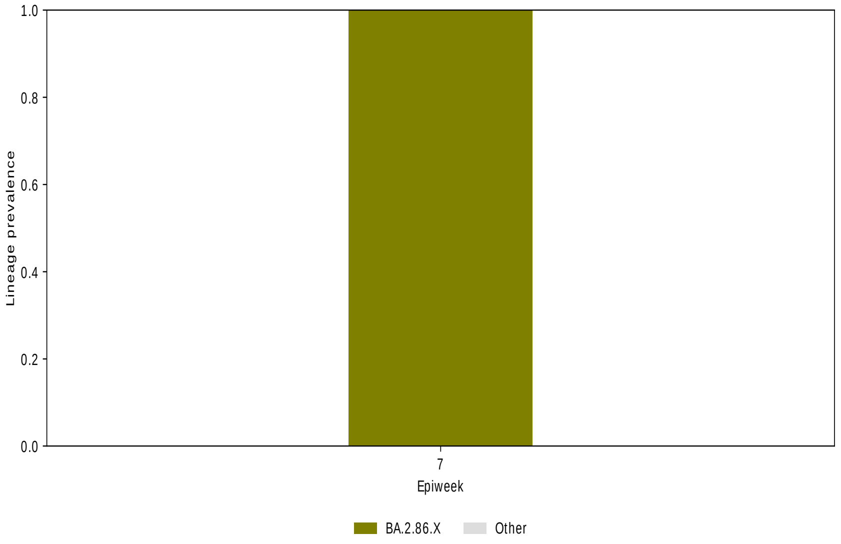
Mpumalanga – Ehlanzeni



★Chloroform start and end date

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

Kingstonvale



Komatipoort

***No sequencing data currently available**

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

*** SARS-CoV-2 wastewater sample collection and testing at Kingstonvale began in Epi week 4, 2024.**

SARS-CoV-2 levels and Genomic Results in Epi week 16:

- At Komatipoort samples collected between Epi week 2 and Epi week 10 results were negative for SARS-CoV-2. Levels were low in Epi week 11 and remain low in Epi week 16.
- At Kingstonvale SARS-CoV-2 levels remain below 0.01 log genome copies/ml from Epi week 4 to Epi week 8. Levels remain low in Epi week 16.

**** Sequencing data ending in Epi week 13 in Kingstonvale***

- No lineages detected in Kingstonvale during Epiweek 2 – 6 due to low coverage and negative results. In Epiweek 7, BA.2.86.X was detected.

COLLABORATORS

Scripps Research

GCRO Gauteng City-Region Observatory

FUNDERS

NATIONAL INSTITUTE FOR COMMUNICABLE DISEASES
Division of the National Health Laboratory Service

WATER RESEARCH COMMISSION

BILL & MELINDA GATES foundation

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