Division of the National Health Laboratory Service

Weekly respiratory pathogens report Week 2 of 2023

<u>Highlights</u>

- In 2023 to date, 3 influenza cases have been detected from all surveillance programmes.
- In 2023 to date, 7 respiratory syncytial virus (RSV) cases have been detected in the pneumonia surveillance and Viral Watch programmes and activity remains below seasonal threshold in all surveillance programmes.
- In 2023 to date, we detected 4 cases of *Bordetella pertussis*; 25% (1) from Western Cape, 50% (2) from Mpumalanga, and 25% (1) from North West provinces. Detection rates for *B. pertussis* cases in influenza-like illness surveillance (ILI) and pneumonia surveillance thus far are lower in January compared to December 2022 at 24% (January, ILI 0/19 and pneumonia surveillance 4/169) vs 4% (December, ILI 4/73 and pneumonia surveillance 17/416) and remain elevated compared to June 2022 detection levels at 0.1% (ILI 0/189, pneumonia surveillance 1/800).
- In 2023 to date, a total of 10 COVID-19 cases were detected from all surveillance programmes.
- Of the 2 SARS-CoV-2 specimens sequenced, none were assigned a variant due to a low viral load or insufficient sample.

Programme Descriptions

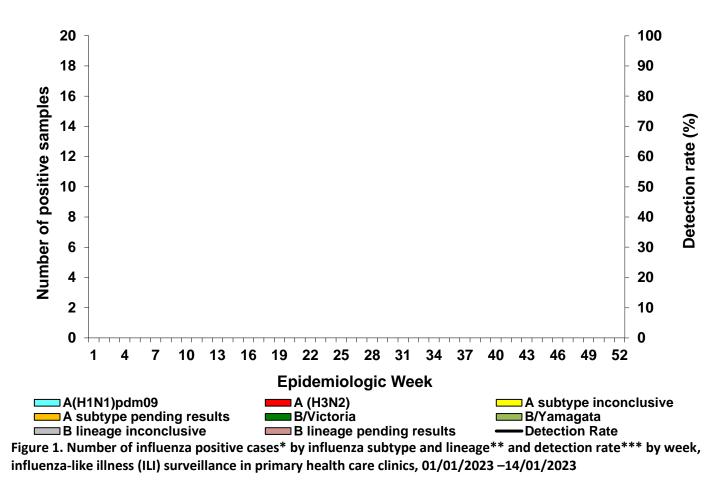
Programme	Influenza-like illness (ILI)	Viral Watch	National syndromic surveillance for pneumonia		
Start year	2012	1984	2009		
Provinces*	ΚZ	EC	EC		
	NW	FS	GP		
	WC	GP	KZ		
	MP	LP	MP		
		MP	NW		
		NC	WC		
		NW	we		
		WC			
Type of site	Primary health care clinics	General practitioners	Public hospitals		
Case definition	ILI: An acute respiratory illness with a	ILI: An acute respiratory illness with a	SRI: Acute (symptom onset≤10 days) or		
case deminion	temperature (\geq 38°C) and cough, & onset	temperature (\geq 38°C) and cough, & onset	chronic (symptom onset >10) lower		
	≤10 days	≤10 days	respiratory tract infection		
	Suspected pertussis		Suspected pertussis		
	Any person with an acute cough illness		Any person with an acute cough illness		
	lasting \geq 14 days (or cough illness of any		lasting \geq 14 days (or cough illness of any		
	duration for children <1 year), without a				
			duration for children <1 year), without a		
	more likely diagnosis AND one or more of		more likely diagnosis AND one or more o		
	the following signs or symptoms:		the following signs or symptoms:		
	 paroxysms of coughing, 		 paroxysms of coughing, 		
	 or inspiratory "whoop", 		 or inspiratory "whoop", 		
	 or post-tussive vomiting 		 or post-tussive vomiting 		
	 or apnoea in children <1 year; OR 		 or apnoea in children <1 year; OR 		
	Any person in whom a clinician suspects pertussis		Any person in whom a clinician suspects pertussis.		
	Suspected SARS-CoV-2	Suspected SARS-CoV-2	Suspected SARS-CoV-2		
	Any person presenting with an acute	Any person presenting with an acute	Any person admitted with a physician-		
	(≤14 days) respiratory tract infection or	(≤14 days) respiratory tract infection or	diagnosis of suspected COVID-19 and		
	other clinical illness compatible with	other clinical illness compatible with	not meeting SRI case definition.		
	COVID-19**	COVID-19**			
Specimens collected	Oropharyngeal & nasopharyngeal swabs	Throat and/or nasal swabs or Nasopharyngeal swabs	Oropharyngeal & nasopharyngeal swabs		
Main pathogens	INF	INF	INF		
tested***	RSV	RSV	RSV		
	BP	BP	BP		
	SARS-CoV-2	SARS-CoV-2	SARS-CoV-2		
Testing Methods	INF and RSV	INF and RSV	INF and RSV		
	- Fast-Track Diagnostics multiplex real-	- Fast-Track Diagnostics multiplex real-	- Fast Track Diagnostics multiplex real-		
	time reverse transcription polymerase	time reverse transcription polymerase	time reverse transcription polymerase		
	chain reaction (until 31 March 2021)	chain reaction (until 31 March 2021)	chain reaction (until 31 March 2021)		
	B. pertussis	B. pertussis	B. pertussis		
	-	•	Multiplex real-time PCR (Tatti <i>et al., J Clir</i>		
	Multiplex real-time PCR (Tatti <i>et al., J Clin</i>	Multiplex real-time PCR (Tatti <i>et al., J Clin</i>	· · · · · · · · · · · · · · · · · · ·		
	Microbiol 2011) and culture (if PCR cycle	Microbiol 2011) and culture (if PCR cycle	Microbiol 2011) and culture (if PCR cycle		
	threshold ≤25)	threshold ≤25)	threshold ≤25)		
	SARS-CoV-2	SARS-CoV-2	SARS-CoV-2		
	1 April 2020 – 31 March 2021: Roche E	1 April 2020 – 31 March 2021: Roche E	1 April 2020 – 31 March 2021: Roche E		
	gene real-time PCR essay (Corman et al.,	gene real-time PCR essay Corman et al.,	gene real-time PCR essay (Corman et al.,		
			Fund (um 2020)		
	Euro Surv 2020)	Euro Surv 2020)	Euro Surv 2020)		
		Euro Surv 2020) 1 April 2021 to date: Allplex™ SARS-CoV-			
	Euro Surv 2020)	,			
	Euro Surv 2020) 1 April 2021 to date: Allplex™ SARS-CoV-	1 April 2021 to date: Allplex™ SARS-CoV-	1 April 2021 to date: Allplex [™] SARS-CoV-		
	Euro Surv 2020) 1 April 2021 to date: Allplex™ SARS-CoV- 2/FluA/FluB/RSV PCR kit	1 April 2021 to date: Allplex™ SARS-CoV- 2/FluA/FluB/RSV PCR kit	1 April 2021 to date: Allplex™ SARS-CoV- 2/FluA/FluB/RSV PCR kit		

Epidemic Threshold

Thresholds are calculated using the Moving Epidemic Method (MEM), a sequential analysis using the R Language, available from: http://CRAN.R-project.org/web/package=mem) designed to calculate the duration, start and end of the annual influenza epidemic. MEM uses the 40th, 90th and 97.5th percentiles established from available years of historical data to calculate thresholds of activity. Thresholds of activity for influenza and RSV are defined as follows: Below seasonal threshold, Low activity, Moderate activity, High activity. Very high activity. For influenza, thresholds from outpatient influenza like illness (ILI in primary health care clinics) are used as an indicator of disease transmission in the community and thresholds from pneumonia surveillance are used as an indicator of impact of disease. For RSV, thresholds from pneumonia surveillance, using data from children aged < 5 years are used to define the start and end of the season.

* EC: Eastern Cape; FS: Free State; GP: Gauteng; KZ: KwaZulu-Natal; LP: Limpopo; MP: Mpumalanga: NC: Northern Cape; NW: North West; WC: Western Cape **Symptoms include ANV of the following respiratory symptoms: cough, sore throat, shortness of breath, anosmia (loss of sense of smell) or dysgeusia (alteration of the sense of taste), with or without other symptoms (which may include fever, weakness, myalgia, or diarrhoea). Testing for SARS-CoV-2 was initiated in all three surveillance programmes in week 10 of 2020 (week starting 2 March 2020).***INF: influenza virus; RSV: respiratory syncytial virus; BP: Bordetella pertusis; SARS-CoV-2: severe acute respiratory syndrome coronavirus 2

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*Specimens from patients with influenza-like illnesses at 5 sentinel sites in 4 provinces

**No cases who met suspected SARS-CoV-2 or B. pertussis case definition but did not meet influenza-like illness (ILI) case definition.

***Only reported for weeks with >10 specimens submitted

Inconclusive: insufficient viral load in sample and unable to characterise further

Two dual infections of influenza B(Victoria) + influenza A(H1N1)pdm09 in week 24 and B(Victoria) + influenza A(H3N2) in week 39 not included in the epidemiological curve.

Table 1. Number of laboratory-confirmed influenza* cases by subtype and lineage and total number of samples
tested by clinic and province, influenza-like illness (ILI) surveillance in primary health care clinics, 01/01/2023 –
14/01/2023

Clinic (Province)	A(H1N1) pdm09	A(H3N2)	A subtype in- conclusive* *	A subtype pending results** *	B/ Victoria	B/ Yamagat a	B lineag e in- conclu sive*	B lineage pending results* **	Total sample s
Agincourt (MP)	0	0	0	0	0	0	0	0	0
Eastridge (WC)	0	0	0	0	0	0	0	0	0
Edendale Gateway (KZ)	0	0	0	0	0	0	0	0	17
Jouberton (NW)	0	0	0	0	0	0	0	0	5
Mitchell's Plain (WC)	0	0	0	0	0	0	0	0	0
Total:	0	0	0	0	0	0	0	0	22

KZ: KwaZulu-Natal; NW: North West; WC: Western Cape; MP: Mpumalanga

*No cases who met suspected SARS-CoV-2 or B. pertussis case definition but did not meet influenza-like illness (ILI) case definition.

**Inconclusive: insufficient viral load in sample and unable to characterise further

***Influenza A subtype or B lineage results are pending

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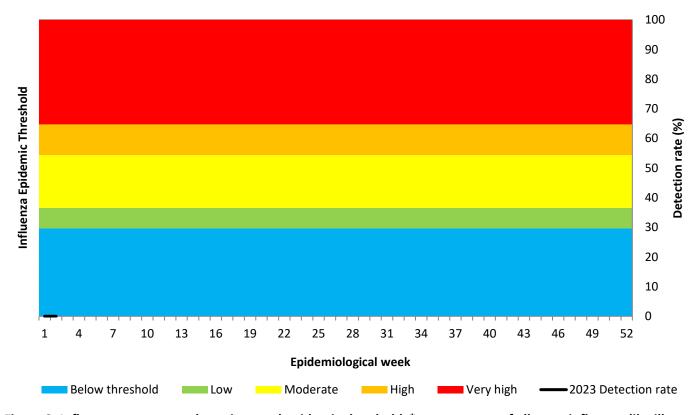


Figure 2. Influenza percentage detections and epidemic thresholds* among cases of all ages, influenza-like illness (ILI) surveillance in primary health care clinics, 01/01/2023 –14/01/2023 *Thresholds based on 2012-2019 data

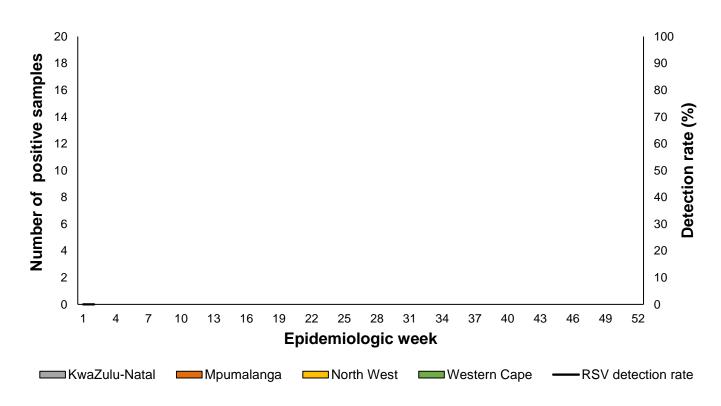


Figure 3. Number of patients testing positive for respiratory syncytial virus* by province and detection rate by week, influenza-like illness (ILI) surveillance in primary health care clinics, 01/01/2023 –14/01/2023

* No cases who met suspected SARS-CoV-2 or *B. pertussis* case definition but did not meet influenza-like illness (ILI) case definition.

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^{**}Only reported for weeks with >10 specimens submitted

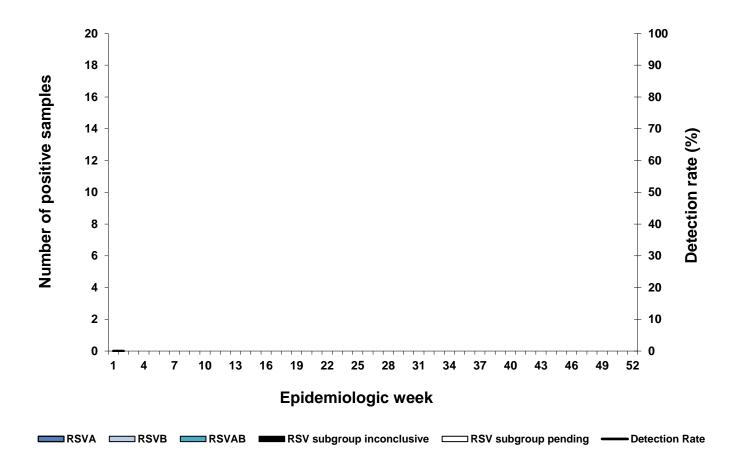


Figure 4. Number of patients testing positive for respiratory syncytial virus* by subgroup and detection rate by week, influenza-like illness (ILI) surveillance in primary health care clinics, 01/01/2023 –14/01/2023

* No cases who met suspected SARS-CoV-2 or B. pertussis case definition but did not meet influenza-like illness (ILI) case definition

**Only reported for weeks with >10 specimens submitted

RSV AB: Both RSV A and B subgroups identified.

Inconclusive: insufficient viral load in sample and unable to characterise further

Table 2. Number of patients testing positive for respiratory syncytial virus (RSV)* by subgroups identified and total number of samples tested by clinic and province, influenza-like illness (ILI) surveillance in primary health care clinics, 01/01/2023 –14/01/2023

Clinic (Province)	RSVA	RSVB	RSVAB**	RSV subgroup inconclusive* **	RSV subgroup pending** **	Total samples
Agincourt (MP)	0	0	0	0	0	0
Eastridge (WC)	0	0	0	0	0	0
Edendale Gateway (KZ)	0	0	0	0	0	17
Jouberton (NW)	0	0	0	0	0	5
Mitchell's Plain (WC)	0	0	0	0	0	0
Total	0	0	0	0	0	22

KZ: KwaZulu-Natal; NW: North West; WC: Western Cape; MP: Mpumalanga

* No cases who met suspected SARS-CoV-2 or *B. pertussis* case definition but did not meet influenza-like illness (ILI) case definition.

**RSV AB: Both RSV A and B subgroups identified

***Inconclusive: insufficient viral load in sample and unable to characterise further

****RSV results for subgroups are pending

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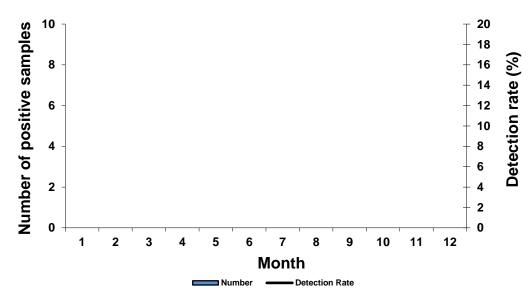


Figure 5. Number of patients testing positive for *B. pertussis** and detection rate by month, influenza-like illness (ILI) surveillance primary health care clinics**, 01/01/2023 –14/01/2023

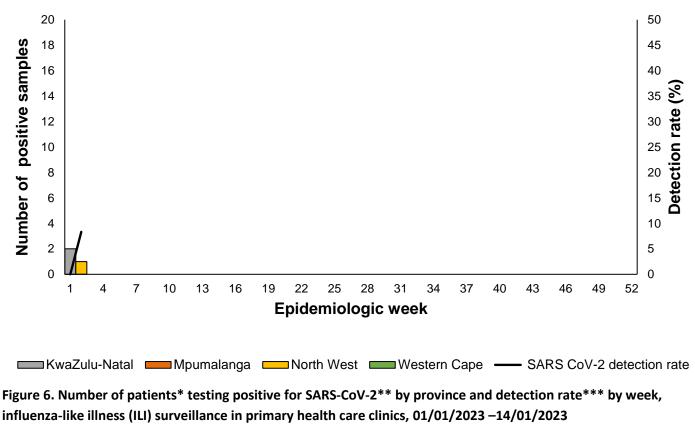
* No cases who met the suspected SARS-CoV-2 or B. pertussis case definition but did not meet influenza-like illness (ILI) case definition ** Specimens from patients with influenza-like illnesses at 5 sentinel sites in 4 provinces

Table 3. Number of patients testing positive for *B. pertussis** identified and total number of samples tested by province, influenza-like illness (ILI) surveillance primary health care clinics, 01/01/2023 –14/01/2023

Clinic (Province)	<i>B. pertussis</i> Positive	Total samples
Agincourt (MP)	0	0
Eastridge (WC)	0	0
Edendale Gateway (KZ)	0	17
Jouberton (NW)	0	2
Mitchell's Plain (WC)	0	0
Total:	0	19

KZ: KwaZulu-Natal; NW: North West; WC: Western Cape; MP: Mpumalanga

* No cases who met the suspected SARS-CoV-2 or *B. pertussis* case definition but did not meet influenza-like illness (ILI) case definition. NB: Results pending for 10 samples.



*Specimens from patients with influenza-like illnesses at 5 sentinel sites in 4 provinces

** No cases who met suspected SARS-CoV-2 or B. pertussis case definition but did not meet influenza-like illness (ILI) case definition.

***Only reported for weeks with >10 specimens submitted

Table 4. Number of patients positive for SARS-CoV-2* identified and total number of samples tested by clinic and province, influenza-like illness (ILI) surveillance primary health care clinics, 01/01/2023 –14/01/2023

Clinic (Province)	SARS-CoV-2 positive	Total samples tested
Agincourt (MP)	0	0
Eastridge (WC)	0	0
Edendale Gateway (KZ)	2	17
Jouberton (NW)	1	5
Mitchell's Plain (WC)	0	0
Total:	3	22

KZ: KwaZulu-Natal; NW: North West; WCP: Western Cape; MP: Mpumalanga

* No cases who met suspected SARS-CoV-2 or B. pertussis case definition but did not meet influenza-like illness (ILI) case definition.

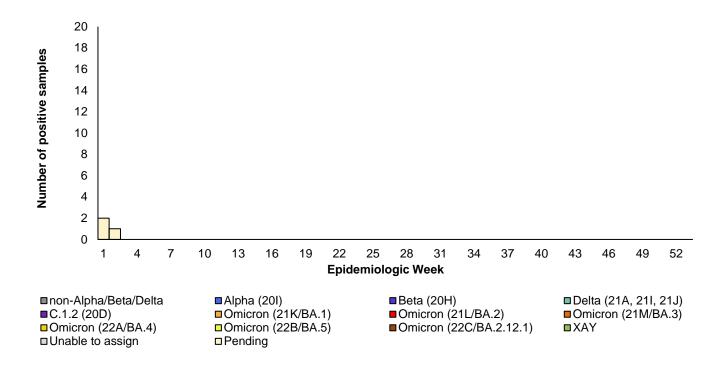


Figure 7. Number and detection rate of laboratory-confirmed SARS-CoV-2* cases by variant type (variant PCR/sequencing) and week, influenza-like illness (ILI) surveillance in primary health care clinics, 01/01/2023-14/01/2023

*Specimens are from patients with influenza-like illness at 5 sentinel sites in 4 provinces who met influenza-like illness (ILI), suspected SARS-CoV-2 or *B. pertussis* case definition

Unable to assign: no lineage assigned due to poor- sequence quality OR low viral load (Ct≥35) OR variant PCR could not assign variant and no sequencing result Pending: outstanding variant results

Table 5. Number of cases positive for SARS-CoV-2^{*} by variant^{**} (variant PCR and/or sequencing) identified and total number of samples tested by clinic and province, influenza-like illness (ILI) surveillance primary health care clinics, 01/01/2023-14/01/2023

Clinic (Province)	Delta (21A, 21I, 21J)	Omicron (21K/BA.1)	Omicron (21L/BA.2)	Omicron (21M/BA.3)	Omicron (22A/BA.4)	Omicron (22B/BA.5)	Omicron (22C/ BA.2.12.1)	Unable to assign	Pending	Total SARS- CoV-2 positive	Total samples tested
Agincourt (MP)	0	0	0	0	0	0	0	0	0	0	0
Eastridge (WC)	0	0	0	0	0	0	0	0	0	0	0
Edendale	0	0	0	0	0	0	0	0	2	2	17
Gateway (KZ)											
Jouberton (NW)	0	0	0	0	0	0	0	0	1	1	5
Mitchell's Plain	0	0	0	0	0	0	0	0	0	0	0
(WC)											
Total:	0	0	0	0	0	0	0	0	3	3	22

KZ: KwaZulu-Natal; NW: North West; WCP: Western Cape; MP: Mpumalanga

*Specimens are from patients with influenza-like illness at 5 sentinel sites in 4 provinces who met influenza-like illness (ILI), suspected SARS-CoV-2 or *B. pertussis* case definition

**No cases of Alpha, Beta or 20D (C.1.2) variants detected.

Unable to assign: no lineage assigned due to poor- sequence quality OR low viral load (Ct ≥35) OR variant PCR could not assign variant and no sequencing result Pending: outstanding variant results

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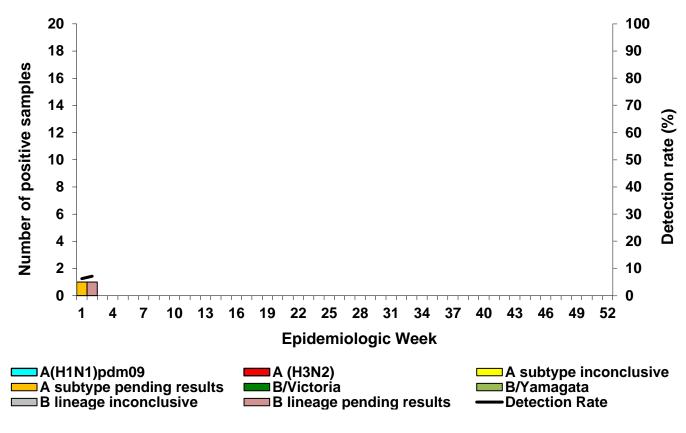


Figure 8. Number of positive patients* by influenza subtype and lineage and detection rate** by week, ILI surveillance - Viral Watch, 01/01/2023-14/01/2023

*Specimens from patients with influenza-like illnesses at 92 sentinel sites in 8 provinces **Only reported for weeks with >10 specimens submitted. Inconclusive: insufficient viral load in sample and unable to characterise further

Table 6. Number of laboratory-confirmed influenza cases by influenza subtype and lineage and total number of samples tested by province, ILI surveillance - Viral Watch, 01/01/2023-14/01/2023

			A subtures	Aculatura			В			
Province	A(H1N1) pdm09	A(H3N2)	A subtype inconclusiv e	A subtype pending results*	B/Victor ia	B/Yamag ata	B lineage inconclus ive	lineage pending results*	Total samples	
Eastern Cape	0	0	0	0	0	0	0	0	0	
Free State	0	0	0	0	0	0	0	0	0	
Gauteng	0	0	0	0	0	0	0	1	27	
Limpopo	0	0	0	0	0	0	0	0	0	
Mpumalanga	0	0	0	0	0	0	0	0	0	
North West	0	0	0	0	0	0	0	0	0	
Northern Cape	0	0	0	0	0	0	0	0	0	
Western Cape	0	0	0	1	0	0	0	0	3	
Total:	0	0	0	1	0	0	0	1	30	

*Inconclusive: insufficient viral load in sample and unable to characterise further **Influenza A subtype or B lineage results are pending

Data are provisional as reported to date (Data for this report drawn on 17/01/2023). Number of consultations/specimens are reported/analysed by date of consultation/specimen collection. Data cleaning is ongoing and this may result in some changes in subsequent reports.

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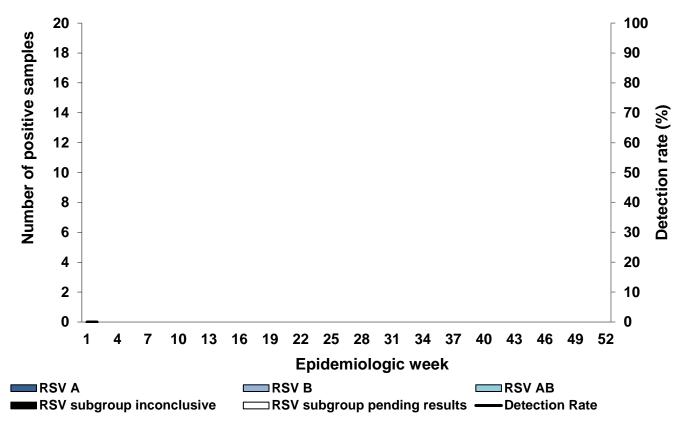


Figure 9. Number of RSV positive cases testing positive for respiratory syncytial virus (RSV)* by subgroup and detection rate** by week, ILI surveillance - Viral Watch, 01/01/2023-14/01/2023

*Specimens from patients with Influenza-like illnesses at 92 sentinel sites in 8 provinces **Only reported for weeks with >10 specimens submitted.

Table 7. Number of RSV positive cases identified and total number of samples tested by province, ILI surveillance - Viral Watch, 01/01/2023-14/01/2023

Province	RSV A	RSV B	RSV AB*	RSV subgroup inconclusive **	RSV subgroup pending results***	Total samples tested
Eastern Cape	0	0	0	0	0	0
Free State	0	0	0	0	0	0
Gauteng	0	0	0	0	0	27
Limpopo	0	0	0	0	0	0
Mpumalanga	0	0	0	0	0	0
North West	0	0	0	0	0	0
Northern Cape	0	0	0	0	0	0
Western Cape	0	0	0	0	0	3
Total:	0	0	0	0	0	30

*RSV AB: Both RSV A and B subgroup identified

**Inconclusive: insufficient viral load in sample and unable to characterise further

***RSV results for subgroups are pending

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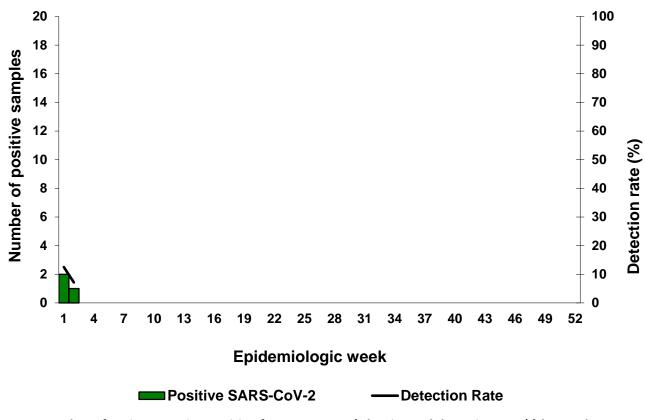


Figure 10. Number of patients testing positive for SARS-CoV-2*, by site and detection rate** by week, ILI surveillance - Viral Watch, 01/01/2023-14/01/2023

*Specimens from patients with influenza-like illnesses at 92 sentinel sites in 8 provinces **Only reported for weeks with >10 specimens submitted.

Table 8. Number of SARS-CoV-2 positive cases identified and total number tested by province, ILI surveillance- Viral Watch, 01/01/2023-14/01/2023

Province	SARS-CoV-2 positive	Total samples tested		
Eastern Cape	0	0		
Free State	0	0		
Gauteng	2	27		
Limpopo	0	0		
Mpumalanga	0	0		
North West	0	0		
Northern Cape	0	0		
Western Cape	1	3		
Total:	3	30		

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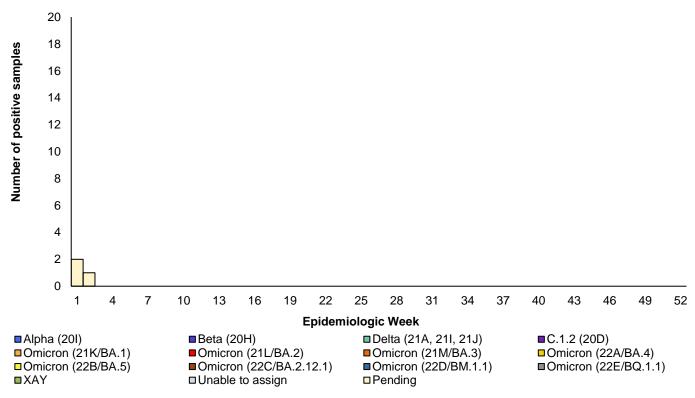


Figure 11. Number and detection rate of laboratory confirmed SARS-CoV-2* cases by variant type (variant PCR/sequencing) and week, ILI surveillance - Viral Watch, 01/01/2023-14/01/2023

Table 9. Number of SARS-CoV-2^{*} positive cases by variant (variant PCR and/or sequencing) identified and total number of samples tested by province, ILI surveillance - Viral Watch, 01/01/2023-14/01/2023

Clinic (Province)	Omicr on (21K/ BA.1)	Omicro n (21L/B A.2)	Omicro n (21M/ BA.3)	Omicro n (22A/B A.4)	Omicro n (22B/B A.5)	Omicro n (22C/ BA.2.1 2.1)	Omicro n (22D/B M.1.1)	Omicro n (22E/B Q.1.1)	ХАҮ	Unable to assign	Pendin g	Total SARS- CoV-2 positiv e	Total sample s tested
Eastern Cape	0	0	0	0	0	0	0	0	0	0	0	0	0
Free State	0	0	0	0	0	0	0	0	0	0	0	0	0
Gauteng	0	0	0	0	0	0	0	0	0	0	2	2	27
Limpopo	0	0	0	0	0	0	0	0	0	0	0	0	0
Mpumalanga	0	0	0	0	0	0	0	0	0	0	0	0	0
North West	0	0	0	0	0	0	0	0	0	0	0	0	0
Northern	0	0	0	0	0	0	0	0	0	0	0	0	0
Cape													
Western Cape	0	0	0	0	0	0	0	0	0	0	1	1	3
Total:	0	0	0	0	0	0	0	0	0	0	3	3	30

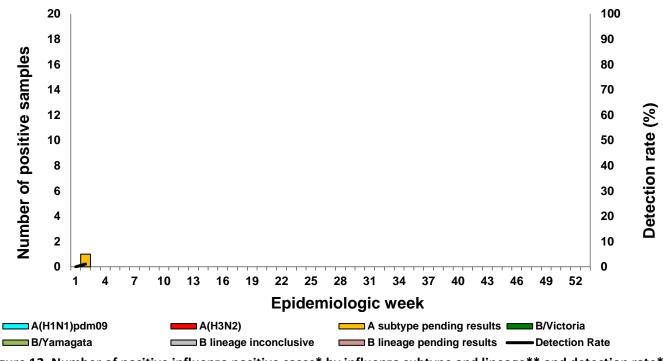
*Specimens from patients with influenza-like illnesses at 92 sentinel sites in 8 provinces

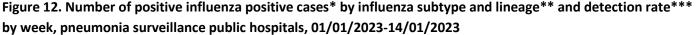
**No cases of Alpha, Beta or 20D (C.1.2) variants detected.

Unable to assign: no lineage assigned due to poor- sequence quality OR low viral load (Ct≥35) OR variant PCR could not assign variant and no sequencing result Pending: outstanding variant results

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^{*}Specimens from patients with influenza-like illnesses at 92 sentinel sites in 8 provinces **Unable to assign**: no lineage assigned due to poor- sequence quality **OR** low viral load ($C_t \ge 35$) **OR** variant PCR could not assign variant and no sequencing result **Pending**: outstanding variant results





Inconclusive: insufficient viral load in sample and unable to characterise further

*Specimens from patients hospitalised with pneumonia at 11 sentinel sites in 6 provinces

**No cases who met suspected the SARS-CoV-2 or *B. pertussis* case definition but did not meet pneumonia (SRI) case definition.

***Only reported for weeks with >10 specimens submitted

Table 10. Number of laboratory confirmed influenza cases by subtype and lineage* and total number of samples tested by hospital, pneumonia surveillance public hospitals, 01/01/2023-14/01/2023

Hospital (Province)	A(H1N1)pd m09	A(H3N2)	A subtype inconclusive	A subtype pending results***	B/Victoria	B/Yamagat a	B lineage inconclusive	B lineage pending results***	Total samples
Edendale (KZ)	0	0	0	0	0	0	0	0	27
Helen Joseph-Rahima Moosa (GP)	0	0	0	0	0	0	0	0	26
Khayelitsha (WC)	0	0	0	0	0	0	0	0	9
Klerksdorp-Tshepong (NW)	0	0	0	0	0	0	0	0	15
Livingstone (EC)	0	0	0	1	0	0	0	0	33
Mapulaneng- Matikwana (MP)	0	0	0	0	0	0	0	0	17
Mitchell's Plain (WC)	0	0	0	0	0	0	0	0	10
Red Cross (WC)	0	0	0	0	0	0	0	0	22
Tambo Memorial (GP)	0	0	0	0	0	0	0	0	0
Tembisa (GP)	0	0	0	0	0	0	0	0	17
Tintswalo (MP)	0	0	0	0	0	0	0	0	0
Tygerberg (WC)	0	0	0	0	0	0	0	0	1
Total:	0	0	0	1	0	0	0	0	177

* No cases who met suspected SARS-CoV-2 or *B. pertussis* case definition but did not meet pneumonia (SRI) case definition.

**Inconclusive: insufficient viral load in sample and unable to characterise further

***Influenza A subtype or B lineage results are pending

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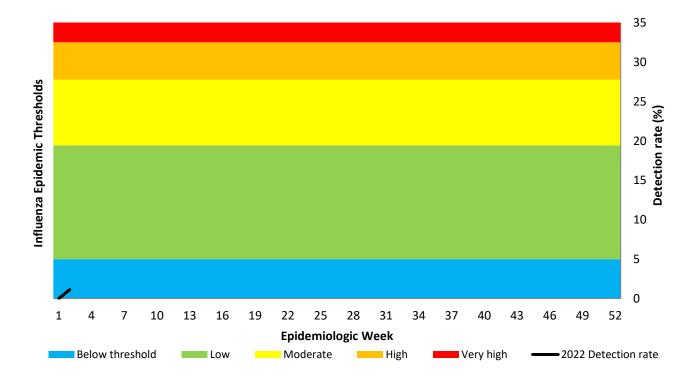
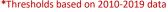


Figure 13. Influenza percentage detections and epidemic thresholds* among cases of all ages, pneumonia surveillance public hospitals, 01/01/2023-14/01/2023 *Thresholds based on 2010-2019 data



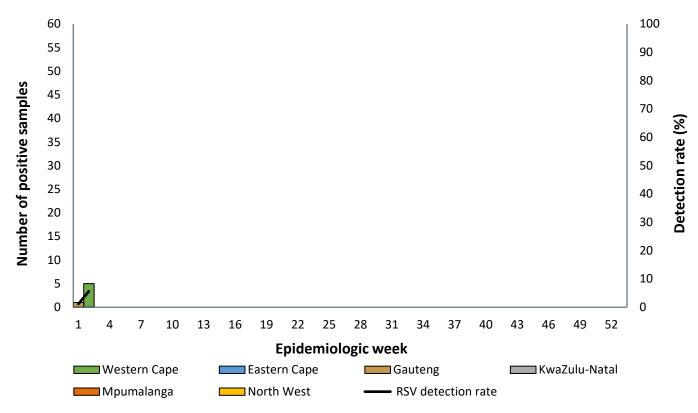


Figure 14. Number of patients (all ages) testing positive for respiratory syncytial virus* by province and detection rate by week, pneumonia surveillance public hospitals, 01/01/2023-14/01/2023

Specimens from patients hospitalised with pneumonia at 11 sentinel sites in 6 provinces.

* No cases who met suspected SARS-CoV-2 or B. pertussis case definition but did not meet pneumonia (SRI) case definition.

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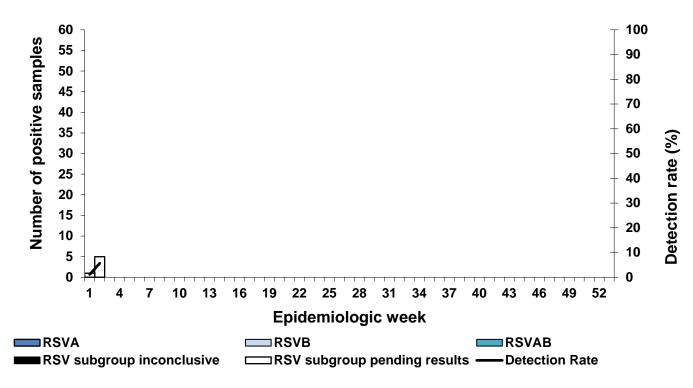


Figure 15. Number of patients (<u>all ages</u>) testing positive for respiratory syncytial virus* by subgroup and detection rate by week, pneumonia surveillance public hospitals, 01/01/2023-14/01/2023

Specimens from patients hospitalised with pneumonia at 11 sentinel sites in 6 provinces.

Inconclusive: insufficient viral load in sample and unable to characterise further

RSV AB: Both RSV A and B subgroup identified

RSV subgroup pending: RSV results for subgroups are pending

* No cases who met suspected SARS-CoV-2 or *B. pertussis* case definition but did not meet pneumonia (SRI) case definition.

Table 11. Number of patients (all ages) positive for respiratory syncytial virus subgroups* by subgroups identified and total number of samples tested by hospital, pneumonia surveillance public hospitals, 01/01/2023-14/01/2023

Hospital (Province)	RSVA	RSVB	RSVAB**	RSV subgroup inconclusive** *	RSV subgroup pending** **	Total samples
Edendale (KZ)	0	0	0	0	0	27
Helen Joseph-Rahima Moosa (GP)	0	0	0	0	1	26
Khayelitsha (WC)	0	0	0	0	0	9
Klerksdorp-Tshepong (NW)	0	0	0	0	0	15
Livingstone (EC)	0	0	0	0	0	33
Mapulaneng-Matikwana (MP)	0	0	0	0	0	17
Mitchell's Plain (WC)	0	0	0	0	0	10
Red Cross (WC)	0	0	0	0	5	22
Tambo Memorial (GP)	0	0	0	0	0	0
Tembisa (GP)	0	0	0	0	0	17
Tintswalo (MP)	0	0	0	0	0	0
Tygerberg (WC)	0	0	0	0	0	1
Total:	0	0	0	0	6	177

* No cases who met suspected SARS-CoV-2 or B. pertussis case definition but did not meet pneumonia (SRI) case definition.

**RSV AB: Both RSV A and B subgroup identified

***Inconclusive: insufficient viral load in sample and unable to characterise further

****RSV results for subgroups are pending

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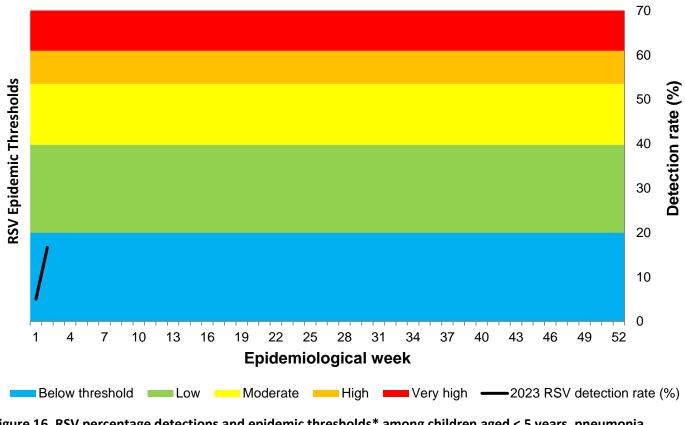


Figure 16. RSV percentage detections and epidemic thresholds* among children aged < 5 years, pneumonia surveillance public hospitals, 01/01/2023-14/01/2023 *Thresholds based on 2010-2019 data

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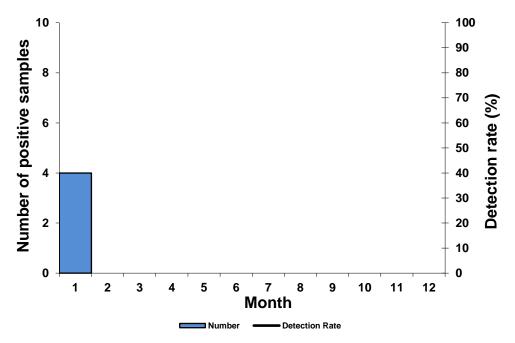


Figure 17. Number of patients testing positive for *B. pertussis** and detection rate by month, pneumonia surveillance public hospitals**, 01/01/2023-14/01/2023

* No cases who met the suspected SARS-CoV-2 or *B. pertussis* case definition but did not meet Pneumonia Surveillance case definition. *Specimens from patients hospitalised with pneumonia at 11 sentinel sites in 6 provinces.

Table 12. Number of patients testing positive for <i>B. pertussis</i> * identified and total number of samples tested
by hospital and province, pneumonia surveillance public hospitals, 01/01/2023-14/01/2023

Hospital (Province)	<i>B. pertussis</i> Positive	Total samples		
Edendale (KZ)	0	27		
Helen Joseph-Rahima Moosa (GP)	0	24		
Khayelitsha (WC)	1	9		
Klerksdorp-Tshepong(NW)	1	14		
Livingstone (EC)	0	32		
Mapulaneng-Matikwana (MP)	2	16		
Mitchell's Plain (WC)	0	9		
Red Cross (WC)	0	21		
Tambo Memorial (GP)	0	0		
Tembisa (GP)	0	17		
Tintswalo (MP)	0	0		
Tygerberg (WC)	0	1		
Total:	4	170		

* No cases who met the suspected SARS-CoV-2 or *B. pertussis* case definition but did not meet the pneumonia (SRI) case definition. These are not included in the table.

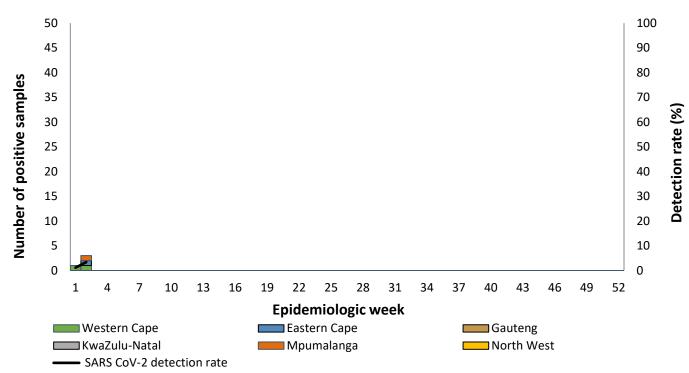


Figure 18. Number of patients testing positive for SARS-CoV-2^{**} by province and detection rate by week, pneumonia surveillance public hospitals, 01/01/2023-14/01/2023

*Specimens from patients hospitalized with pneumonia at 11 sentinel sites in 6 provinces. **No cases met suspected SARS-CoV-2 or *B. pertussis* case definition but did not meet pneumonia (SRI) case definition.

Table 13. Number of patients positive for SARS-CoV-2* and total number of samples tested by hospital, pneumonia surveillance public hospitals, 01/01/2023-14/01/2023

Hospital (Province)	SARS-CoV-2 positive	Total samples tested		
Edendale (KZ)	0	27		
Helen Joseph-Rahima Moosa (GP)	0	26		
Khayelitsha (WC)	0	9		
Klerksdorp-Tshepong (NW)	0	15		
Livingstone (EC)	1	33		
Mapulaneng-Matikwana (MP)	1	17		
Mitchell's Plain (WC)	0	10		
Red Cross (WC)	2	22		
Tambo Memorial (GP)	0	0		
Tembisa (GP)	0	17		
Tintswalo (MP)	0	0		
Tygerberg (WC)	0	1		
Total:	4	177		

* No cases who met suspected SARS-CoV-2 or *B. pertussis* case definition but did not meet pneumonia (SRI) case definition.

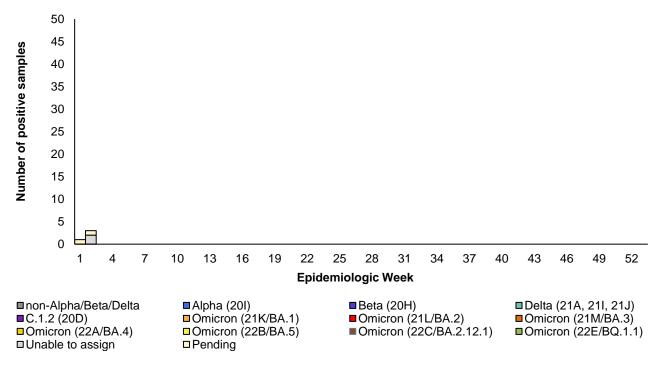


Figure 19. Number and detection rate of laboratory-confirmed SARS-CoV-2 cases* by variant type (variant

PCR/sequencing), pneumonia surveillance public hospitals, 01/01/2023-14/01/2023

*Specimens are from hospitalized patients at 11 sentinel sites in 6 provinces who met the pneumonia (SRI), suspected SARS-CoV-2 or *B. pertussis* case definition Unable to assign: no lineage assigned due to poor- sequence quality OR low viral load (Ct235) OR variant PCR could not assign variant and no sequencing result Pending: outstanding variant results

Hospital (Province)	Delta (21A, 21I, 21J)	Omicro n (21K/B A.1)	Omicro n (21L/BA .2)	Omicro n (21M/B A.3)	Omicro n (22A/B A.4)	Omicro n (22B/B A.5)	Omicro n (22C/ BA.2.12 .1)	Omicro n (22E/B Q.1.1)	Unable to assign	Pending	Total SARS- CoV-2 positive	Total sample s tested
Edendale (KZ)	0	0	0	0	0	0	0	0	0	0	0	27
Helen Joseph-	0	0	0	0	0	0	0	0	0	0	0	26
Rahima Moosa (GP)												
Khayelitsha (WC)	0	0	0	0	0	0	0	0	0	0	0	9
Klerksdorp-	0	0	0	0	0	0	0	0	0	0	0	15
Tshepong (NW)												
Livingstone (EC)	0	0	0	0	0	0	0	0	1	0	1	33
Mapulaneng-	0	0	0	0	0	0	0	0	1	0	1	17
Matikwana (MP)												
Mitchell's Plain	0	0	0	0	0	0	0	0	0	0	0	10
(WC)												
Red Cross (WC)	0	0	0	0	0	0	0	0	0	2	2	22
Tambo Memorial	0	0	0	0	0	0	0	0	0	0	0	0
(GP)												
Tembisa (GP)	0	0	0	0	0	0	0	0	0	0	0	17
Tintswalo (MP)	0	0	0	0	0	0	0	0	0	0	0	0
Tygerberg (WC)	0	0	0	0	0	0	0	0	0	0	0	1
Total:	0	0	0	0	0	0	0	0	2	2	4	177

Table 14. Number of SARS-CoV-2 positive cases* by variant (variant PCR and/or sequencing) identified and total number of samples tested by hospital, pneumonia surveillance public hospitals, 01/01/2023-14/01/2023

*Specimens are from hospitalized patients at 11 sentinel sites in 6 provinces who met the pneumonia (SRI), suspected SARS-CoV-2 or *B. pertussis* case definition Unable to assign: no lineage assigned due to poor- sequence quality OR low viral load (Ct235) OR variant PCR could not assign variant and no sequencing result Pending: outstanding variant results

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Methods

SARS-CoV-2 Testing

March 2020 – March 2021: SARS-CoV-2 was detected using the Roche E gene real-time PCR assay (Corman et al. *Euro Surveillance* 2020) with cycle threshold (C_t) <40 interpreted as positive for SARS-CoV-2. From April 2021 to date the laboratory changed to the AllplexTM SARS-CoV-2/FluA/FluB/RSV kit (Seegene Inc., Seoul, South Korea), with positivity assigned if the PCR cycle threshold (C_t) was <40 for ≥1 gene targets (N, S or RdRp).

A confirmed SARS-CoV-2 case is a person of any age enrolled in surveillance with laboratory confirmation of SARS-CoV-2 infection by PCR. Only positive SARS-CoV-2 specimens on PCR are further tested to determine variant/lineage type by variant PCR or genomic sequencing. Variant PCR

AllplexTM SARS-CoV-2 Variants I PCR detects Alpha and Beta/Gamma variants. The assay was conducted on all SARS-CoV-2-positive samples from 1 March 2020 – 30 June 2021.

AllplexTM SARS-CoV-2 Variants II PCR detects Delta variant and distinguishes Beta from Gamma. The assay was conducted on SARS-CoV-2-positive samples from 1 Jan to 30 June 2021.

Extraction: Total nucleic acids were extracted from 200µl NP/OP samples in universal or viral transport medium using a MagNA Pure 96 automated extractor and DNA/Viral NA Small Volume v2.0 extraction kit (Roche Diagnostics, Mannheim, Germany).

SARS-CoV-2 genomic surveillance

SARS-CoV-2 Whole-Genome Sequencing and Genome Assembly

RNA Extraction

RNA was extracted either manually or automatically in batches, using the QIAamp viral RNA mini kit (QIAGEN, CA, USA) or the Chemagic 360 using the CMG-1049 kit (PerkinElmer, MA, USA). A modification was done on the manual extractions by adding 280 µl per sample, in order to increase yields. 300 µl of each sample was used for automated magnetic bead-based extraction using the Chemagic 360. RNA was eluted in 60 µl of the elution buffer. Isolated RNA was stored at -80 °C prior to use.

PCR and Library Preparation

Sequencing was performed using the Illumina COVIDSeq protocol (Illumina Inc., CA, USA) or nCoV-2019 ARTIC network sequencing protocol v3 (https://artic.network/ncov-2019). These are amplicon-based next-generation sequencing approaches. Briefly, for the nCoV-2019 ARTIC network sequencing protocol, the first strand synthesis was carried out on extracted RNA samples using random hexamer primers from the SuperScript IV reverse transcriptase synthesis kit (Life Technologies, CA, USA) or LunaScript RT SuperMix Kit (New England Biolabs (NEB), MA, USA). The synthesized cDNA was amplified using multiplex polymerase chain reactions (PCRs) using ARTIC nCoV-2019 v3 primers. For the COVIDSeq protocol, the first strand synthesis was carried out using random hexamer primers from Illumina and the synthesized cDNA underwent two separate multiplex PCR reactions.

For Illumina sequencing using the nCoV-2019 ARTIC network sequencing protocol, the pooled PCR products underwent bead-based tagmentation using the Nextera Flex DNA library preparation kit (Illumina Inc., CA, USA). The adapter-tagged amplicons were cleaned up using AmpureXP purification beads (Beckman Coulter, High Wycombe, UK) and amplified using one round of PCR. The PCRs were indexed using the Nextera CD indexes (Illumina Inc., CA, USA) according to the manufacturer's instructions. For COVIDSeq sequencing protocol, pooled PCR amplified products were processed for tagmentation and adapter ligation using IDT for Illumina Nextera UD Indexes. Further enrichment and clean-up was performed as per protocols provided by the manufacturer (Illumina Inc., CA, USA). Pooled samples from both COVIDSeq protocol and nCoV-2019 ARTIC network protocol were quantified using Qubit 3.0 or 4.0 fluorometer (Invitrogen Inc., MA, USA) using the Qubit dsDNA High Sensitivity assay according to manufacturer's instructions. The fragment sizes were analyzed using TapeStation 4200 (Invitrogen Inc., MA, USA). The pooled libraries were further normalized to 4nM concentration and 25 µl of each normalized pool containing unique index adapter sets were combined in a new tube. The final library pool was denatured and neutralized with 0.2 N sodium hydroxide and 200 mM Tris-HCL (pH7), respectively. 1.5 pM sample library was spiked with 2% PhiX. Libraries were loaded onto a 300-cycle NextSeq 500/550 HighOutput Kit v2 and run on the Illumina NextSeq 550 instrument (Illumina Inc., CA, USA).

Assembly, Processing and Quality Control of Genomic Sequences

Raw reads from Illumina sequencing were assembled using the Exatype NGS SARS-CoV-2 pipeline v1.6.1, (<u>https://sars-cov-2.exatype.com/</u>). The resulting consensus sequence was further manually polished by considering and correcting indels in homopolymer regions that break the open reading frame (probably sequencing errors) using Aliview v1.27, (<u>http://ormbunkar.se/aliview/</u>) (Larsson, 2014). Mutations resulting in mid-gene stop codons and frameshifts were reverted to wild type. All assemblies determined to have acceptable quality (defined as having at least 1 000 000 reads and at least 40 % 10 X coverage) were deposited on GISAID (<u>https://www.gisaid.org/</u>) (Elbe & Buckland-Merrett, 2017; Shu & McCauley, 2017).

Classification of Lineage, Clade and Associated Mutations

Assembled genomes were assigned lineages using the 'Phylogenetic Assignment of Named Global Outbreak Lineages' (PANGOLIN) software suite (<u>https://github.com/hCoV-2019/pangolin</u>) (Rambaut et al., 2020), a tool used for dynamic SARS-CoV-2 lineage classification. The SARS-CoV-2 genomes in our dataset were also classified using the clade classification proposed by NextStrain (<u>https://nextstrain.org/</u>), a tool built for real-time tracking of the pathogen evolution (Hadfield et al., 2018).