Division of the National Health Laboratory Service

Weekly respiratory pathogens report Week 26 of 2023

<u>Highlights</u>

- The 2023 influenza season started in week 17 (week starting 24 April 2023) when the influenza detection rate (3-week moving average) breached the seasonal threshold and peaked in week 22 (week starting on 4 June 2023). Influenza transmission is currently below seasonal threshold, while impact is at the low level and circulation continues to decline.
- In 2023 to date, 917 influenza cases have been detected from all surveillance programmes, of which 98% (819/842) of those with typing information available were influenza A(H3N2). The majority of cases were reported from Western Cape (n=311), followed by Gauteng (n=249), North West (n=127), KwaZulu-Natal (n=94), Mpumalanga (n=94), Eastern Cape (n=38), Limpopo (n=2) and Free State (n=2) sentinel surveillance sites.
- In 2023 to date, 711 respiratory syncytial virus (RSV) cases have been detected from all surveillance programmes. The RSV season ended in week 21 (week starting 22 May 2023), although circulation of RSV continues.
- In 2023 to date, 135 cases of *Bordetella pertussis* were detected, of which 26% (35/135) were from Gauteng Province, 21% (29/135) North West Province, 17% (23/135) from Mpumalanga Province 16% (22/135) Western Cape Province, 15% (20/135) from KwaZulu-Natal Province and 4% (6/135) from Eastern Cape Province.
- In 2023 to date, 254 COVID-19 cases were detected from all surveillance programmes. Of the 226 specimens sequenced, a variant could be assigned in 62.4% (141/226). Of these, 98% (138/141) were assigned the Omicron variant, of which 60% (82/138) were Omicron (23A/XBB.1.5), 12% (17/138) each were Omicron (22E/BQ.1.1) and Omicron (22B/BA.5), 10% (14/138) Omicron (22F/BA.2.10.1), 3% (4/138) Omicron (23B/XBB.1.16), and 2% (2/138) each Omicron (21K/BA.1) and Omicron (22D/BM.1.1). One (0.4%, 1/226) was assigned XAY, XBF and XBL each respectively, while for the remaining 38% (85/226), a variant could not be assigned due to a low viral load or insufficient sample.

Programme Descriptions

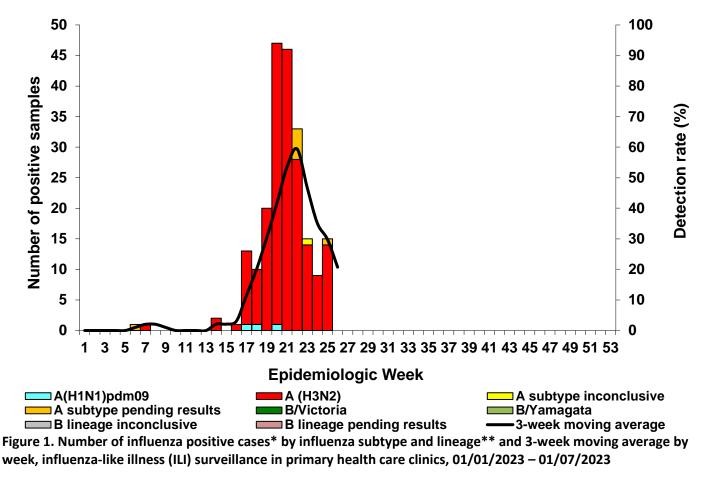
Influenza-like illness (ILI)	Viral Watch	National Syndromic Surveillance for Pneumonia
2012	1984	2009
KZ	EC	EC
NW	FS	GP
WC	GP	KZ
MP	LP	MP
	 MP	NW
	NC	WC
Primary health care clinics	General practitioners	Public hospitals
	ILI: An acute respiratory illness with a	SRI: Acute (symptom onset≤10 days) or
		chronic (symptom onset >10) lower
≤10 days	≤10 days	respiratory tract illness
Suspected pertussis		Suspected pertussis
Any person with an acute cough illness		Any person with an acute cough illness
lasting ≥14 days (or cough illness of any		lasting ≥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 of
the following signs or symptoms:		the following signs or symptoms:
 paroxysms of coughing, 		 paroxysms of coughing,
		 or inspiratory "whoop",
		 or post-tussive vomiting
		 or apnoea in children <1 year;
OR		OR
Any person in whom a clinician suspects pertussis		Any person in whom a clinician suspects pertussis.
Suspected SARS-CoV-2	Surgested CARS CoV 2	Suspected SARS-CoV-2
	•	Any person admitted with a physician-
		diagnosis of suspected COVID-19 and
		not meeting SRI case definition.
		not meeting shi case demitton.
		Oropharyngeal & nasopharyngeal swabs
Oropharyngeal & nasopharyngeal swabs	Throat and/or hasar swabs of	
Oropharyngeal & nasopharyngeal swabs	Nasopharyngeal swabs	.,,.
Oropharyngeal & nasopharyngeal swabs	-	INF
	Nasopharyngeal swabs	
INF	Nasopharyngeal swabs INF	INF
INF RSV	Nasopharyngeal swabs INF RSV	INF RSV
INF RSV BP SARS-CoV-2 INF and RSV	Nasopharyngeal swabs INF RSV	INF RSV BP
INF RSV BP SARS-CoV-2	Nasopharyngeal swabs INF RSV SARS-CoV-2	INF RSV BP SARS-CoV-2
INF RSV BP SARS-CoV-2 INF and RSV	Nasopharyngeal swabs INF RSV SARS-CoV-2 INF and RSV	INF RSV BP SARS-CoV-2 INF and RSV
INF RSV BP SARS-CoV-2 INF and RSV - Fast-Track Diagnostics multiplex real-	Nasopharyngeal swabs INF RSV SARS-CoV-2 INF and RSV - Fast-Track Diagnostics multiplex real-	INF RSV BP SARS-CoV-2 INF and RSV - Fast Track Diagnostics multiplex real-
INF RSV BP SARS-CoV-2 INF and RSV - Fast-Track Diagnostics multiplex real- time reverse transcription polymerase	Nasopharyngeal swabs INF RSV SARS-CoV-2 INF and RSV - Fast-Track Diagnostics multiplex real- time reverse transcription polymerase	INF RSV BP SARS-CoV-2 INF and RSV - Fast Track Diagnostics multiplex real- time reverse transcription polymerase
INF RSV BP SARS-CoV-2 INF and RSV - Fast-Track Diagnostics multiplex real- time reverse transcription polymerase chain reaction (until 31 March 2021)	Nasopharyngeal swabs INF RSV SARS-CoV-2 INF and RSV - Fast-Track Diagnostics multiplex real- time reverse transcription polymerase chain reaction (until 31 March 2021)	INF RSV BP SARS-CoV-2 INF and RSV - Fast Track Diagnostics multiplex real- time reverse transcription polymerase chain reaction (until 31 March 2021) B. pertussis
INF RSV BP SARS-CoV-2 INF and RSV - Fast-Track Diagnostics multiplex real- time reverse transcription polymerase chain reaction (until 31 March 2021) <i>B. pertussis</i>	Nasopharyngeal swabs INF RSV SARS-CoV-2 INF and RSV - Fast-Track Diagnostics multiplex real- time reverse transcription polymerase chain reaction (until 31 March 2021) <i>B. pertussis</i>	INF RSV BP SARS-CoV-2 INF and RSV - Fast Track Diagnostics multiplex real- time reverse transcription polymerase chain reaction (until 31 March 2021) B. pertussis
INF RSV BP SARS-CoV-2 INF and RSV - Fast-Track Diagnostics multiplex real- time reverse transcription polymerase chain reaction (until 31 March 2021) B. pertussis Multiplex real-time PCR (Tatti <i>et al., J Clin</i>	Nasopharyngeal swabs INF RSV SARS-CoV-2 INF and RSV - Fast-Track Diagnostics multiplex real- time reverse transcription polymerase chain reaction (until 31 March 2021) <i>B. pertussis</i> Multiplex real-time PCR (Tatti <i>et al., J Clin</i>	INF RSV BP SARS-CoV-2 INF and RSV - Fast Track Diagnostics multiplex real- time reverse transcription polymerase chain reaction (until 31 March 2021) B. pertussis Multiplex real-time PCR (Tatti <i>et al., J Clin</i>
INF RSV BP SARS-CoV-2 INF and RSV - Fast-Track Diagnostics multiplex real- time reverse transcription polymerase chain reaction (until 31 March 2021) <i>B. pertussis</i> Multiplex real-time PCR (Tatti <i>et al., J Clin</i> <i>Microbiol</i> 2011) and culture (if PCR cycle	Nasopharyngeal swabs INF RSV SARS-CoV-2 INF and RSV - Fast-Track Diagnostics multiplex real- time reverse transcription polymerase chain reaction (until 31 March 2021) <i>B. pertussis</i> Multiplex real-time PCR (Tatti <i>et al., J Clin</i> <i>Microbiol</i> 2011) and culture (if PCR cycle	INF RSV BP SARS-CoV-2 INF and RSV - Fast Track Diagnostics multiplex real- time reverse transcription polymerase chain reaction (until 31 March 2021) B. pertussis Multiplex real-time PCR (Tatti <i>et al., J Clin</i> <i>Microbiol</i> 2011) and culture (if PCR cycle
INF RSV BP SARS-CoV-2 INF and RSV - Fast-Track Diagnostics multiplex real- time reverse transcription polymerase chain reaction (until 31 March 2021) <i>B. pertussis</i> Multiplex real-time PCR (Tatti <i>et al., J Clin</i> <i>Microbiol</i> 2011) and culture (if PCR cycle threshold ≤25) SARS-CoV-2	Nasopharyngeal swabs INF RSV SARS-CoV-2 INF and RSV - Fast-Track Diagnostics multiplex real- time reverse transcription polymerase chain reaction (until 31 March 2021) B. pertussis Multiplex real-time PCR (Tatti et al., J Clin Microbiol 2011) and culture (if PCR cycle threshold ≤25)	INF RSV BP SARS-CoV-2 INF and RSV - Fast Track Diagnostics multiplex real- time reverse transcription polymerase chain reaction (until 31 March 2021) B. pertussis Multiplex real-time PCR (Tatti <i>et al., J Clir</i> <i>Microbiol</i> 2011) and culture (if PCR cycle threshold ≤25) SARS-CoV-2
INF RSV BP SARS-CoV-2 INF and RSV - Fast-Track Diagnostics multiplex real- time reverse transcription polymerase chain reaction (until 31 March 2021) <i>B. pertussis</i> Multiplex real-time PCR (Tatti <i>et al., J Clin</i> <i>Microbiol</i> 2011) and culture (if PCR cycle threshold ≤25) SARS-CoV-2 1 April 2020 – 31 March 2021: Roche E	Nasopharyngeal swabs INF RSV SARS-CoV-2 INF and RSV - Fast-Track Diagnostics multiplex real- time reverse transcription polymerase chain reaction (until 31 March 2021) B. pertussis Multiplex real-time PCR (Tatti et al., J Clin Microbiol 2011) and culture (if PCR cycle threshold ≤25) SARS-CoV-2 1 April 2020 – 31 March 2021: Roche E	INF RSV BP SARS-CoV-2 INF and RSV - Fast Track Diagnostics multiplex real- time reverse transcription polymerase chain reaction (until 31 March 2021) B. pertussis Multiplex real-time PCR (Tatti <i>et al., J Clir</i> <i>Microbiol</i> 2011) and culture (if PCR cycle threshold ≤25) SARS-CoV-2 1 April 2020 – 31 March 2021: Roche E
INF RSV BP SARS-CoV-2 INF and RSV - Fast-Track Diagnostics multiplex real- time reverse transcription polymerase chain reaction (until 31 March 2021) <i>B. pertussis</i> Multiplex real-time PCR (Tatti <i>et al., J Clin</i> <i>Microbiol</i> 2011) and culture (if PCR cycle threshold ≤25) SARS-CoV-2 1 April 2020 – 31 March 2021: Roche E gene real-time PCR essay (Corman <i>et al.,</i>	Nasopharyngeal swabs INF RSV SARS-CoV-2 INF and RSV - Fast-Track Diagnostics multiplex real- time reverse transcription polymerase chain reaction (until 31 March 2021) B. pertussis Multiplex real-time PCR (Tatti et al., J Clin Microbiol 2011) and culture (if PCR cycle threshold ≤25) SARS-CoV-2 1 April 2020 – 31 March 2021: Roche E gene real-time PCR essay Corman et al.,	INF RSV BP SARS-CoV-2 INF and RSV - Fast Track Diagnostics multiplex real- time reverse transcription polymerase chain reaction (until 31 March 2021) <i>B. pertussis</i> Multiplex real-time PCR (Tatti <i>et al., J Clir</i> <i>Microbiol</i> 2011) and culture (if PCR cycle threshold ≤25) SARS-CoV-2 1 April 2020 – 31 March 2021: Roche E gene real-time PCR essay (Corman <i>et al.,</i>
INF RSV BP SARS-CoV-2 INF and RSV - Fast-Track Diagnostics multiplex real- time reverse transcription polymerase chain reaction (until 31 March 2021) <i>B. pertussis</i> Multiplex real-time PCR (Tatti <i>et al., J Clin</i> <i>Microbiol</i> 2011) and culture (if PCR cycle threshold ≤25) SARS-CoV-2 1 April 2020 – 31 March 2021: Roche E gene real-time PCR essay (Corman <i>et al.,</i> <i>Euro Surv</i> 2020)	Nasopharyngeal swabs INF RSV SARS-CoV-2 INF and RSV - Fast-Track Diagnostics multiplex real- time reverse transcription polymerase chain reaction (until 31 March 2021) B. pertussis Multiplex real-time PCR (Tatti et al., J Clin Microbiol 2011) and culture (if PCR cycle threshold ≤25) SARS-CoV-2 1 April 2020 – 31 March 2021: Roche E gene real-time PCR essay Corman et al., Euro Surv 2020)	INF RSV BP SARS-CoV-2 INF and RSV - Fast Track Diagnostics multiplex real- time reverse transcription polymerase chain reaction (until 31 March 2021) B. pertussis Multiplex real-time PCR (Tatti <i>et al., J Clir</i> <i>Microbiol</i> 2011) and culture (if PCR cycle threshold ≤25) SARS-CoV-2 1 April 2020 – 31 March 2021: Roche E gene real-time PCR essay (Corman <i>et al.,</i> <i>Euro Surv</i> 2020)
INF RSV BP SARS-CoV-2 INF and RSV - Fast-Track Diagnostics multiplex real- time reverse transcription polymerase chain reaction (until 31 March 2021) <i>B. pertussis</i> Multiplex real-time PCR (Tatti <i>et al., J Clin</i> <i>Microbiol</i> 2011) and culture (if PCR cycle threshold ≤25) SARS-CoV-2 1 April 2020 – 31 March 2021: Roche E gene real-time PCR essay (Corman <i>et al.,</i> <i>Euro Surv</i> 2020) 1 April 2021 to date: Allplex [™] SARS-CoV-	Nasopharyngeal swabs INF RSV SARS-CoV-2 INF and RSV - Fast-Track Diagnostics multiplex real- time reverse transcription polymerase chain reaction (until 31 March 2021) B. pertussis Multiplex real-time PCR (Tatti et al., J Clin Microbiol 2011) and culture (if PCR cycle threshold ≤25) SARS-CoV-2 1 April 2020 – 31 March 2021: Roche E gene real-time PCR essay Corman et al., Euro Surv 2020) 1 April 2021 to date: Allplex [™] SARS-CoV-	INF RSV BP SARS-CoV-2 INF and RSV - Fast Track Diagnostics multiplex real- time reverse transcription polymerase chain reaction (until 31 March 2021) B. pertussis Multiplex real-time PCR (Tatti <i>et al., J Clir</i> <i>Microbiol</i> 2011) and culture (if PCR cycle threshold ≤25) SARS-CoV-2 1 April 2020 – 31 March 2021: Roche E gene real-time PCR essay (Corman <i>et al.,</i> <i>Euro Surv</i> 2020) 1 April 2021 to date: Allplex [™] SARS-CoV-
INF RSV BP SARS-CoV-2 INF and RSV - Fast-Track Diagnostics multiplex real- time reverse transcription polymerase chain reaction (until 31 March 2021) <i>B. pertussis</i> Multiplex real-time PCR (Tatti <i>et al., J Clin</i> <i>Microbiol</i> 2011) and culture (if PCR cycle threshold ≤25) SARS-CoV-2 1 April 2020 – 31 March 2021: Roche E gene real-time PCR essay (Corman <i>et al.,</i> <i>Euro Surv</i> 2020) 1 April 2021 to date: Allplex [™] SARS-CoV- 2/FluA/FluB/RSV PCR kit	Nasopharyngeal swabs INF RSV SARS-CoV-2 INF and RSV - Fast-Track Diagnostics multiplex real- time reverse transcription polymerase chain reaction (until 31 March 2021) B. pertussis Multiplex real-time PCR (Tatti et al., J Clin Microbiol 2011) and culture (if PCR cycle threshold ≤25) SARS-CoV-2 1 April 2020 – 31 March 2021: Roche E gene real-time PCR essay Corman et al., Euro Surv 2020) 1 April 2021 to date: Allplex™ SARS-CoV- 2/FluA/FluB/RSV PCR kit	INF RSV BP SARS-CoV-2 INF and RSV - Fast Track Diagnostics multiplex real- time reverse transcription polymerase chain reaction (until 31 March 2021) <i>B. pertussis</i> Multiplex real-time PCR (Tatti <i>et al., J Clin</i> <i>Microbiol</i> 2011) and culture (if PCR cycle threshold ≤25) SARS-CoV-2 1 April 2020 – 31 March 2021: Roche E gene real-time PCR essay (Corman <i>et al.,</i> <i>Euro Surv</i> 2020) 1 April 2021 to date: Allplex [™] SARS-CoV- 2/FluA/FluB/RSV PCR kit
INF RSV BP SARS-CoV-2 INF and RSV - Fast-Track Diagnostics multiplex real- time reverse transcription polymerase chain reaction (until 31 March 2021) <i>B. pertussis</i> Multiplex real-time PCR (Tatti <i>et al., J Clin</i> <i>Microbiol</i> 2011) and culture (if PCR cycle threshold ≤25) SARS-CoV-2 1 April 2020 – 31 March 2021: Roche E gene real-time PCR essay (Corman <i>et al.,</i> <i>Euro Surv</i> 2020) 1 April 2021 to date: Allplex [™] SARS-CoV-	Nasopharyngeal swabs INF RSV SARS-CoV-2 INF and RSV - Fast-Track Diagnostics multiplex real- time reverse transcription polymerase chain reaction (until 31 March 2021) B. pertussis Multiplex real-time PCR (Tatti et al., J Clin Microbiol 2011) and culture (if PCR cycle threshold ≤25) SARS-CoV-2 1 April 2020 – 31 March 2021: Roche E gene real-time PCR essay Corman et al., Euro Surv 2020) 1 April 2021 to date: Allplex [™] SARS-CoV-	INF RSV BP SARS-CoV-2 INF and RSV - Fast Track Diagnostics multiplex real- time reverse transcription polymerase chain reaction (until 31 March 2021) B. pertussis Multiplex real-time PCR (Tatti <i>et al., J Clir</i> <i>Microbiol</i> 2011) and culture (if PCR cycle threshold ≤25) SARS-CoV-2 1 April 2020 – 31 March 2021: Roche E gene real-time PCR essay (Corman <i>et al.,</i> <i>Euro Surv</i> 2020) 1 April 2021 to date: Allplex [™] SARS-CoV-
	2012 KZ NW WC MP Primary health care clinics ILI: An acute respiratory illness with a temperature (≥38°C) and cough, & onset ≤10 days Suspected pertussis Any person with an acute cough illness of any duration for children <1 year), without a more likely diagnosis AND one or more of the following signs or symptoms: paroxysms of coughing, or inspiratory "whoop", or apnoea in children <1 year; OR Any person in whom a clinician suspects pertussis Suspected SARS-CoV-2 Any person presenting with an acute (≤14 days) respiratory tract infection or other clinical illness compatible with COVID-19** 	2012 1984 KZ EC NW FS WC GP MP LP MP LP MP KC Superiod SARS-Coved General practitioners ILI: An acute respiratory illness with a temperature (≥38°C) and cough, & onset ≤10 days ILI: An acute respiratory illness with a temperature (≥38°C) and cough, & onset ≤10 days Suspected pertussis Any person with an acute cough illness lasting ≥14 days (or cough illness of any duration for children <1 year), without a more likely diagnosis AND one or more of the following signs or symptoms: paroxysms of coughing, or inspiratory "whoop", or apnoea in children <1 year; OR Any person in whom a clinician suspects pertussis Suspected SARS-CoV-2 Any person presenting with an acute (S14 days) respiratory tract infection or other clinical illness compatible with Suspected SARS-CoV-2 Any person presenting with an acute

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

Page **2** of **20**



*Specimens from patients with influenza-like illnesses at 5 sentinel sites in 4 provinces ** Influenza A(H3N2) was detected in 10/21, 48% of specimens from patients 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

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 – 01/07/2023

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	39	0	1	0	0	0	0	158
Eastridge (WC)	1	29	0	4	0	0	0	0	184
Edendale Gateway (KZ)	2	50	1	1	0	0	0	0	326
Jouberton (NW)	0	75	0	0	0	0	0	0	233
Mitchell's Plain (WC)	0	9	0	1	0	0	0	0	82
Total:	3	202	1	7	0	0	0	0	983

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

* Influenza A(H3N2) was detected in 10/21, 48% of specimens from patients 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

Page **3** of **20**

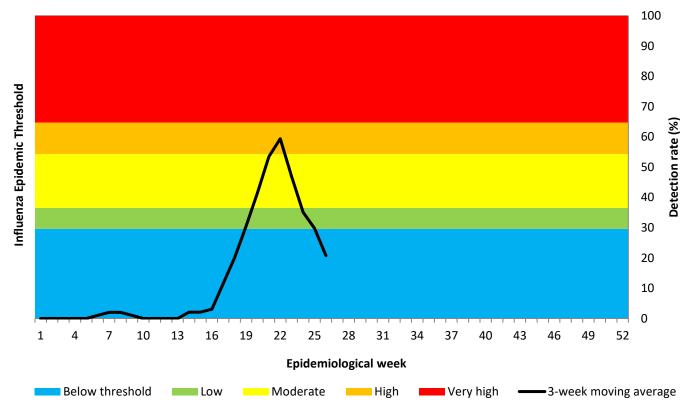


Figure 2. Influenza percentage detections and epidemic thresholds* among cases of all ages, influenza-like illness (ILI) surveillance in primary health care clinics, 01/01/2023 –01/07/2023 *Thresholds based on 2012-2019 data

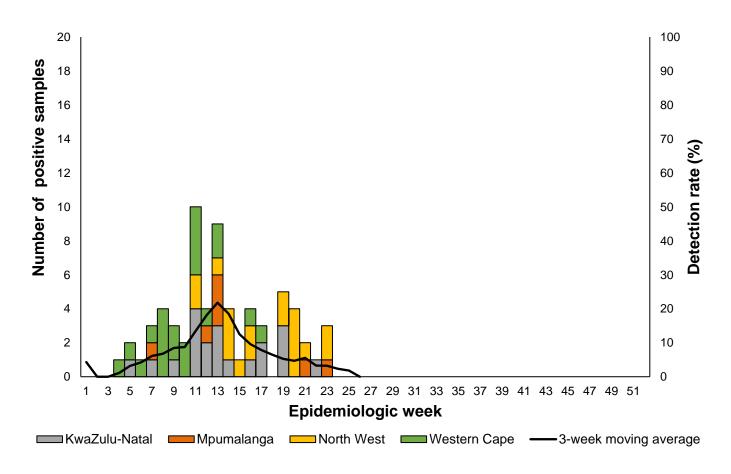


Figure 3. Number of patients testing positive for respiratory syncytial virus* by province and 3-week moving average by week, influenza-like illness (ILI) surveillance in primary health care clinics, 01/01/2023 –01/07/2023

*RSV was detected in 1/21, 5% of specimens of patients who met suspected SARS-CoV-2 or *B. pertussis* case definition but did not meet influenza-like illness (ILI) case definition.

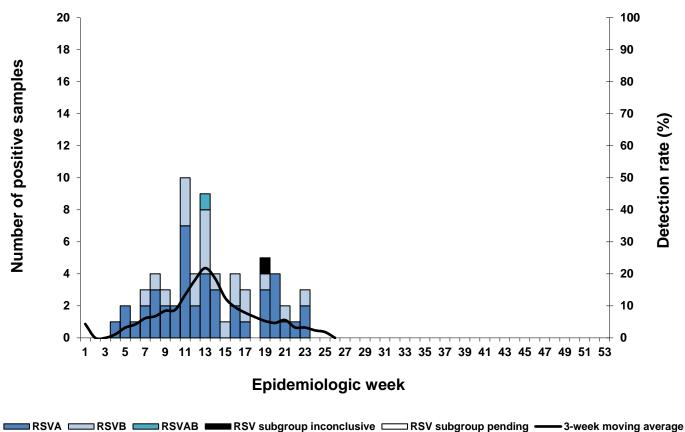


Figure 4. Number of patients testing positive for respiratory syncytial virus* by subgroup and 3-week moving average by week, influenza-like illness (ILI) surveillance in primary health care clinics, 01/01/2023 –01/07/2023

*RSV was detected in 1/21, 5% of specimens of patients 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

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 –01/07/2023

Clinic (Province)	RSVA	RSVB	RSVAB**	RSV subgroup inconclusive* **	RSV subgroup pending** **	Total samples
Agincourt (MP)	2	4	1	0	0	158
Eastridge (WC)	18	2	0	0	0	184
Edendale Gateway (KZ)	8	11	0	1	0	326
Jouberton (NW)	14	4	0	0	0	233
Mitchell's Plain (WC)	1	0	0	0	0	82
Total	43	21	1	1	0	983

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

*RSV was detected in 1/21, 5% of specimens of patients 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

Page 5 of 20

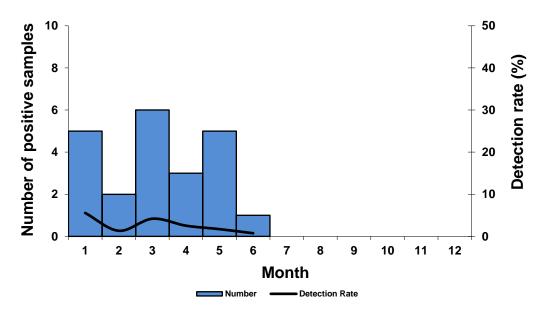


Figure 5. Number of patients testing positive for *B. pertussis** and detection rate by month, influenza-like illness (ILI) surveillance primary health care clinics**, 01/01/2023 –01/07/2023

*B. pertussis was detected in 1/21, 5% of specimens of patients who met the suspected SARS-CoV-2 or B. pertussis case definition but did not meet influenza-like illness (ILI) case definition. These are not included in the epidemiological curve.

** 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 –01/07/2023

Clinic (Province)	<i>B. pertussis</i> Positive	Total samples
Agincourt (MP)	4	158
Eastridge (WC)	2	175
Edendale Gateway (KZ)	6	310
Jouberton (NW)	10	228
Mitchell's Plain (WC)	0	77
Total:	22	948

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

*B. pertussis was detected in 1/21, 5% of specimens of patients who met the suspected SARS-CoV-2 or B. pertussis case definition but did not meet influenza-like illness (ILI) case definition. These are not included in the epidemiological curve.

NB: Results pending for 17 samples.

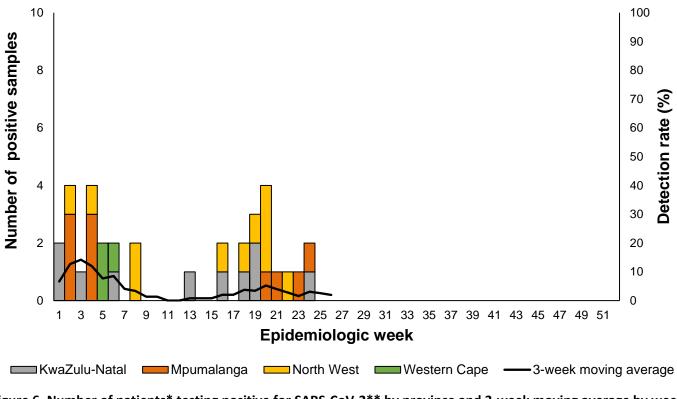


Figure 6. Number of patients* testing positive for SARS-CoV-2** by province and 3-week moving average by week, influenza-like illness (ILI) surveillance in primary health care clinics, 01/01/2023 –01/07/2023

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

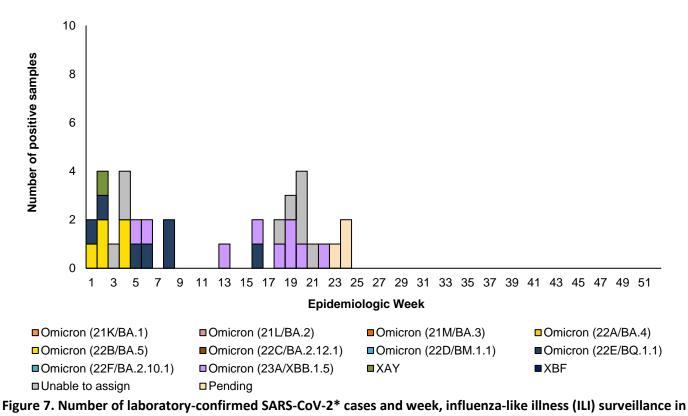
**SARS-CoV-2 was not detected in 21 specimens from patients who met suspected SARS-CoV-2 or *B. pertussis* case definition but did not meet influenza-like illness (ILI) case definition. These are not included in the epidemiological curve.

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 –01/07/2023

Clinic (Province)	SARS-CoV-2 positive	Total samples tested
Agincourt (MP)	10	158
Eastridge (WC)	1	184
Edendale Gateway (KZ)	10	326
Jouberton (NW)	11	233
Mitchell's Plain (WC)	2	82
Total:	34	983

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

*SARS-CoV-2 was not detected in 21 specimens from patients who met suspected SARS-CoV-2 or *B. pertussis* case definition but did not meet influenza-like illness (ILI) case definition. These are not included in the epidemiological curve.



primary health care clinics, 01/01/2023-01/07/2023

*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, 01/01/2023-01/07/2023

Province	Omicron (21L/BA.2)	Omicron (21M/ BA.3)	Omicron (22A/BA.4)	Omicron (22B/ BA.5)	Omicron (22C/BA.2.12. 11	Omicron (22D/BM.1.1)	Omicron (22E/BQ.1.1)	Omicron (22F/BA.2.10. 11	Omicron (23A/XBB.1.5)	ХАҮ	Unable to assign**	Pending***	SARS-CoV-2 positive	Total samples tested
Agincourt	0	0	0	4	0	0	0	0	1	1	2	2	10	158
Clinic (MP)														
Eastridge	0	0	0	0	0	0	0	0	1	0	0	0	1	184
Clinic (WC)														
Edendale	0	0	0	1	0	0	3	0	4	0	1	1	10	344
Clinic (KZ)														
Jouberton	0	0	0	0	0	0	3	0	2	0	6	0	11	236
Clinic (NW)														
Mitchell's	0	0	0	0	0	0	1	0	1	0	0	0	2	82
Plain Clinic														
(WC)														
Total:	0	0	0	5	0	0	7	0	9	1	9	3	34	1004

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

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

Page 8 of 20

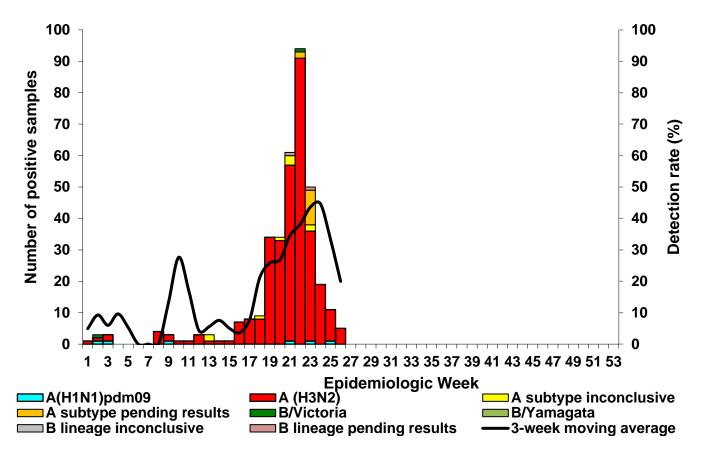


Figure 8. Number of positive patients* by influenza subtype and lineage and 3-week moving average by week, ILI surveillance - Viral Watch, 01/01/2023-01/07/2023

*Specimens from patients with influenza-like illnesses at 92 sentinel sites in 8 provinces Inconclusive: insufficient viral load in sample and unable to characterise further

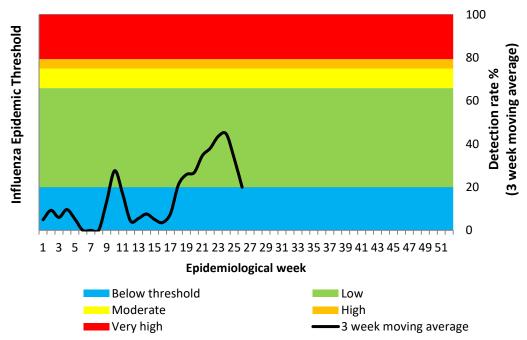


Figure 8b. Influenza percentage detections and epidemic thresholds* among cases of all ages, ILI surveillance -Viral Watch, 01/01/2023-01/07/2023

*Thresholds based on 2015-2019 data

Page **9** of **20**

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, 01/01/2023-01/07/2023

Province	A(H1N1) pdm09	A(H3N2)	A subtype inconclusive	A subtype pending results*	B/Victori a	B/Yamaga ta	B lineage inconclusi ve	B lineage pending results*	Total samples
Eastern Cape	0	16	0	0	0	0	0	0	33
Free State	0	2	0	0	0	0	0	0	2
Gauteng	2	115	4	2	1	0	1	1	517
Limpopo	0	2	0	0	0	0	0	0	6
Mpumalanga	1	18	2	0	0	0	0	0	36
North West	0	3	0	0	0	0	0	0	3
Northern Cape	0	0	0	0	0	0	0	0	0
Western Cape	3	168	3	11	1	0	1	1	327
Total:	6	324	9	13	2	0	2	2	924

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

**Influenza A subtype or B lineage results are pending

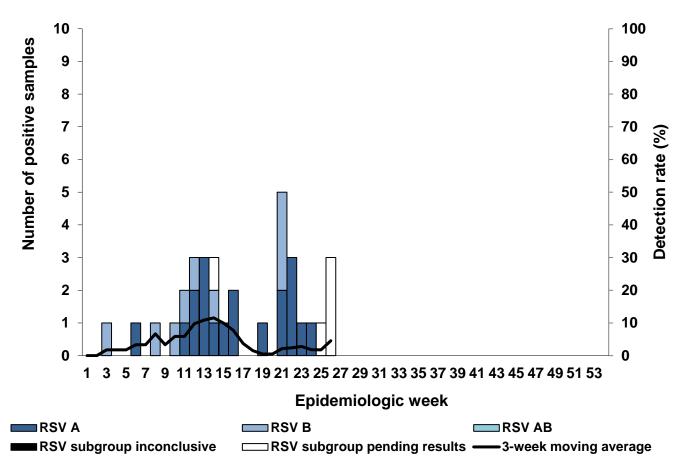


Figure 9. Number of RSV positive cases testing positive for respiratory syncytial virus (RSV)* by subgroup and 3week moving average by week, ILI surveillance - Viral Watch, 01/01/2023-01/07/2023 *Specimens from patients with Influenza-like illnesses at 92 sentinel sites in 8 provinces

Page **10** of **20**

Table 7. Number of RSV positive cases identified and total number of samples tested by province, ILIsurveillance - Viral Watch, 01/01/2023-01/07/2023

Province	RSV A	RSV B	RSV AB*	RSV subgroup inconclusive **	RSV subgroup pending results***	Total samples tested
Eastern Cape	1	1	0	0	0	33
Free State	0	0	0	0	0	2
Gauteng	10	5	0	0	4	517
Limpopo	0	0	0	0	0	6
Mpumalanga	0	0	0	0	1	36
North West	0	0	0	0	0	3
Northern Cape	0	0	0	0	0	0
Western Cape	8	3	0	0	0	327
Total:	19	9	0	0	5	924

*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

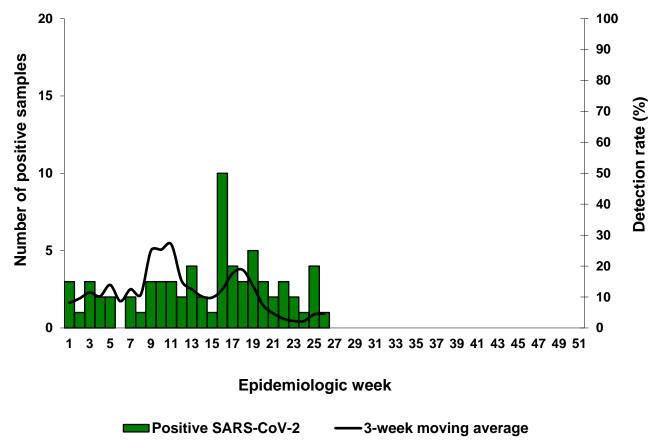


Figure 10. Number of patients testing positive for SARS-CoV-2*, by site and 3-week moving average** by week, ILI surveillance - Viral Watch, 01/01/2023-01/07/2023

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

Data are provisional as reported to date (Data for this report drawn on 05/07/2023). 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.

Page **11** of **20**

Table 8. Number of SARS-CoV-2 positive cases identified and total number tested by province, ILI surveillance - Viral Watch, 01/01/2023-01/07/2023

Province	SARS-CoV-2 positive	Total samples tested
Eastern Cape	4	33
Free State	0	2
Gauteng	46	517
Limpopo	0	6
Mpumalanga	2	36
North West	0	3
Northern Cape	0	0
Western Cape	18	327
Total:	70	924

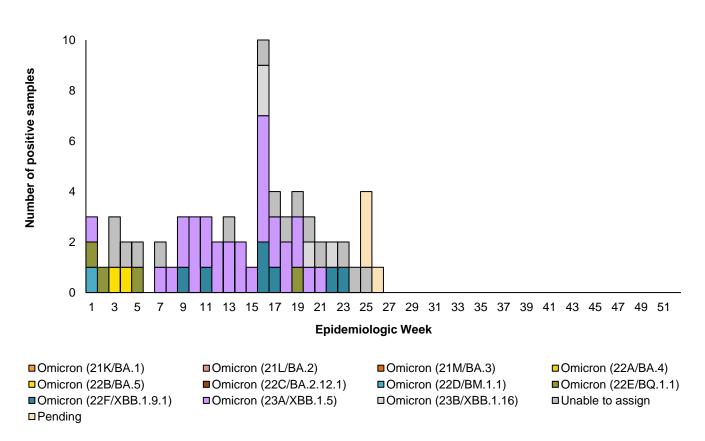


Figure 11. Number of laboratory confirmed SARS-CoV-2* cases and week, ILI surveillance - Viral Watch, 01/01/2023-01/07/2023

*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

Page 12 of 20

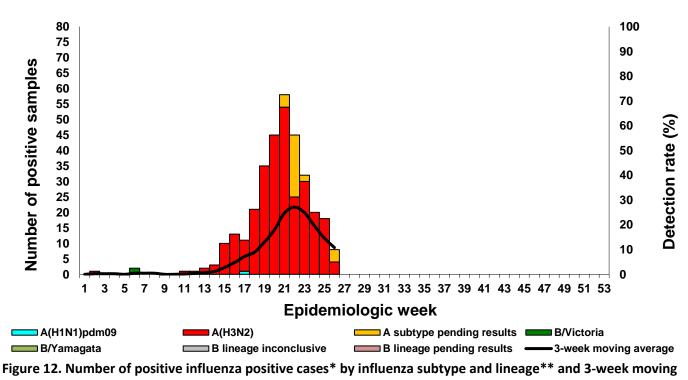
Table 9. Number of SARS-CoV-2^{*} positive cases by variant (variant PCR and/or sequencing) identified and total number of samples tested by province, ILI surveillance - Viral Watch, 01/01/2023-01/07/2023

Clinic (Province)	Omicron (21L/BA.2)	Omicron (21M/BA.3)	Omicron (22A/BA.4)	Omicron (22B/BA.5)	Omicron (22C/ BA.2.12.1)	Omicron (22D/BM.1.1)	Omicron (22E/BQ.1.1)	Omicron (22F/XBB.1.9.1)	Omicron (23A/XBB.1.5)	Omicron (23B/XBB.1.16)	Unable to assign**	Pending***	Total SARS-CoV- 2 positive	Total samples tested
Eastern	0	0	0	0	0	0	0	1	2	0	1	0	4	33
Cape														
Free State	0	0	0	0	0	0	0	0	0	0	0	0	0	2
Gauteng	0	0	0	2	0	1	3	4	22	2	5	4	43	517
Limpopo	0	0	0	0	0	0	0	0	0	0	0	0	0	6
Mpumala nga	0	0	0	0	0	0	0	0	1	0	1	0	2	36
North West	0	0	0	0	0	0	0	0	0	0	0	0	0	3
Northern	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cape														
Western	0	0	0	0	0	0	1	2	5	2	8	0	18	327
Cape														
Total:	0	0	0	2	0	1	4	7	30	4	15	4	67	924

*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



average by week, pneumonia surveillance public hospitals, 01/01/2023-01/07/2023

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

*Specimens from patients hospitalised with pneumonia at 15 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.

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	29	1	0	1	0	0	0	433
Helen Joseph- Rahima Moosa (GP)	1	57	1	5	0	0	0	0	753
Khayelitsha (WC)	0	23	1	6	1	0	0	0	364
Klerksdorp- Tshepong (NW)	0	46	1	1	0	0	0	0	321
Livingstone (EC)	0	21	1	0	0	0	0	0	404
Mapulaneng- Matikwana (MP)	0	17	1	4	0	0	0	0	275
Mitchell's Plain (WC)	0	11	0	4	0	0	1	0	280
Red Cross (WC)	0	19	1	4	0	0	0	0	566
Tambo Memorial (GP)	0	27	1	5	0	0	0	0	305
Tembisa (GP)	0	25	2	0	0	0	0	0	308
Tintswalo (MP)	0	10	0	0	0	0	0	0	178
Tygerberg (WC)	0	8	0	1	0	0	0	0	99
Total:	1	293	10	30	2	0	1	0	4286

Table 10. Number of laboratory confirmed influenza cases by subtype and lineage* and total number of samples tested by hospital, pneumonia surveillance public hospitals, 01/01/2023-25/06/2023

* 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

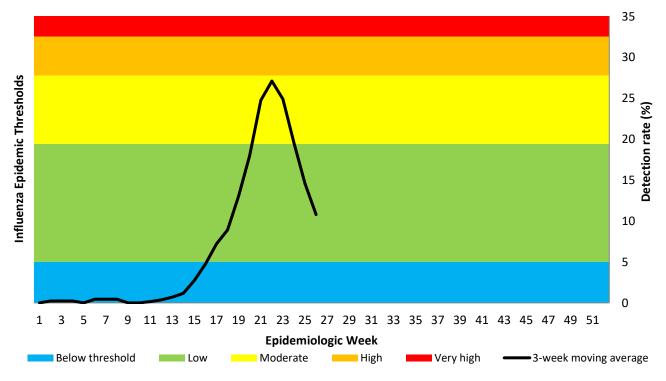


Figure 13. Influenza percentage detections and epidemic thresholds* among cases of all ages, pneumonia surveillance public hospitals, 01/01/2023-01/07/2023

*Thresholds based on 2010-2019 data

Data are provisional as reported to date (Data for this report drawn on 05/07/2023). 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.

Page **14** of **20**

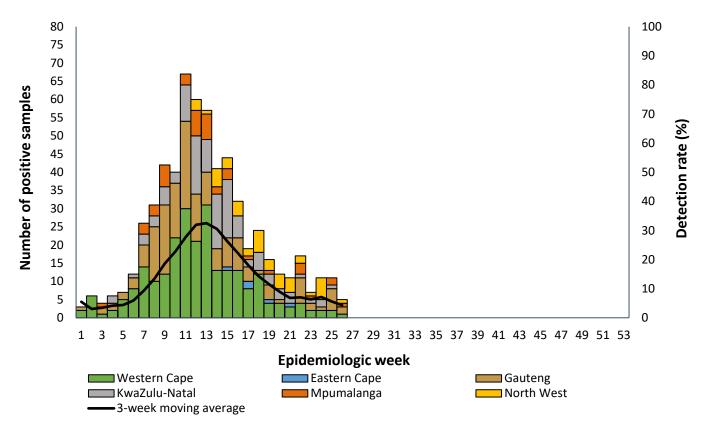


Figure 14. Number of patients (all ages) testing positive for respiratory syncytial virus* by province and 3-week moving average by week, pneumonia surveillance public hospitals, 01/01/2023-01/07/2023

Specimens from patients hospitalised with pneumonia at 15 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.

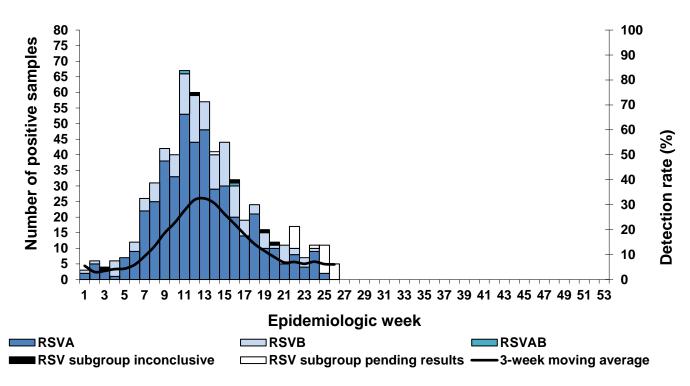


Figure 15. Number of patients (<u>all ages</u>) testing positive for respiratory syncytial virus* by subgroup and 3-week moving average by week, pneumonia surveillance public hospitals, 01/01/2023-01/07/2023

Specimens from patients hospitalised with pneumonia at 15 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

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

Page 15 of 20

RSV subgroup pending: RSV results for subgroups are pending

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, 01/01/2023-01/07/2023

Hospital (Province)	RSVA	RSVB	RSVAB**	RSV subgroup inconclusive** *	RSV subgroup pending** **	Total samples
Edendale (KZ)	36	71	1	1	1	433
Helen Joseph-Rahima Moosa (GP)	135	9	0	1	7	753
Khayelitsha (WC)	4	3	0	0	2	364
Klerksdorp-Tshepong (NW)	41	2	0	1	1	321
Livingstone (EC)	3	2	0	0	0	404
Mapulaneng-Matikwana (MP)	11	5	1	0	2	275
Mitchell's Plain (WC)	63	9	0	0	1	280
Red Cross (WC)	131	25	0	2	3	566
Tambo Memorial (GP)	2	1	0	0	1	305
Tembisa (GP)	1	1	0	0	4	308
Tintswalo (MP)	24	0	0	0	1	178
Tygerberg (WC)	1	1	0	0	0	99
Total:	452	129	2	5	23	4286

*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

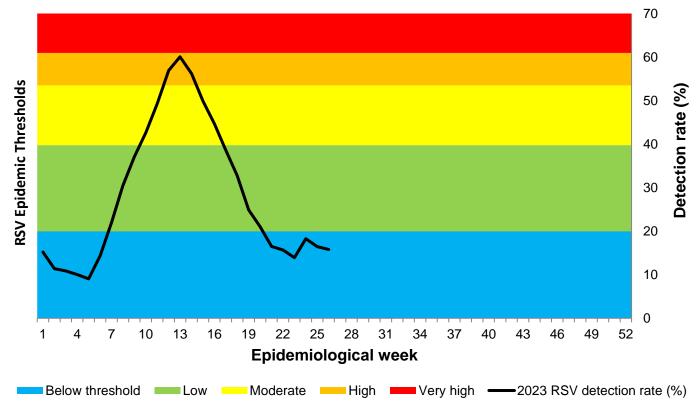


Figure 16. RSV percentage 3-week moving average and epidemic thresholds* among children aged < 5 years, pneumonia surveillance public hospitals, 01/01/2023-01/07/2023 *Thresholds based on 2010-2019 data

Data are provisional as reported to date (Data for this report drawn on 05/07/2023). 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.

Page 16 of 20

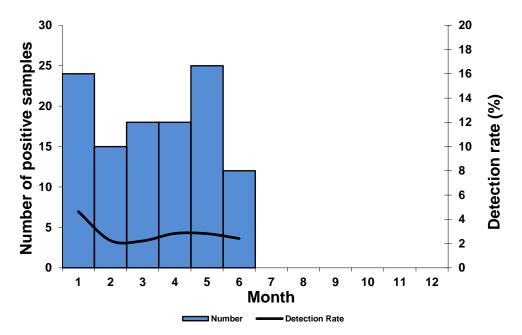


Figure 17. Number of patients testing positive for *B. pertussis** and detection rate by month, pneumonia surveillance public hospitals**, 01/01/2023-01/07/2023

*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 15 sentinel sites in 6 provinces.

Table 12. Number of patients testing positive for *B. pertussis** identified and total number of samples tested by hospital and province, pneumonia surveillance public hospitals, 01/01/2023-01/07/2023

Hospital (Province)	<i>B. pertussis</i> Positive	Total samples		
Edendale (KZ)	14	412		
Helen Joseph-Rahima Moosa (GP)	23	712		
Khayelitsha (WC)	3	340		
Klerksdorp-Tshepong(NW)	18	310		
Livingstone (EC)	6	375		
Mapulaneng-Matikwana (MP)	16	252		
Mitchell's Plain (WC)	2	271		
Red Cross (WC)	13	538		
Tambo Memorial (GP)	6	267		
Tembisa (GP)	6	280		
Tintswalo (MP)	3	167		
Tygerberg (WC)	2	91		
Total:	112	4015		

*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.

NB: Results pending for 98 samples

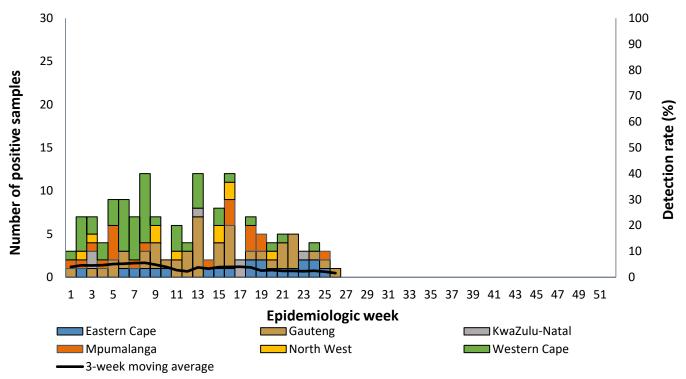


Figure 18. Number of patients testing positive for SARS-CoV-2^{**} by province and 3-week moving average by week, pneumonia surveillance public hospitals, 01/01/2023-01/07/2023

*Specimens from patients hospitalized with pneumonia at 15 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, 01/01/2023-01/07/2023

Hospital (Province)	SARS-CoV-2 positive	Total samples tested		
Edendale (KZ)	6	433		
Helen Joseph-Rahima Moosa (GP)	19	753		
Khayelitsha (WC)	16	364		
Klerksdorp-Tshepong (NW)	10	321		
Livingstone (EC)	21	404		
Mapulaneng-Matikwana (MP)	15	275		
Mitchell's Plain (WC)	15	280		
Red Cross (WC)	12	566		
Tambo Memorial (GP)	17	305		
Tembisa (GP)	10	308		
Tintswalo (MP)	5	178		
Tygerberg (WC)	4	99		
Total:	150	4286		

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

Page **18** of **20**

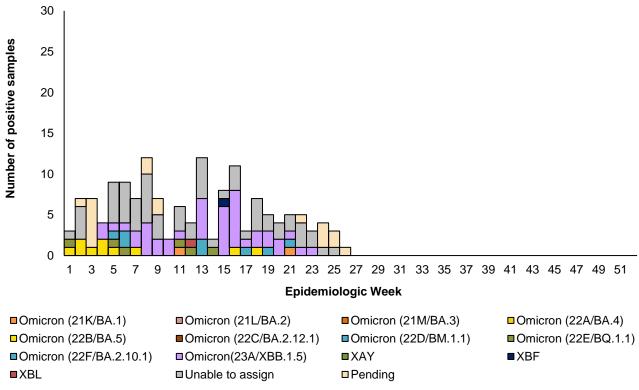


Figure 19. Number and 3-week moving average of laboratory-confirmed SARS-CoV-2 cases and weeks, pneumonia surveillance public hospitals, 01/01/2023-01/07/2023

*Specimens are from hospitalized patients at 15 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

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, 01/01/2023-01/07/2023

Hospital (Province)	Omicron (21K/BA.1 11	Omicron (22A/BA.4 1	Omicron (22B/BA.5 1	Omicron (22C/ 84 2 12 11	Omicron (22D/BM. 1 11	Omicron(2 2E/BQ.1.1	Omicron (22F/BA.2. 1011	Omicron(2 3A/XBB.1. בו	XBF	XBL	Unable to assign**	Pending** *	Total SARS-CoV-	
Edendale (KZ)	0	0	0	0	0	0	1	1	0	0	2	2	6	433
Helen Joseph-	1	0	1	0	0	1	2	9	0	0	4	1	19	753
Rahima Moosa (GP)														
Khayelitsha (WC)	1	0	0	0	0	0	0	5	0	0	9	1	16	364
Klerksdorp-	0	0	1	0	0	0	0	5	0	0	2	2	10	321
Tshepong (NW)														
Livingstone (EC)	0	0	1	0	1	0	0	4	1	0	9	5	21	404
Mapulaneng- Matikwana (MP)	0	0	3	0	0	1	0	2	0	0	8	1	15	275
Mitchell's Plain (WC)	0	0	1	0	0	0	2	5	0	0	6	1	15	280
Red Cross (WC)	0	0	3	0	0	0	1	2	0	1	4	1	12	566
Tambo Memorial (GP)	0	0	0	0	0	3	0	9	0	0	4	1	17	305
Tembisa (GP)	0	0	0	0	0	1	1	0	0	0	7	1	10	308
Tintswalo (MP)	0	0	0	0	0	0	0	0	0	0	5	0	5	178
Tygerberg (WC)	0	0	0	0	0	0	0	1	0	0	1	2	4	99
Total:	2	0	10	0	1	6	7	43	1	1	61	18	150	4286

*Specimens are from hospitalized patients at 15 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

Page **19** of **20**

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).