



NATIONAL INSTITUTE FOR COMMUNICABLE DISEASES

Division of the National Health Laboratory Service

Weekly respiratory pathogens report

Week 22 of 2023

Highlights

- 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. Influenza transmission is currently in the high level and impact is high with circulation increasing week on week.
- In 2023 to date, 566 influenza cases have been detected from all surveillance programmes, of which 83% (333/399) of those with typing information available were influenza A(H3N2). The majority of cases were reported from Gauteng (n=159), followed by Western Cape (n=155), North West (n=110), KwaZulu-Natal (n=74), Eastern Cape (n=30), Mpumalanga (n=36) and Free State (n=2) sentinel surveillance sites.
- In 2023 to date, 656 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, 122 cases of *Bordetella pertussis* were detected, of which 22% (27/122) were from Gauteng Province, 18% (22/122) Western Cape Province, 20% (25/122) North West Province, 18% (22/122) from Mpumalanga Province, 16% (20/122) from KwaZulu-Natal Province and 5% (6/122) from Eastern Cape Province.
- In 2023 to date, 225 COVID-19 cases were detected from all surveillance programmes. Of the 175 specimens sequenced, a variant could be assigned in 59% (104/175). Of these, 97% (101/104) were assigned the Omicron variant, of which 60% (61/101) were Omicron (23A/XBB.1.5), 14% (14/101) were Omicron (22E/BQ.1.1), 11% (11/101) each Omicron (22B/BA.5) and Omicron (22F/BA.2.10.1), 2% (2/101) Omicron (23B/XBB.1.16) and 1% (1/55) each Omicron (21K/BA.1) and Omicron(22D/BM.1.1). One (1%, 1/170) was assigned XAY, XBF and XBL each respectively, while for the remaining 41% (71/170), a variant could not be assigned 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*	KZ NW WC MP	EC FS GP LP MP NC NW WC	EC GP KZ MP NW WC
Type of site	Primary health care clinics	General practitioners	Public hospitals
Case definition	<p>ILI: An acute respiratory illness with a temperature ($\geq 38^{\circ}\text{C}$) and cough, & onset ≤ 10 days</p> <p>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:</p> <ul style="list-style-type: none"> • paroxysms of coughing, • or inspiratory "whoop", • or post-tussive vomiting • or apnoea in children < 1 year; <p>OR</p> <p>Any person in whom a clinician suspects pertussis</p> <p>Suspected SARS-CoV-2 Any person presenting with an acute (≤ 14 days) respiratory tract infection or other clinical illness compatible with COVID-19**</p>	<p>ILI: An acute respiratory illness with a temperature ($\geq 38^{\circ}\text{C}$) and cough, & onset ≤ 10 days</p> <p>Suspected SARS-CoV-2 Any person presenting with an acute (≤ 14 days) respiratory tract infection or other clinical illness compatible with COVID-19**</p>	<p>SRI: Acute (symptom onset ≤ 10 days) or chronic (symptom onset > 10) lower respiratory tract illness</p> <p>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:</p> <ul style="list-style-type: none"> • paroxysms of coughing, • or inspiratory "whoop", • or post-tussive vomiting • or apnoea in children < 1 year; <p>OR</p> <p>Any person in whom a clinician suspects pertussis.</p> <p>Suspected SARS-CoV-2 Any person admitted with a physician-diagnosis of suspected COVID-19 and not meeting SRI case definition.</p>
Specimens collected	Oropharyngeal & nasopharyngeal swabs	Throat and/or nasal swabs or Nasopharyngeal swabs	Oropharyngeal & nasopharyngeal swabs
Main pathogens tested***	INF RSV BP SARS-CoV-2	INF RSV SARS-CoV-2	INF RSV BP SARS-CoV-2
Testing Methods	<p>INF and RSV - Fast-Track Diagnostics multiplex real-time reverse transcription polymerase chain reaction (until 31 March 2021)</p> <p>B. pertussis Multiplex real-time PCR (Tatti <i>et al.</i>, <i>J Clin Microbiol</i> 2011) and culture (if PCR cycle threshold ≤ 25)</p> <p>SARS-CoV-2 1 April 2020 – 31 March 2021: Roche E gene real-time PCR assay (Corman <i>et al.</i>, <i>Euro Surv</i> 2020) 1 April 2021 to date: Allplex™ SARS-CoV-2/FluA/FluB/RSV PCR kit</p> <p>- positivity assigned if PCR cycle threshold is < 40 for ≥ 1 gene targets (N, S, OR RdRp)</p>	<p>INF and RSV - Fast-Track Diagnostics multiplex real-time reverse transcription polymerase chain reaction (until 31 March 2021)</p> <p>B. pertussis Multiplex real-time PCR (Tatti <i>et al.</i>, <i>J Clin Microbiol</i> 2011) and culture (if PCR cycle threshold ≤ 25)</p> <p>SARS-CoV-2 1 April 2020 – 31 March 2021: Roche E gene real-time PCR assay Corman <i>et al.</i>, <i>Euro Surv</i> 2020) 1 April 2021 to date: Allplex™ SARS-CoV-2/FluA/FluB/RSV PCR kit</p> <p>- positivity assigned if PCR cycle threshold is < 40 for ≥ 1 gene targets (N, S, OR RdRp)</p>	<p>INF and RSV - Fast Track Diagnostics multiplex real-time reverse transcription polymerase chain reaction (until 31 March 2021)</p> <p>B. pertussis Multiplex real-time PCR (Tatti <i>et al.</i>, <i>J Clin Microbiol</i> 2011) and culture (if PCR cycle threshold ≤ 25)</p> <p>SARS-CoV-2 1 April 2020 – 31 March 2021: Roche E gene real-time PCR assay (Corman <i>et al.</i>, <i>Euro Surv</i> 2020) 1 April 2021 to date: Allplex™ SARS-CoV-2/FluA/FluB/RSV PCR kit</p> <p>- positivity assigned if PCR cycle threshold is < 40 for ≥ 1 gene targets (N, S, OR RdRp)</p>

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 ANY 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 pertussis*; SARS-CoV-2: severe acute respiratory syndrome coronavirus 2

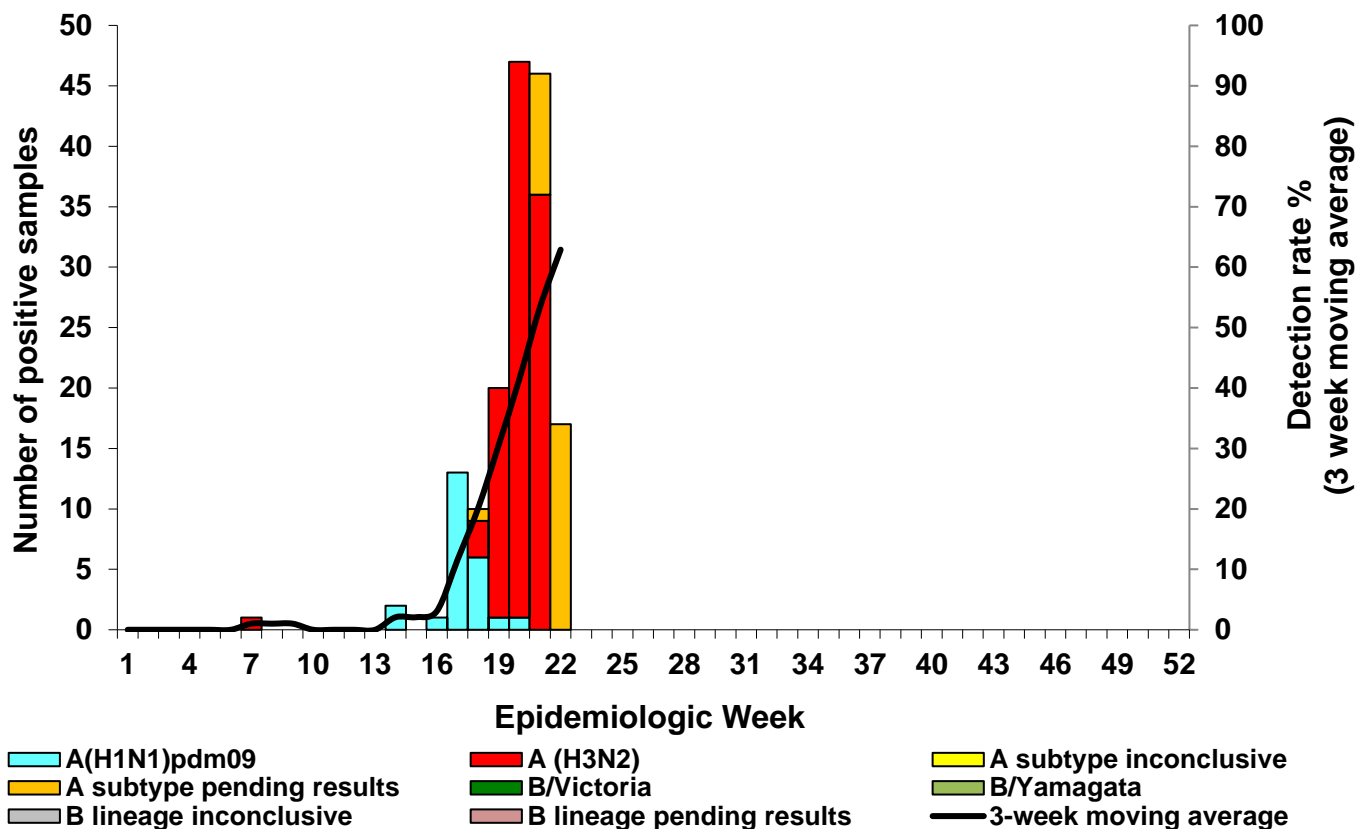


Figure 1. Number of influenza positive cases* by influenza subtype and lineage and 3-week moving average by week, influenza-like illness (ILI) surveillance in primary health care clinics, 01/01/2023 – 04/06/2023**

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

** Influenza was detected in 10/22, 45% 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 – 04/06/2023

Clinic (Province)	A(H1N1) pdm09	A(H3N2)	A subtype inconclusive*	A subtype pending results**	B/Victoria	B/Yamagata	B lineage inconclusive*	B lineage pending results**	Total samples
Agincourt (MP)	2	14	0	2	0	0	0	0	119
Eastridge (WC)	7	11	0	6	0	0	0	0	156
Edendale Gateway (KZ)	14	24	0	2	0	0	0	0	247
Jouberton (NW)	1	50	0	16	0	0	0	0	205
Mitchell's Plain (WC)	0	6	0	2	0	0	0	0	71
Total:	24	105	0	28	0	0	0	0	798

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

* Influenza was detected in 10/22, 45% 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

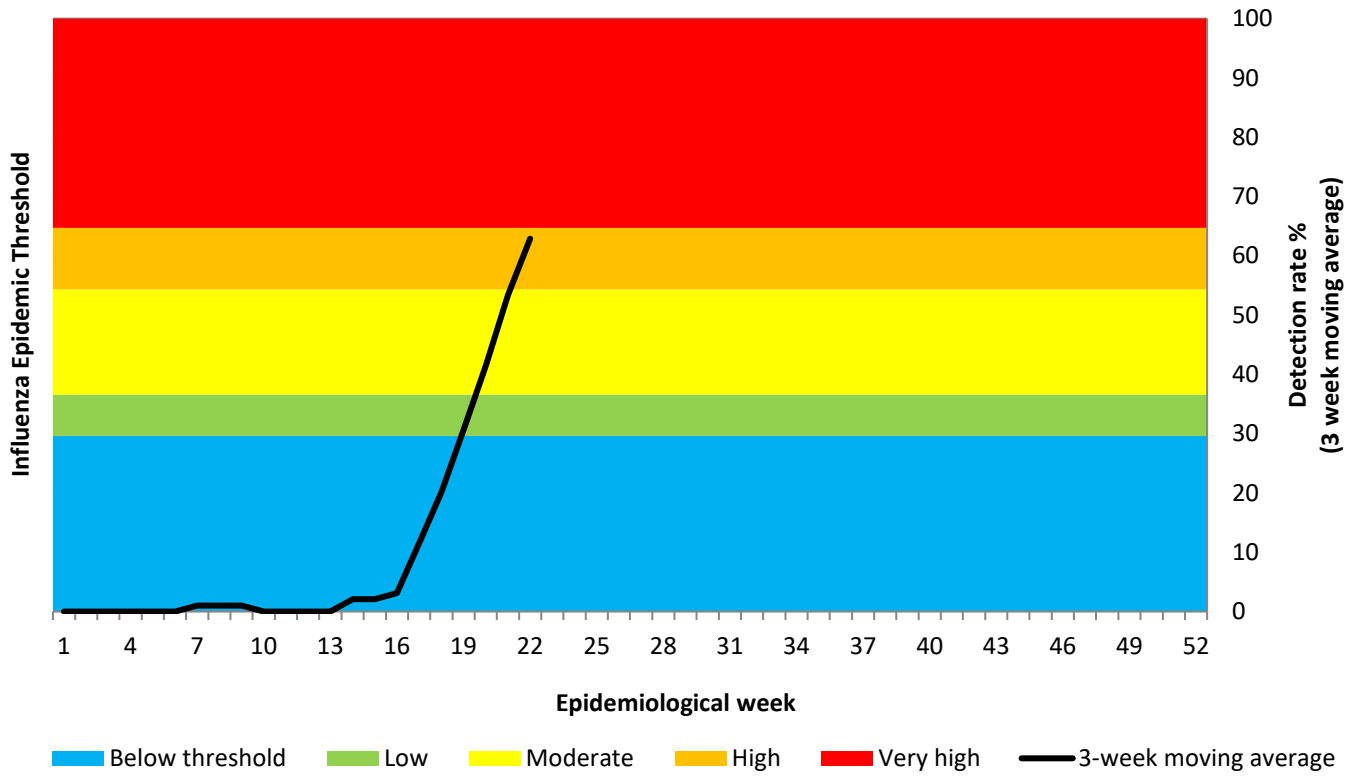


Figure 2. Influenza percentage detections and epidemic thresholds* among cases of all ages, influenza-like illness (ILI) surveillance in primary health care clinics, 01/01/2023 –04/06/2023

*Thresholds based on 2012-2019 data

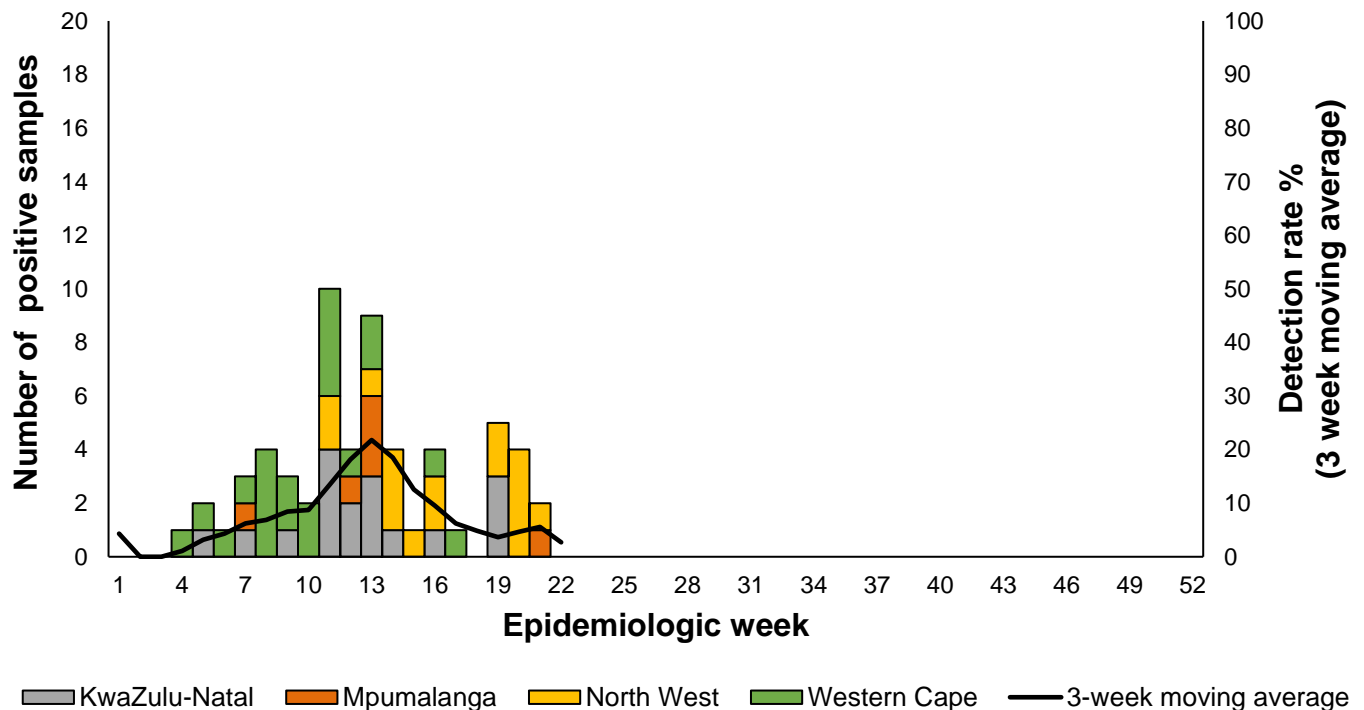


Figure 3. Number of patients testing positive for respiratory syncytial virus* by province and 3-week moving average by week, influenza-like illness (ILI) surveillance in primary health care clinics, 01/01/2023 –04/06/2023

*RSV was detected in 1/22, 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.

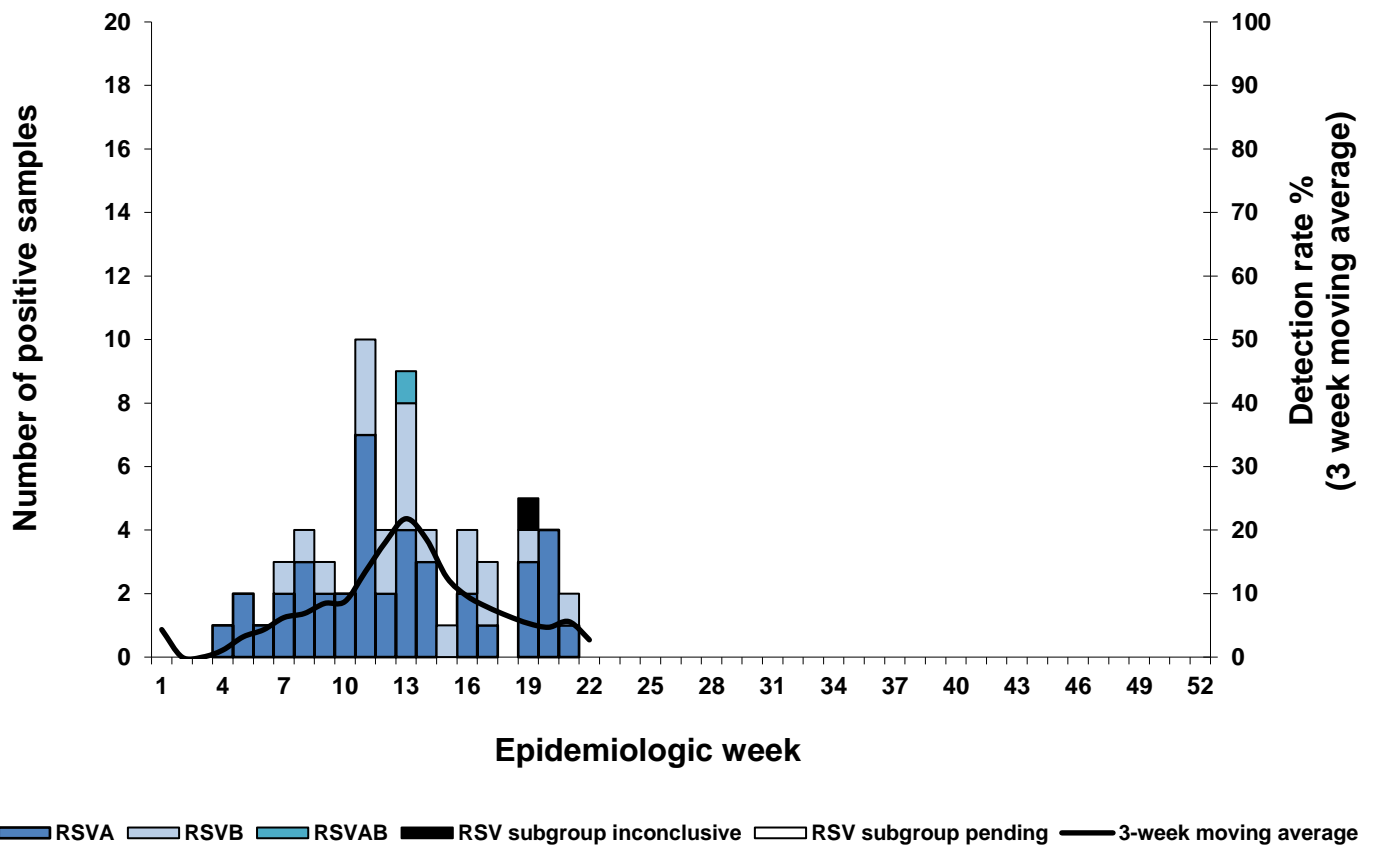


Figure 4. Number of patients testing positive for respiratory syncytial virus* by subgroup and 3-week moving average by week, influenza-like illness (ILI) surveillance in primary health care clinics, 01/01/2023 –04/06/2023

*RSV was detected in 1/22, 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 –04/06/2023

Clinic (Province)	RSVA	RSVB	RSVAB**	RSV subgroup inconclusive* **	RSV subgroup pending** **	Total samples
Agincourt (MP)	2	3	1	0	0	119
Eastridge (WC)	18	2	0	0	0	156
Edendale Gateway (KZ)	7	11	0	1	0	247
Jouberton (NW)	12	4	0	0	4	205
Mitchell's Plain (WC)	1	0	0	0	0	71
Total	40	20	1	1	4	798

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

*RSV was detected in 1/22, 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

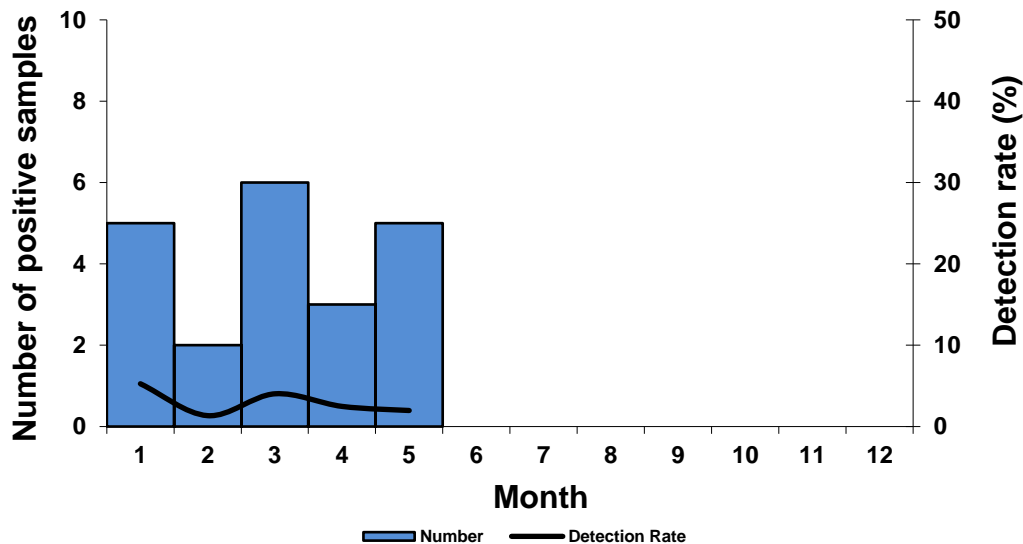


Figure 5. Number of patients testing positive for *B. pertussis and detection rate by month, influenza-like illness (ILI) surveillance primary health care clinics**, 01/01/2023 –04/06/2023**

**B. pertussis* was detected in 1/22, 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 –04/06/2023**

Clinic (Province)	<i>B. pertussis</i> Positive	Total samples
Agincourt (MP)	4	119
Eastridge (WC)	2	149
Edendale Gateway (KZ)	6	239
Jouberton (NW)	9	193
Mitchell's Plain (WC)	0	67
Total:	21	767

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

**B. pertussis* was detected in 1/22, 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 49 samples.

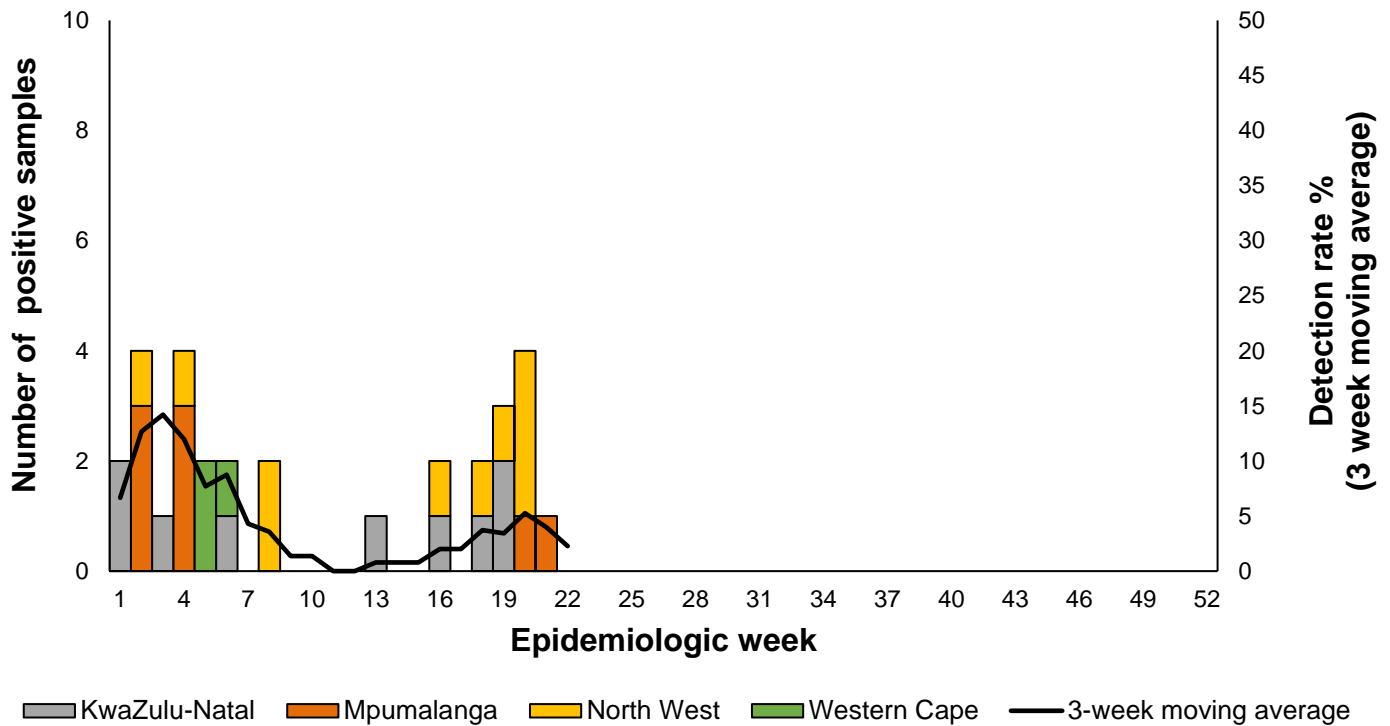


Figure 6. Number of patients* testing positive for SARS-CoV-2 by province and 3-week moving average by week, influenza-like illness (ILI) surveillance in primary health care clinics, 01/01/2023 –04/06/2023**

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

**SARS-CoV-2 was not detected in 22 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 –04/06/2023

Clinic (Province)	SARS-CoV-2 positive	Total samples tested
Agincourt (MP)	8	119
Eastridge (WC)	1	156
Edendale Gateway (KZ)	9	247
Jouberton (NW)	10	205
Mitchell’s Plain (WC)	2	71
Total:	30	798

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

*SARS-CoV-2 was not detected in 22 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.

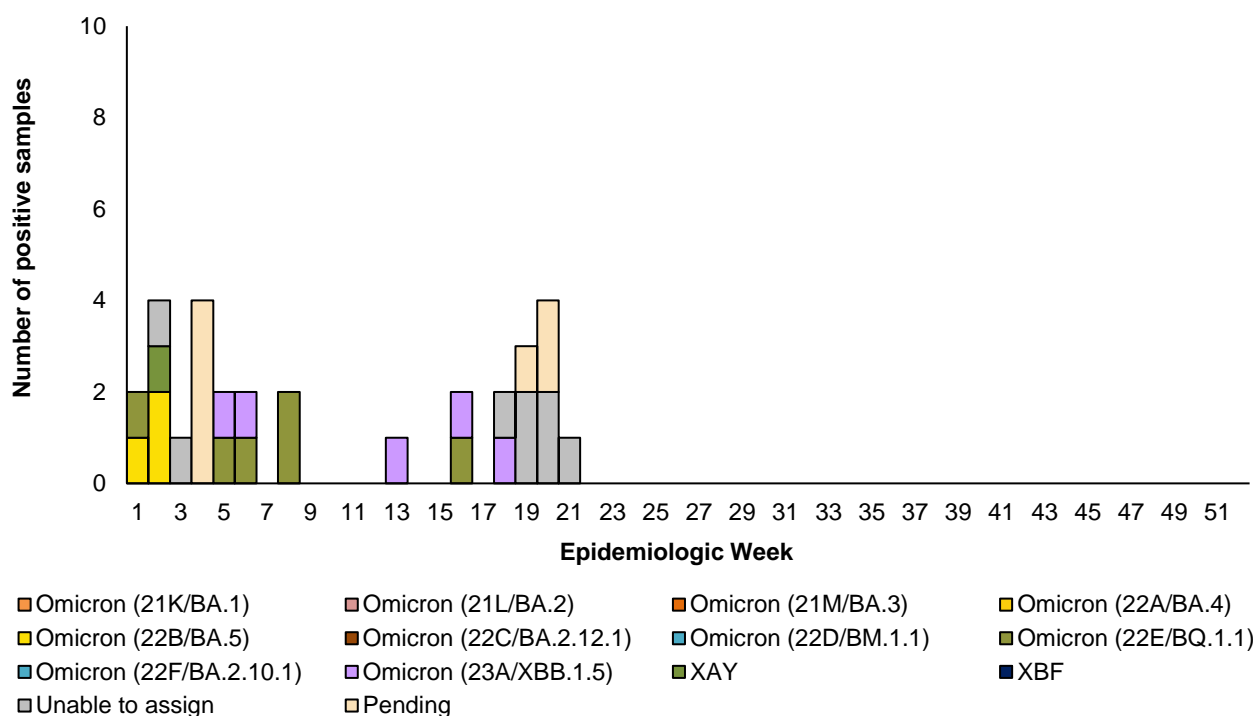


Figure 7. Number of laboratory-confirmed SARS-CoV-2* cases by variant type (variant PCR/sequencing) and week, influenza-like illness (ILI) surveillance in primary health care clinics, 01/01/2023-04/06/2023

*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 ($C_t \geq 35$) **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, 01/01/2023-04/06/2023**

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/BA.2.10.1)	Omicron (23A/XBB.1.5)	XAY	Unable to assign**	Pending***	SARS-CoV-2 positive	Total samples tested
Agincourt Clinic (MP)	0	0	0	2	0	0	0	0	0	1	1	4	8	119
Eastridge Clinic (WC)	0	0	0	0	0	0	0	0	1	0	0	0	1	156
Edendale Clinic (KZ)	0	0	0	1	0	0	3	0	2	0	2	1	9	266
Jouberton Clinic (NW)	0	0	0	0	0	0	2	0	1	0	5	2	10	208
Mitchell's Plain Clinic (WC)	0	0	0	0	0	0	1	0	1	0	0	0	2	71
Total:	0	0	0	3	0	0	6	0	5	1	8	7	30	820

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 ($C_t \geq 35$) **OR** variant PCR could not assign variant and no sequencing result
Pending: outstanding variant results

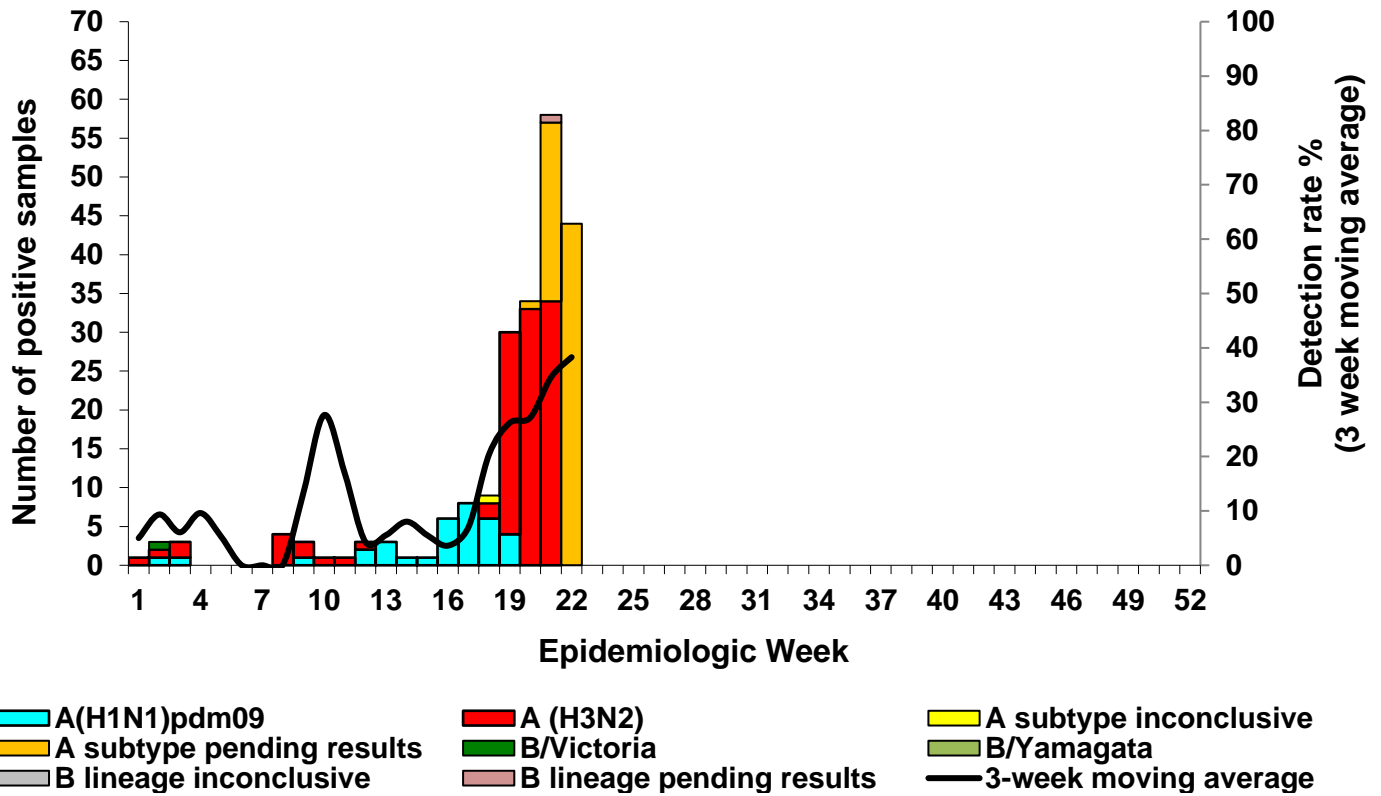


Figure 8. Number of positive patients* by influenza subtype and lineage and 3-week moving average by week, ILI surveillance - Viral Watch, 01/01/2023-04/06/2023

*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

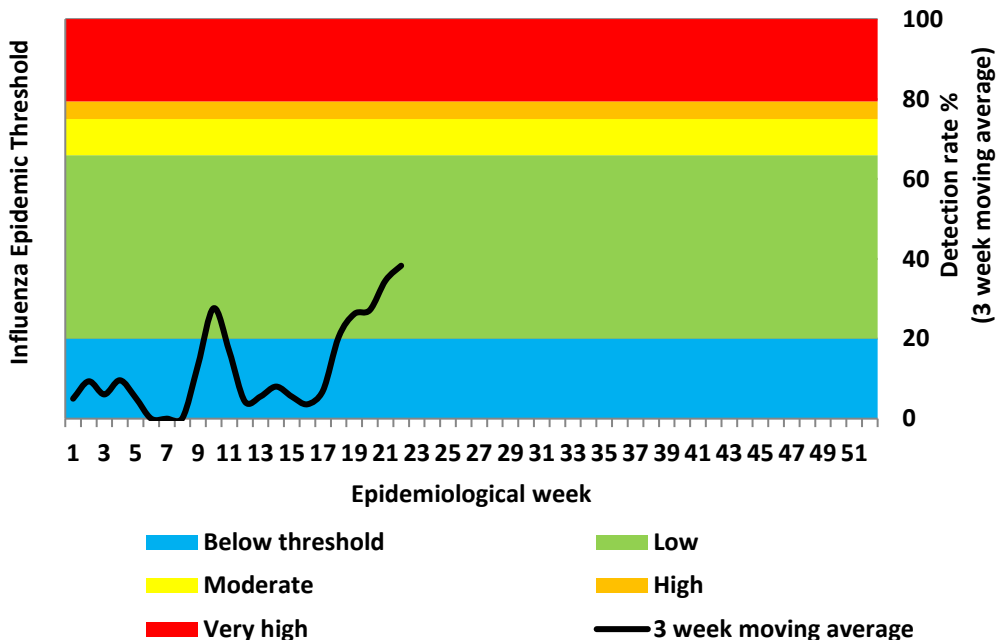


Figure 8b. Influenza percentage detections and epidemic thresholds* among cases of all ages, ILI surveillance - Viral Watch, 01/01/2023-04/06/2023

*Thresholds based on 2015-2019 data

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, 01/01/2023-04/06/2023

Province	A(H1N1) pdm09	A(H3N2)	A subtype inconclusive	A subtype pending results*	B/Victoria	B/Yamagata	B lineage inconclusive	B lineage pending results*	Total samples
Eastern Cape	4	6	0	4	0	0	0	0	27
Free State	0	2	0	0	0	0	0	0	2
Gauteng	18	43	0	19	1	0	0	0	392
Limpopo	0	0	0	0	0	0	0	0	0
Mpumalanga	0	7	1	7	0	0	0	0	23
North West	1	1	0	1	0	0	0	0	3
Northern Cape	0	0	0	0	0	0	0	0	0
Western Cape	11	49	0	37	0	0	0	1	179
Total:	34	108	1	68	1	0	0	1	626

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

**Influenza A subtype or B lineage results are pending

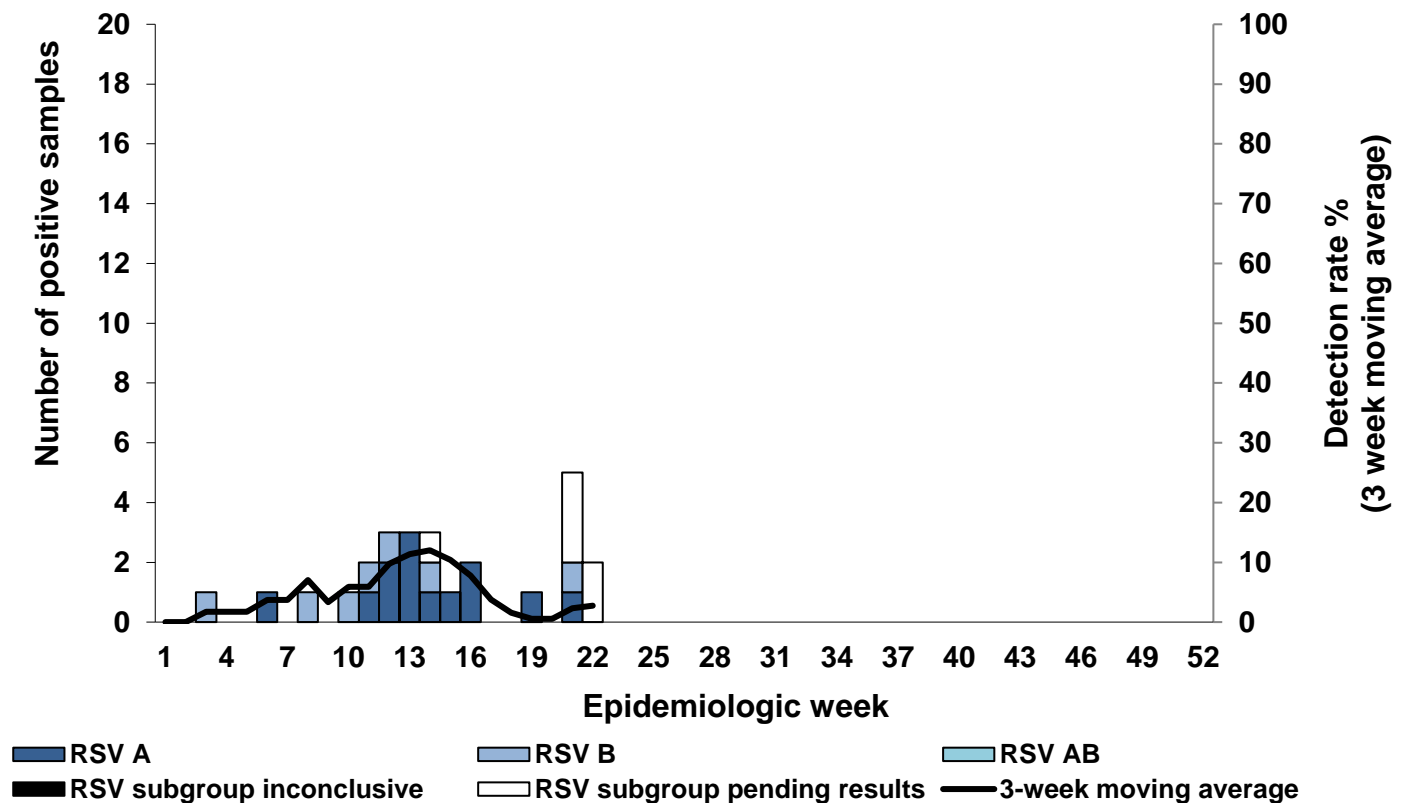


Figure 9. Number of RSV positive cases testing positive for respiratory syncytial virus (RSV)* by subgroup and 3-week moving average by week, ILI surveillance - Viral Watch, 01/01/2023-04/06/2023

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

Table 7. Number of RSV positive cases identified and total number of samples tested by province, ILI surveillance - Viral Watch, 01/01/2023-04/06/2023

Province	RSV A	RSV B	RSV AB*	RSV subgroup inconclusive**	RSV subgroup pending results***	Total samples tested
Eastern Cape	0	1	0	0	0	27
Free State	0	0	0	0	0	2
Gauteng	9	4	0	0	3	392
Limpopo	0	0	0	0	0	0
Mpumalanga	0	0	0	0	0	23
North West	0	0	0	0	0	3
Northern Cape	0	0	0	0	0	0
Western Cape	4	2	0	0	3	179
Total:	13	7	0	0	6	626

*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

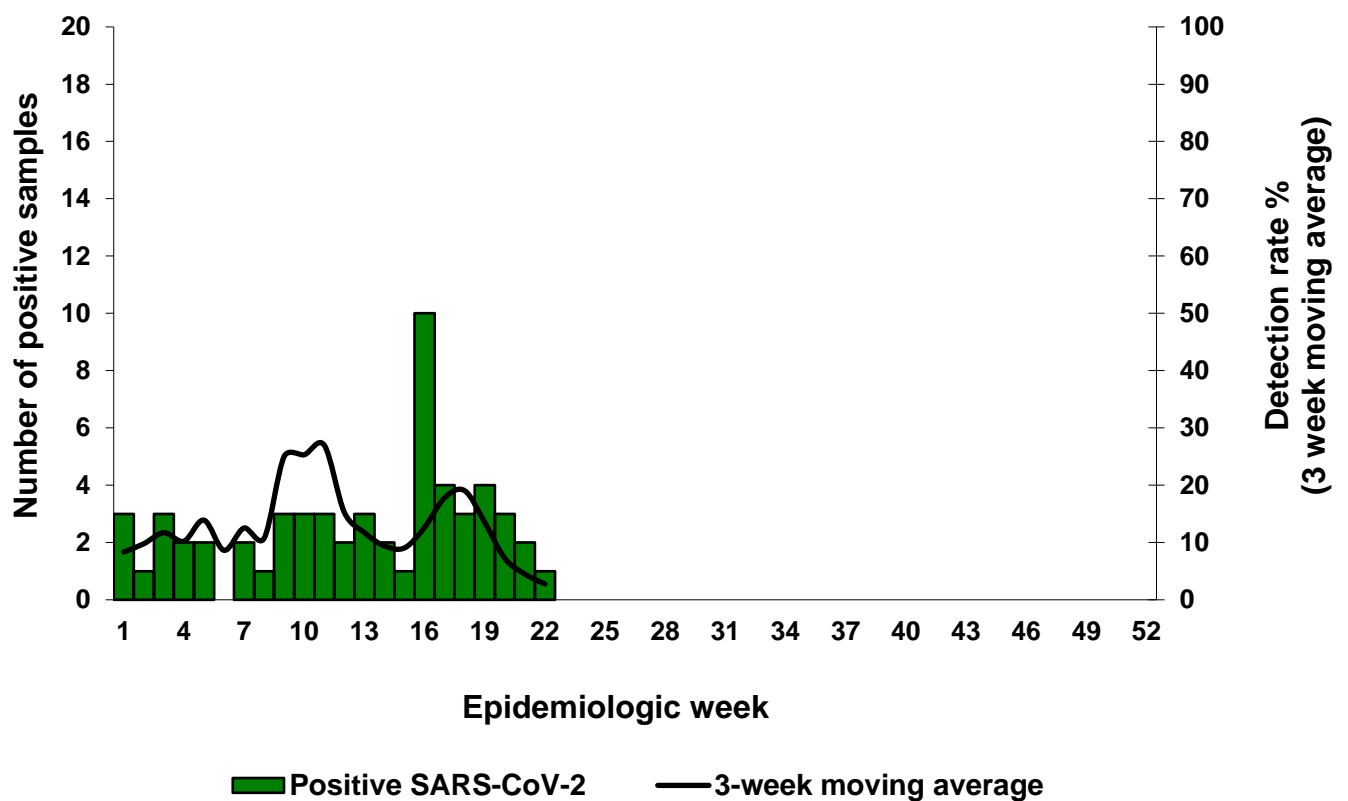


Figure 10. Number of patients testing positive for SARS-CoV-2*, by site and 3-week moving average by week, ILI surveillance - Viral Watch, 01/01/2023-04/06/2023**

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

Table 8. Number of SARS-CoV-2 positive cases identified and total number tested by province, ILI surveillance - Viral Watch, 01/01/2023-04/06/2023

Province	SARS-CoV-2 positive	Total samples tested
Eastern Cape	2	27
Free State	0	2
Gauteng	38	392
Limpopo	0	0
Mpumalanga	2	23
North West	0	3
Northern Cape	0	0
Western Cape	16	179
Total:	58	626

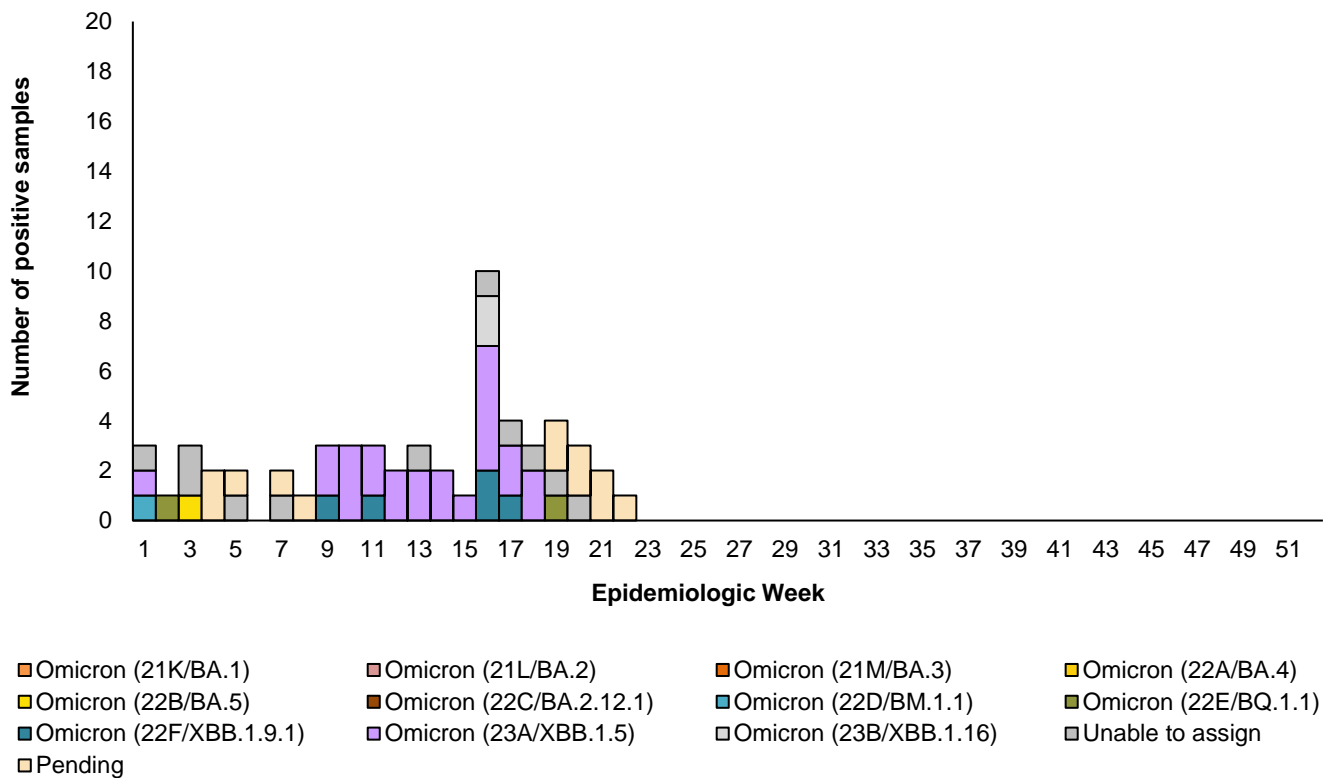


Table 9. Number of SARS-CoV-2* positive cases by variant (variant PCR and/or sequencing) identified and total number of samples tested by province, ILI surveillance - Viral Watch, 01/01/2023-04/06/2023

Clinic (Province)	Omicron (21I/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 Cape	0	0	0	0	0	0	0	0	2	0	0	0	2	27
Free State	0	0	0	0	0	0	0	0	0	0	0	0	0	2
Gauteng	0	0	0	1	0	1	2	4	18	2	4	6	38	392
Limpopo	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Mpumalanga	0	0	0	0	0	0	0	0	1	0	1	0	2	23
North West	0	0	0	0	0	0	0	0	0	0	0	0	0	3
Northern Cape	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Western Cape	0	0	0	0	0	0	0	1	3	0	6	6	16	179
Total:	0	0	0	1	0	1	2	5	24	2	11	12	58	626

*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 ($C_{t} \geq 35$) OR variant PCR could not assign variant and no sequencing result
 Pending: outstanding variant results

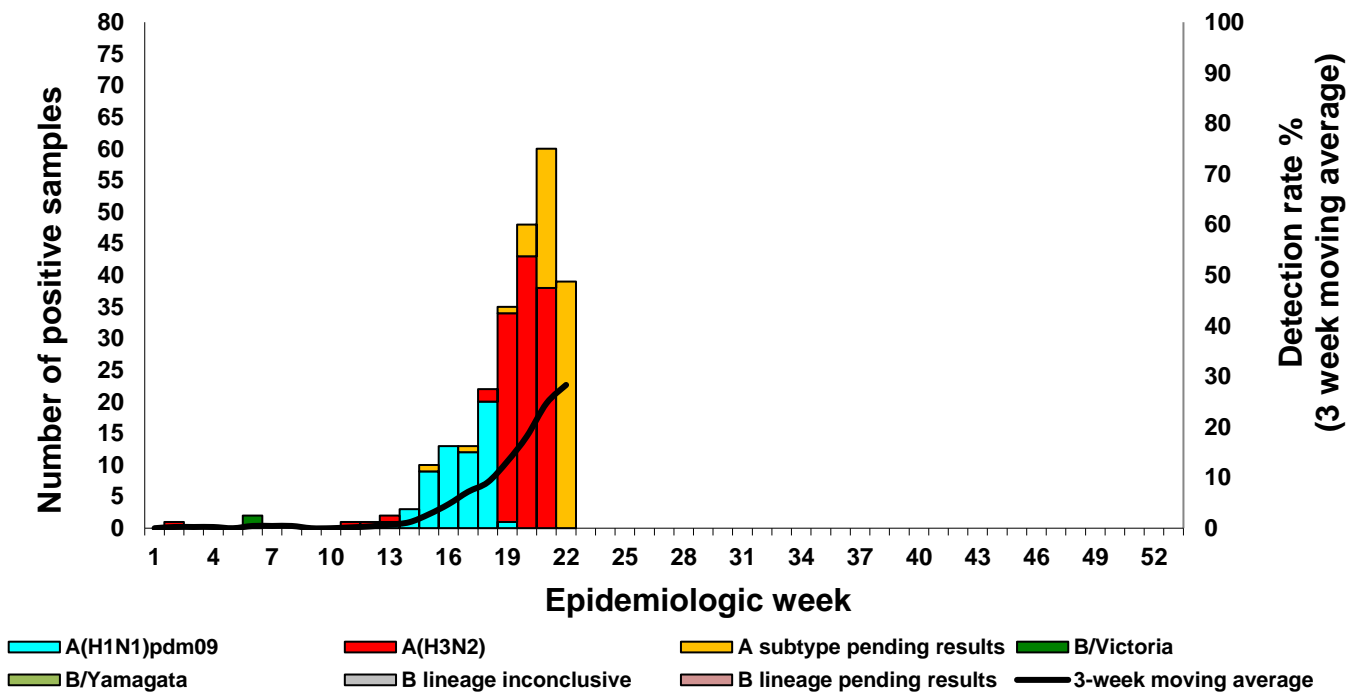


Figure 12. Number of positive influenza positive cases* by influenza subtype and lineage and 3-week moving average by week, pneumonia surveillance public hospitals, 01/01/2023-04/06/2023**

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.

Table 10. Number of laboratory confirmed influenza cases by subtype and lineage* and total number of samples tested by hospital, pneumonia surveillance public hospitals, 01/01/2023-04/06/2023

Hospital (Province)	A(H1N1)pd m09	A(H3N2)	A subtype inconclusive	A subtype pending results***	B/Victoria	B/Yamagata	B lineage inconclusive	B lineage pending results***	Total samples
Edendale (KZ)	5	18	0	1	1	0	0	0	369
Helen Joseph-Rahima Moosa (GP)	23	11	0	15	0	0	0	0	607
Khayelitsha (WC)	2	13	0	10	1	0	0	0	320
Klerksdorp-Tshepong (NW)	2	27	0	11	0	0	0	0	266
Livingstone (EC)	10	10	0	0	0	0	0	0	352
Mapulaneng-Matikwana (MP)	0	2	0	6	0	0	0	0	225
Mitchell's Plain (WC)	0	6	0	5	0	0	0	0	260
Red Cross (WC)	4	6	0	6	0	0	0	0	508
Tambo Memorial (GP)	5	11	0	9	0	0	0	0	235
Tembisa (GP)	8	10	0	2	0	0	0	0	234
Tintswalo (MP)	0	2	0	0	0	0	0	0	146
Tygerberg (WC)	0	4	0	4	0	0	0	0	85
Total:	59	120	0	69	2	0	0	0	3607

* 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

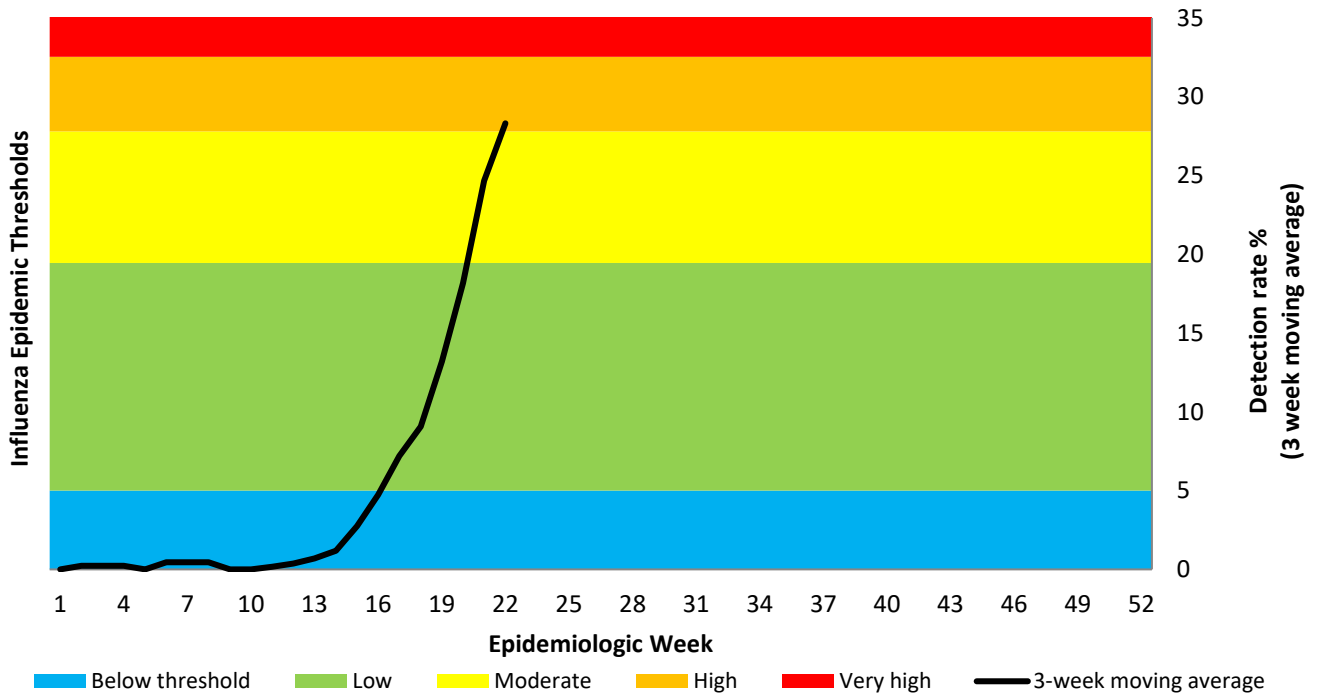


Figure 13. Influenza percentage detections and epidemic thresholds* among cases of all ages, pneumonia surveillance public hospitals, 01/01/2023-04/06/2023

*Thresholds based on 2010-2019 data

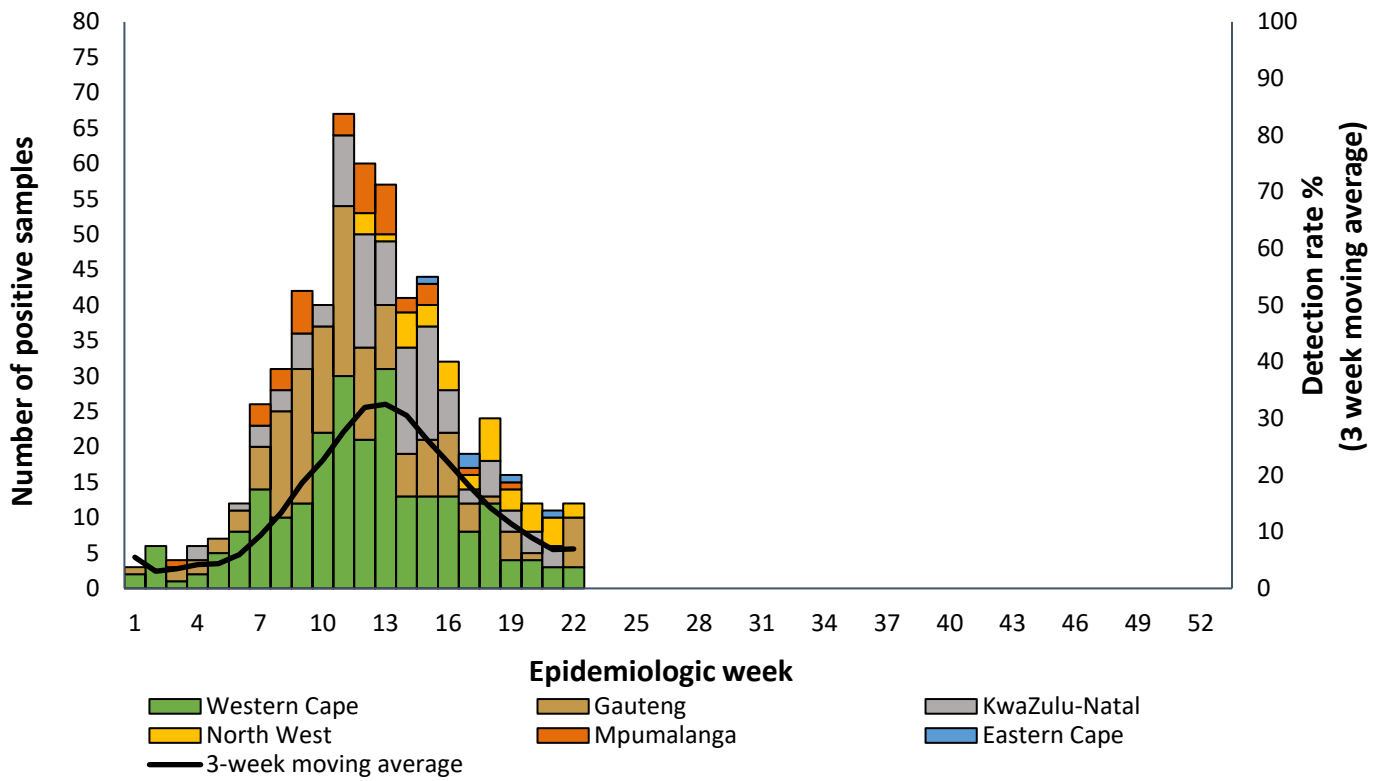


Figure 14. Number of patients (all ages) testing positive for respiratory syncytial virus* by province and 3-week moving average by week, pneumonia surveillance public hospitals, 01/01/2023-04/06/2023

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.

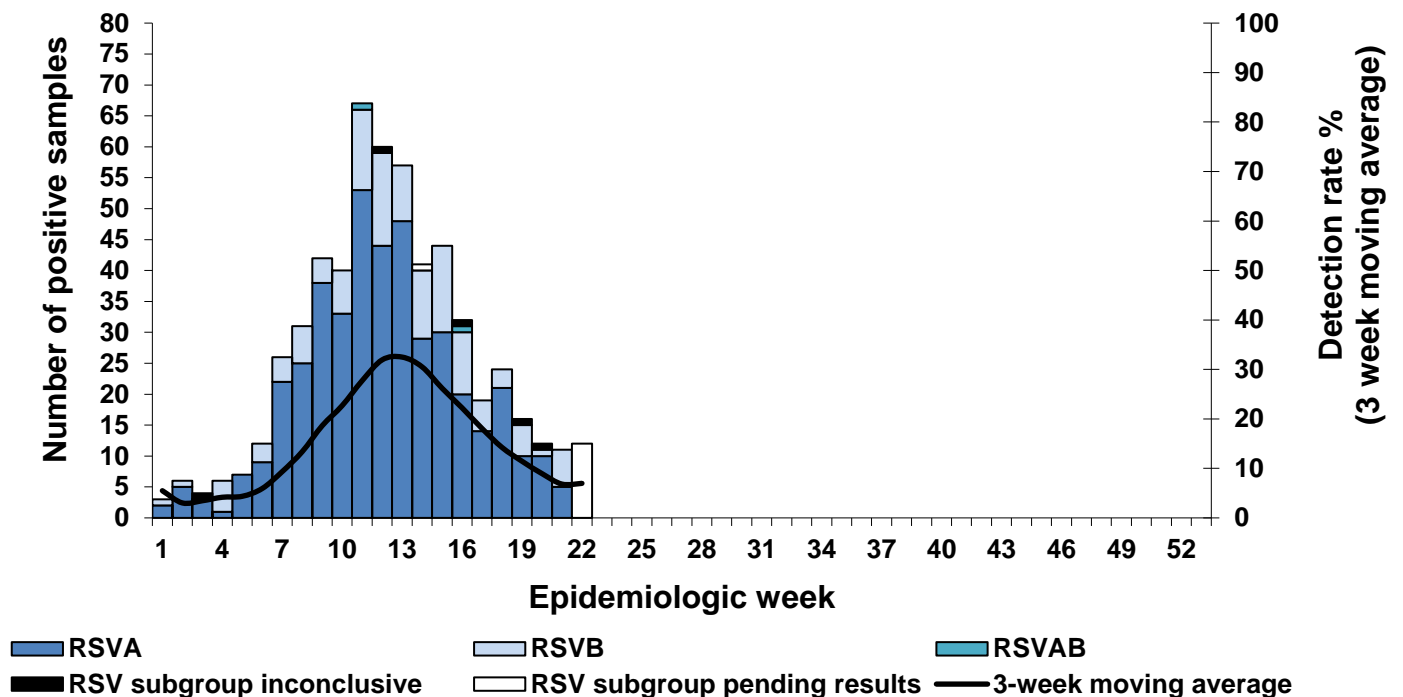


Figure 15. Number of patients (all ages) testing positive for respiratory syncytial virus* by subgroup and 3-week moving average by week, pneumonia surveillance public hospitals, 01/01/2023-04/06/2023

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

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, 01/01/2023-04/06/2023

Hospital (Province)	RSVA	RSVB	RSVAB**	RSV subgroup inconclusive** *	RSV subgroup pending** **	Total samples
Edendale (KZ)	35	68	1	1	0	369
Helen Joseph-Rahima Moosa (GP)	129	9	0	1	5	607
Khayelitsha (WC)	4	3	0	0	1	320
Klerksdorp-Tshepong (NW)	32	2	0	1	2	266
Livingstone (EC)	3	2	0	0	0	352
Mapulaneng-Matikwana (MP)	7	5	1	0	0	225
Mitchell's Plain (WC)	62	9	0	0	1	260
Red Cross (WC)	129	23	0	2	1	508
Tambo Memorial (GP)	2	0	0	0	0	235
Tembisa (GP)	1	1	0	0	3	234
Tintswalo (MP)	24	0	0	0	0	146
Tygerberg (WC)	1	1	0	0	0	85
Total:	429	123	2	5	13	3607

*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

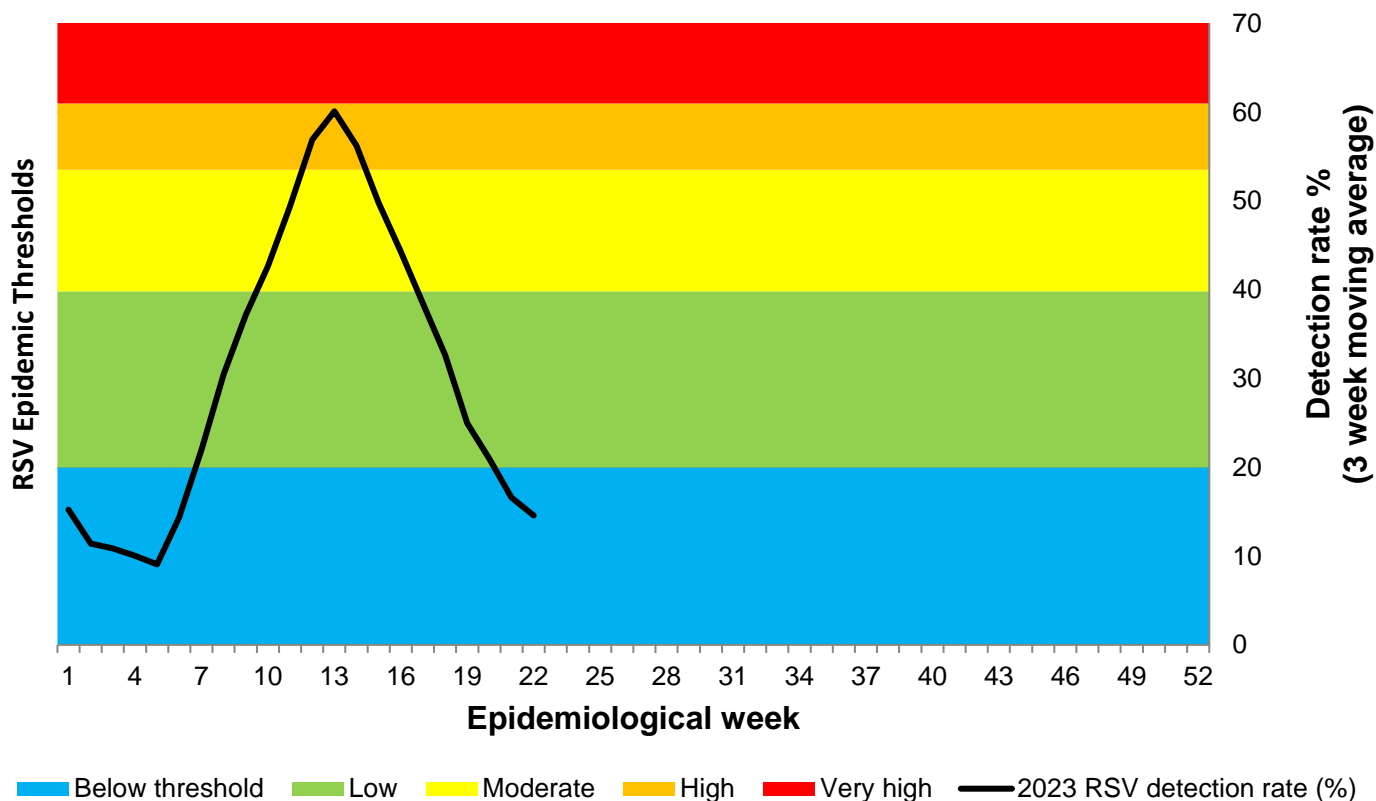


Figure 16. RSV percentage 3-week moving average and epidemic thresholds* among children aged < 5 years, pneumonia surveillance public hospitals, 01/01/2023-04/06/2023

*Thresholds based on 2010-2019 data

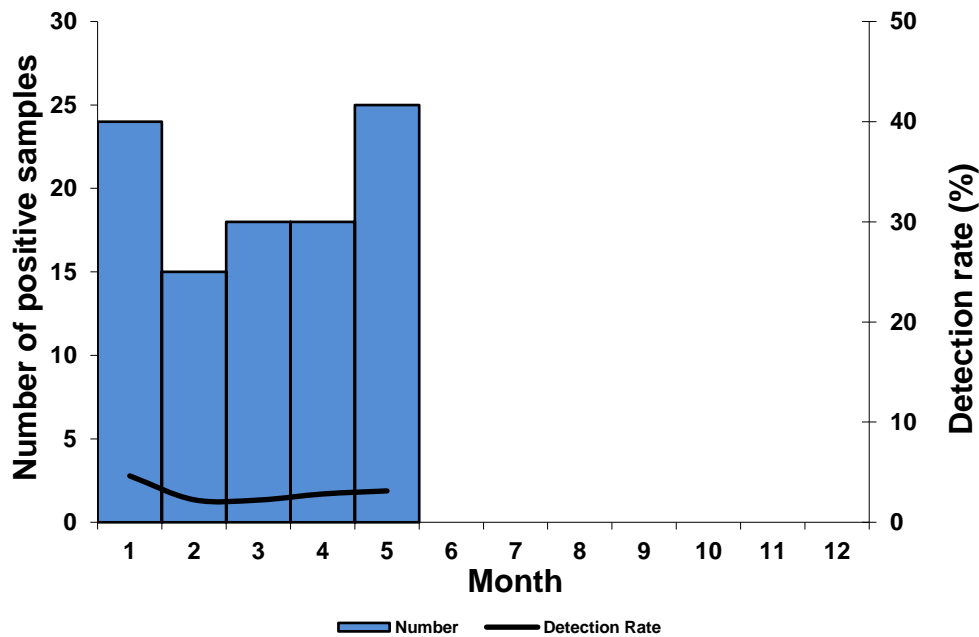


Figure 17. Number of patients testing positive for *B. pertussis and 3-week moving average by month, pneumonia surveillance public hospitals**, 01/01/2023-04/06/2023**

*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, 01/01/2023-04/06/2023**

Hospital (Province)	<i>B. pertussis</i> Positive	Total samples
Edendale (KZ)	14	358
Helen Joseph-Rahima Moosa (GP)	17	585
Khayelitsha (WC)	3	289
Klerksdorp-Tshepong(NW)	15	255
Livingstone (EC)	6	334
Mapulaneng-Matikwana (MP)	15	216
Mitchell's Plain (WC)	2	243
Red Cross (WC)	13	491
Tambo Memorial (GP)	5	216
Tembisa (GP)	5	218
Tintswalo (MP)	3	144
Tygerberg (WC)	2	82
Total:	100	3431

*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.

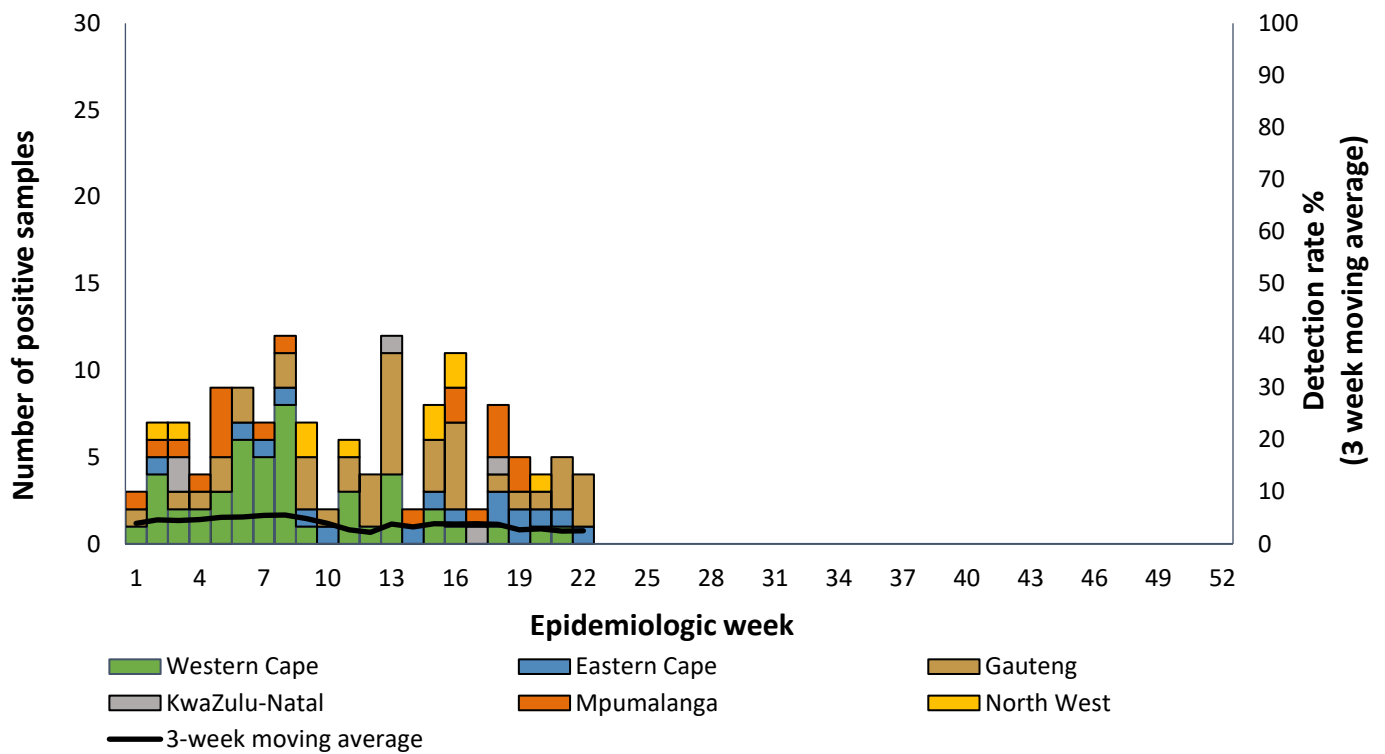


Figure 18. Number of patients testing positive for SARS-CoV-2 by province and 3-week moving average by week, pneumonia surveillance public hospitals, 01/01/2023-04/06/2023**

*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, 01/01/2023-04/06/2023

Hospital (Province)	SARS-CoV-2 positive	Total samples tested
Edendale (KZ)	5	369
Helen Joseph-Rahima Moosa (GP)	17	607
Khayelitsha (WC)	16	320
Klerksdorp-Tshepong (NW)	10	266
Livingstone (EC)	16	352
Mapulaneng-Matikwana (MP)	14	225
Mitchell's Plain (WC)	15	260
Red Cross (WC)	12	508
Tambo Memorial (GP)	15	235
Tembisa (GP)	10	234
Tintswalo (MP)	5	146
Tygerberg (WC)	3	85
Total:	138	3607

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

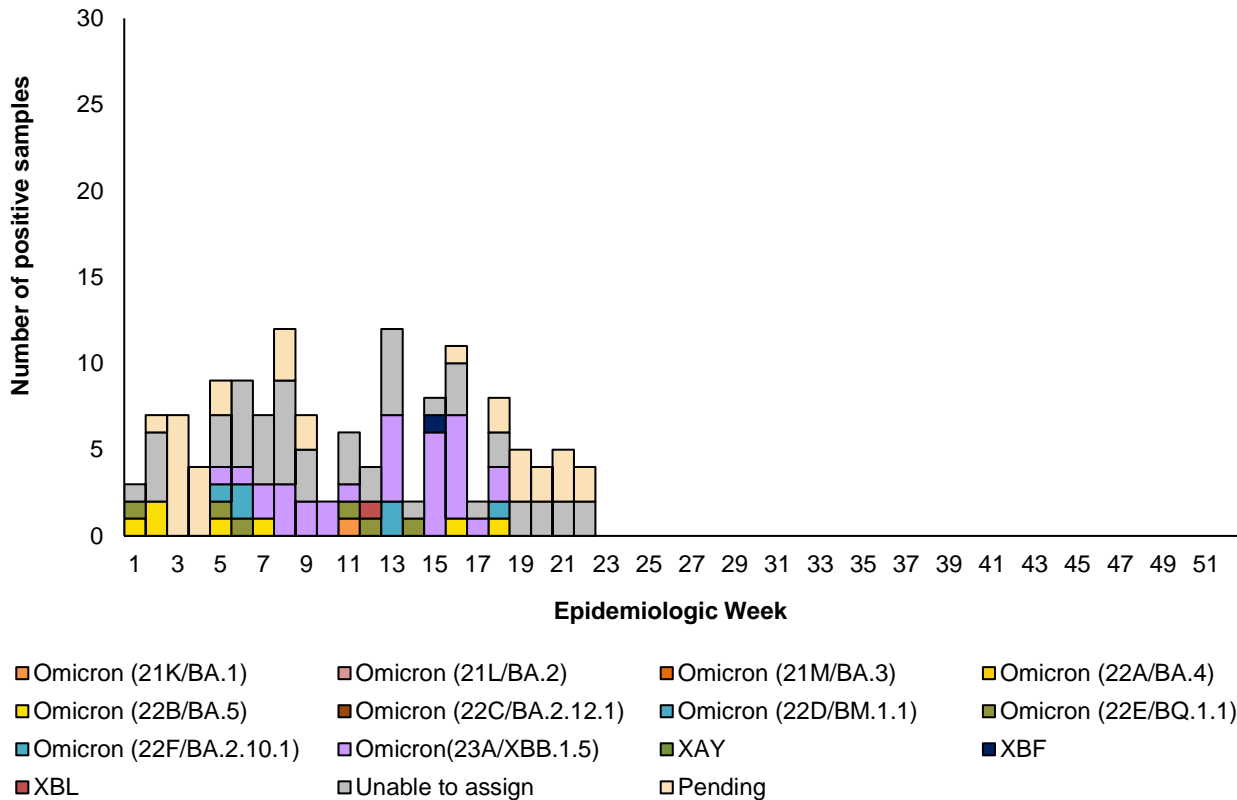


Figure 19. Number and 3-week moving average of laboratory-confirmed SARS-CoV-2 cases* by variant type (variant PCR/sequencing), pneumonia surveillance public hospitals, 01/01/2023-04/06/2023

*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 ($C_t \geq 35$) **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, 01/01/2023-04/06/2023

Hospital (Province)	Omicron (21K/BA.1)	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/BA.2.10.1)	Omicron(23A/XBB.1.5)	XBF	XBL	Unable to assign**	Pending***	Total SARS-CoV-2 positive	Total samples tested
Edendale (KZ)	0	0	0	0	0	0	1	1	0	0	1	2	5	369
Helen Joseph- Rahima Moosa (GP)	1	0	0	0	0	1	2	8	0	0	3	2	17	607
Khayelitsha (WC)	0	0	0	0	0	0	0	4	0	0	9	3	16	320
Klerksdorp- Tshepong (NW)	0	0	1	0	0	0	0	4	0	0	2	3	10	266
Livingstone (EC)	0	0	1	0	0	0	0	1	1	0	7	6	16	352
Mapulaneng- Matikwana (MP)	0	0	1	0	0	1	0	1	0	0	6	5	14	225
Mitchell's Plain (WC)	0	0	1	0	0	0	2	4	0	0	6	2	15	260
Red Cross (WC)	0	0	3	0	0	0	1	1	0	1	4	2	12	508
Tambo Memorial (GP)	0	0	0	0	0	3	0	7	0	0	3	2	15	235
Tembisa (GP)	0	0	0	0	0	1	0	0	0	0	6	3	10	234
Tintswalo (MP)	0	0	0	0	0	0	0	0	0	0	4	1	5	146
Tygerberg (WC)	0	0	0	0	0	0	0	1	0	0	1	1	3	85
Total:	1	0	7	0	0	6	6	32	1	1	52	32	138	3607

*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 ($C_t \geq 35$) **OR** variant PCR could not assign variant and no sequencing result
Pending: outstanding variant results

Data are provisional as reported to date (Data for this report drawn on 06/06/2023). 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.

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 Allplex™ 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

Allplex™ 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.

Allplex™ 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, (<https://sars-cov-2.exatype.com/>). 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, (<http://ormbunkar.se/aliview/>) (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 (<https://www.gisaid.org/>) (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 (<https://github.com/hCoV-2019/pangolin>) (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 (<https://nextstrain.org/>), a tool built for real-time tracking of the pathogen evolution (Hadfield et al., 2018).