

# MONTHLY RESPIRATORY PATHOGENS SURVEILLANCE REPORT

SOUTH AFRICA WEEK 13 2022

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

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## HIGHLIGHTS

- In 2022 to date, 32 sporadic influenza cases have been detected from Gauteng (n=2), Western Cape (n=1), Kwa-Zulu Natal (n=21) and Mpumalanga (n=8) sentinel surveillance sites.

- The 2022 RSV season started in week7 (week starting 14 February 2022) when RSV detection rate among children under five years of age in pneumonia surveillance rose above the seasonal threshold, as determined by the Moving Epidemic Method. In week 13 RSV detection rate among children aged < 5 years reached high threshold.

- In 2022 to date, a total of 253 COVID-19 cases were detected from all surveillance programmes. In week 13, a decline in detection rate of COVID-19 cases has been noted in pneumonia surveillance, whereas an increase was noted in Influenza-like illness (ILI) programme compared to week12. Of the 158 hospitalised COVID-19 cases reported with available data on outcome, 11 (7%) died.

- Of the 215/233 (92%) with variant data from ILI and pneumonia surveillance programmes, Omicron variant dominated, 52% (111/215), <1% (1/215) was Alpha variant and for 48% (103/215) variant was not assigned.

## CUMULATIVE DATA FROM



# INFLUENZA, RESPIRATORY SYNCYTIAL VIRUS AND SARS-CoV-2 SURVEILLANCE REPORT

WEEK 13 2022

## 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	GP KZ MP NW WC
Type of site	Primary health care clinics	General practitioners	Public hospitals
Case definition	<p><b>ILI:</b> An acute respiratory illness with a temperature (<math>\geq 38^{\circ}\text{C}</math>) and cough, &amp; onset <math>\leq 10</math> days</p> <p><b>Suspected pertussis</b> Any person with an acute cough illness lasting <math>\geq 14</math> days (or cough illness of any duration for children <math>&lt; 1</math> year), without a more likely diagnosis AND one or more of the following signs or symptoms:</p> <ul style="list-style-type: none"> <li>• paroxysms of coughing,</li> <li>• or inspiratory “whoop”,</li> <li>• or post-tussive vomiting</li> <li>• or apnoea in children <math>&lt; 1</math> year;</li> </ul> <p>OR</p> <p>Any person in whom a clinician suspects pertussis</p> <p><b>Suspected SARS-CoV-2</b> Any person presenting with an acute (<math>\leq 14</math> days) respiratory tract infection or other clinical illness compatible with COVID-19<sup>§</sup></p>	<p><b>ILI:</b> An acute respiratory illness with a temperature (<math>\geq 38^{\circ}\text{C}</math>) and cough, &amp; onset <math>\leq 10</math> days</p> <p><b>Suspected SARS-CoV-2</b> Any person presenting with an acute (<math>\leq 14</math> days) respiratory tract infection or other clinical illness compatible with COVID-19<sup>§</sup></p>	<p><b>SRI:</b> Acute (symptom onset <math>\leq 10</math> days) or chronic (symptom onset <math>&gt; 10</math>) lower respiratory tract infection</p> <p><b>Suspected pertussis</b> Any person with an acute cough illness lasting <math>\geq 14</math> days (or cough illness of any duration for children <math>&lt; 1</math> year), without a more likely diagnosis AND one or more of the following signs or symptoms:</p> <ul style="list-style-type: none"> <li>• paroxysms of coughing,</li> <li>• or inspiratory “whoop”,</li> <li>• or post-tussive vomiting</li> <li>• or apnoea in children <math>&lt; 1</math> year;</li> </ul> <p>OR</p> <p>Any person in whom a clinician suspects pertussis.</p> <p><b>Suspected SARS-CoV-2</b> 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 BP SARS-CoV-2	INF RSV BP SARS-CoV-2
Testing Methods	<p><b>INF and RSV</b> - Fast-Track Diagnostics multiplex real-time reverse transcription polymerase chain reaction (until 31 March 2021)</p> <p><b>B. pertussis</b> Multiplex real-time PCR (Tatti et al., <i>J Clin Microbiol</i> 2011) and culture (if PCR cycle threshold <math>\leq 25</math>)</p> <p><b>SARS-CoV-2</b> 1 April 2020 – 31 March 2021: Roche E gene real-time PCR essay (Corman et al., <i>Euro Surv</i> 2020) 1 April 2021 to date: Allplex™ SARS-CoV-2/FluA/FluB/RSV PCR kit</p> <ul style="list-style-type: none"> <li>• positivity assigned if PCR cycle threshold is <math>&lt; 40</math> for <math>\geq 1</math> gene targets (N, S, OR RdRp)</li> </ul>	<p><b>INF and RSV</b> - Fast-Track Diagnostics multiplex real-time reverse transcription polymerase chain reaction (until 31 March 2021)</p> <p><b>B. pertussis</b> Multiplex real-time PCR (Tatti et al., <i>J Clin Microbiol</i> 2011) and culture (if PCR cycle threshold <math>\leq 25</math>)</p> <p><b>SARS-CoV-2</b> 1 April 2020 – 31 March 2021: Roche E gene real-time PCR essay (Corman et al., <i>Euro Surv</i> 2020) 1 April 2021 to date: Allplex™ SARS-CoV-2/FluA/FluB/RSV PCR kit</p> <ul style="list-style-type: none"> <li>• positivity assigned if PCR cycle threshold is <math>&lt; 40</math> for <math>\geq 1</math> gene targets (N, S, OR RdRp)</li> </ul>	<p><b>INF and RSV</b> - Fast Track Diagnostics multiplex real-time reverse transcription polymerase chain reaction (until 31 March 2021)</p> <p><b>B. pertussis</b> Multiplex real-time PCR (Tatti et al., <i>J Clin Microbiol</i> 2011) and culture (if PCR cycle threshold <math>\leq 25</math>)</p> <p><b>SARS-CoV-2</b> 1 April 2020 – 31 March 2021: Roche E gene real-time PCR essay (Corman et al., <i>Euro Surv</i> 2020) 1 April 2021 to date: Allplex™ SARS-CoV-2/FluA/FluB/RSV PCR kit</p> <ul style="list-style-type: none"> <li>• positivity assigned if PCR cycle threshold is <math>&lt; 40</math> for <math>\geq 1</math> gene targets (N, S, OR RdRp)</li> </ul>



## 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

\*\*Started in 2019

\*\*\*Started in November 2020

\*\*\*\*INF: influenza virus; RSV: respiratory syncytial virus; BP: *Bordetella pertussis*; SARS-CoV-2: severe acute respiratory syndrome coronavirus 2

§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).

## COMMENTS

### Influenza

In 2022 to date, a total of 32 influenza cases have been reported. In week 13, transmission and impact are below threshold.

**ILI programme:** In 2022 to date, specimens from 410 patients meeting ILI case definition were tested from 4 ILI sites. Influenza was detected in 13 (3%) patients. Of which, 10 (77%) were influenza A(H1N1) pdm09 and three (23%) were influenza A(pending subtype results). (Fig1, Table1).

**Viral Watch programme:** In 2022 to date, specimens from 54 patients from four of the 8 Viral Watch sites participating in surveillance were tested and influenza A(H3N2) was detected in one (2%). (Fig7, Table6)

**Pneumonia surveillance:** Since the beginning of 2022, specimens from 1563 patients with severe respiratory illness (SRI) were tested from the 6 sentinel sites. Influenza was detected in 16 (1%) patients. Of which, 14 (88%) were influenza A(H1N1)pdm09 and two (12%) were influenza A(H3N2). (Fig11, Table10)

In addition, 31 specimens from patients who met suspected SARS-CoV-2 case definition but did not meet the pneumonia/ILI surveillance case definitions were tested for influenza. Two (6%) tested positive, one (50%) was influenza B(Victoria) and influenza A subtype results were pending for one (50%).

### Respiratory syncytial virus

The 2022 RSV season has started in week 7 (week starting 14 February 2022) when RSV detection rate among children under five years of age in pneumonia surveillance rose above the seasonal threshold, as determined by the Moving Epidemic Method.

**ILI programme:** In 2022 to date, 410 specimens from patients meeting the ILI case definition were tested and RSV was detected in 42 (10%) patients. Of which, 37 (88%) were RSV subgroup A, one (2%) was RSV subgroup B, two (5%) were RSV inconclusive and RSV subgroup results were pending for two (5%). (Fig2-3, Table2)

**Viral Watch programme:** In 2022 to date, 54 specimens from Viral Watch patients were tested and RSV subgroup B was detected in one (2%). (Fig8, Table7)

**Pneumonia surveillance:** Since the beginning of 2022, 1563 specimens were tested and RSV was detected in specimens of 249 (16%) patients. Of which, 91 (37%) were RSV subgroup A, 136 (55%) were

RSV subgroup B, one (<1%) was RSV-AB, and RSV subgroup results were inconclusive for four (2%) and pending for 17 (7%). (Fig12-13, Table11)

In addition, 31 specimens from patients who met suspected SARS-CoV-2 case definition but did not meet the pneumonia/ILI surveillance case definitions were tested for RSV and none tested positive for RSV.

### *Bordetella pertussis*

**ILI programme:** From 3 Jan 2022 to date, combined nasopharyngeal and oropharyngeal specimens were tested from 406 patients and *B. pertussis* was not detected. (Fig4, Table3)

**Pneumonia surveillance:** During the same period, combined nasopharyngeal and oropharyngeal specimens were tested from 1552 patients and *B. pertussis* was not detected. (Fig14, Table12).

In addition, *B. pertussis* was not detected in 31 specimens from patients who met suspected SARS-CoV-2 case definition but did not meet the pneumonia/ILI surveillance case definition.

### SARS-CoV-2 (Severe acute respiratory syndrome coronavirus 2)

**ILI programme:** From 3 January 2022 to date, 410 patients were tested and SARS-CoV-2 was detected in 56 (14%) patients. Of the 52 (52/56, 93%) with variant data, Omicron was 29% (16/52) and was variant was not assigned for 69% (36/52). (Fig5-6, Table4-5)

**Viral Watch programme:** From 3 January 2022 to date, 54 patients presenting with ILI were tested and SARS-CoV-2 was detected in 20 (37%). Of the 16 (16/20, 80%) with variant data, majority were Omicron variant (12/16, 75%) and variant was not assigned for 25% (4/16). (Fig9-10, Table8-9)

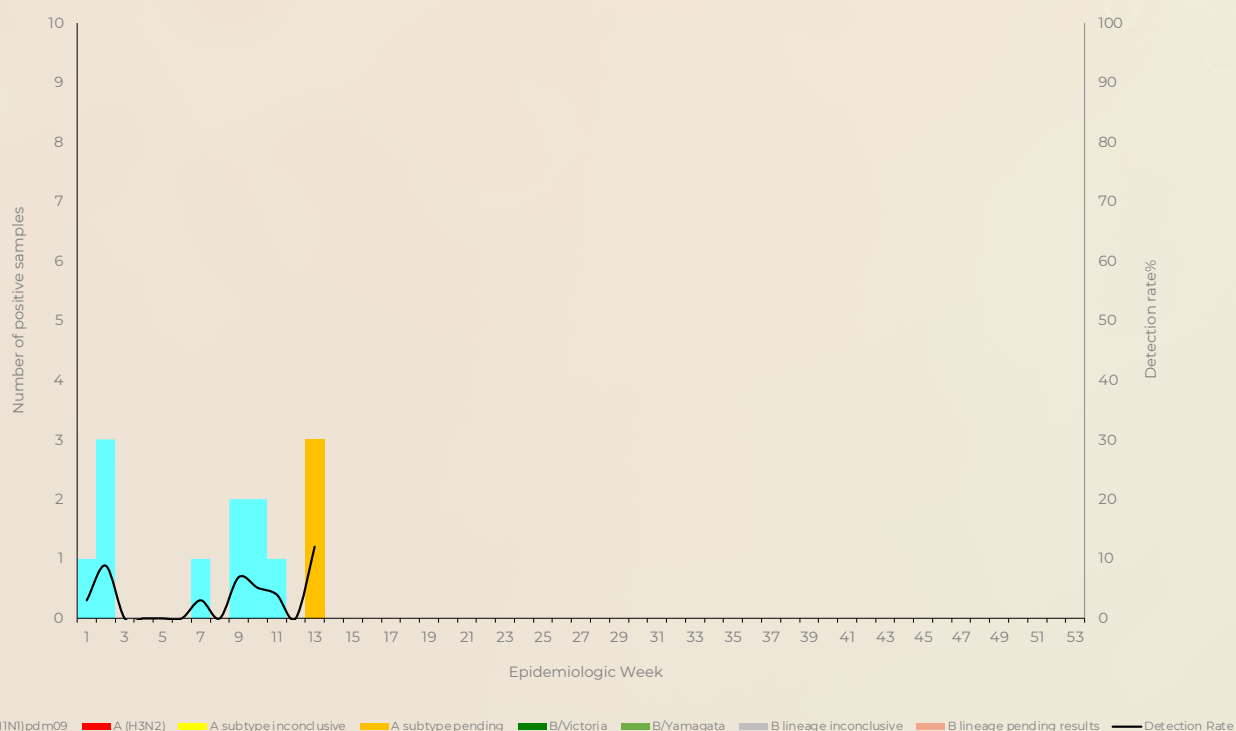
**Pneumonia surveillance:** From 3 January 2022 to date, 1563 patients with severe respiratory illness (SRI) were tested and SARS-CoV-2 was detected in 165 (11%) patients. Of the 151 (151/165, 92%) with variant data, 58% (88/151) were Omicron variant, one (1%) was Alpha variant and variant was not assigned for 41% (62/151). (Fig15-16, Table13-14)

In addition, SARS-CoV-2 was detected in 12 of 31 (39%) specimens from patients who met suspected SARS-CoV-2 case definition but did not meet the pneumonia/ILI surveillance case definitions. Of these, 58% (7/12) were Omicron variant and variant was not assigned for 42% (5/12).

# INFLUENZA, RSV, BORDETELLA PERTUSSIS AND SARS-COV-2 SURVEILLANCE REPORT

WEEK 13 2022

## INFLUENZA-LIKE ILLNESS (ILI) SURVEILLANCE PRIMARY HEALTH CARE CLINICS



**Figure 1.** Number of influenza positive cases\* by influenza subtype and lineage\*\* and detection rate\*\*\* by week, Influenza-like illness (ILI) surveillance primary health care clinics, 03/01/2022 – 03/04/2022

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

\*\*Influenza was detected in two (11%) of 19 specimens from patients who met suspected SARS-CoV-2 case definition but did not meet Influenza-like illness (ILI) case definition, of which one (50%) was influenza B(Victoria) and one (50%) was pending influenza A subtype results. These are not included in the epidemiological curve.

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

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 primary health care clinics, 03/01/2022 – 03/04/2022

Clinic (Province)	A(H1N1)pdm09	A(H3N2)	A subtype inconclusive	A subtype pending results <sup>§</sup>	B/Victoria	B/Yamagata	B lineage inconclusive	B lineage pending results <sup>§</sup>	Total samples
Agincourt (MP)	3	0	0	0	0	0	0	0	75
Eastridge (WC)	0	0	0	0	0	0	0	0	69
Edendale Gateway (KZ)	0	0	0	3	0	0	0	0	127
Jouberton (NW)	7	0	0	0	0	0	0	0	119
Mitchell's Plain (WC)	0	0	0	0	0	0	0	0	20
<b>Total:</b>	<b>10</b>	<b>0</b>	<b>0</b>	<b>3</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>410</b>

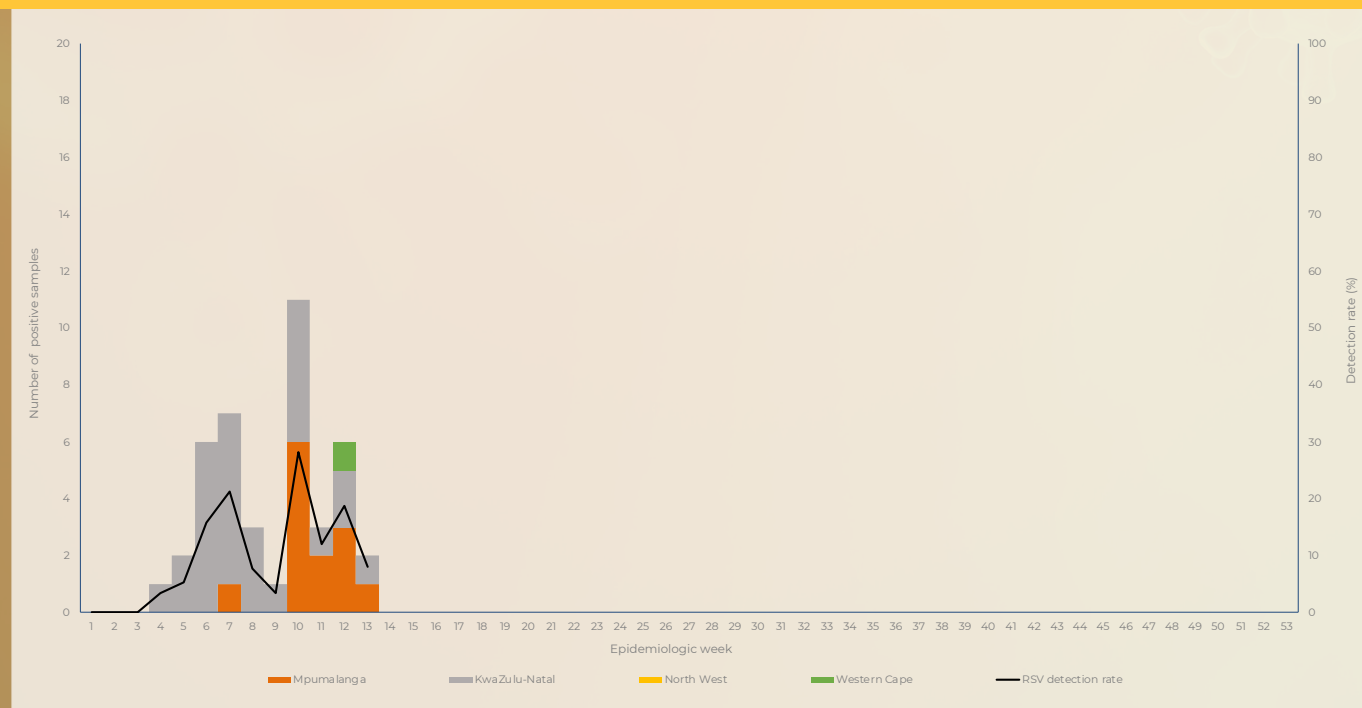
KZ: KwaZulu-Natal; NW: North West; WC: Western Cape; MP: Mpumalanga

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

<sup>§</sup>Influenza A subtype or B lineage results are pending

\*\*Influenza was detected in two (11%) of 19 specimens from patients who met suspected SARS-CoV-2 case definition but did not meet Influenza-like illness (ILI) case definition, of which one (50%) was influenza B(Victoria) and one (50%) was pending influenza A subtype results. These are not included in the table.

## INFLUENZA-LIKE ILLNESS (ILI) SURVEILLANCE PRIMARY HEALTH CARE CLINICS

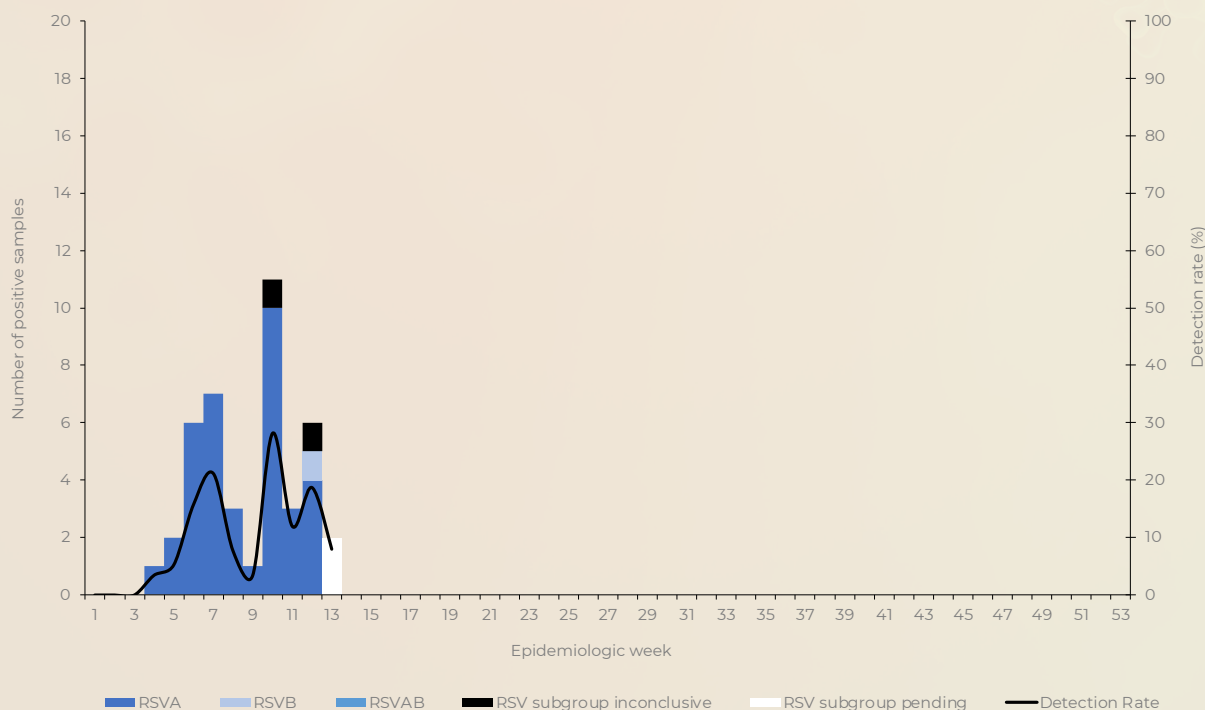


**Figure 2.** Number of patients testing positive for respiratory syncytial virus\* by province and detection rate by week, Influenza-like illness (ILI) surveillance primary health care clinics, 03/01/2022 – 03/04/2022

\*RSV was not detected from 19 patients who met suspected SARS-CoV-2 case definition but did not meet influenza-like illness (ILI) case definition. These are not included in the epidemiological curve.



## INFLUENZA-LIKE ILLNESS (ILI) SURVEILLANCE PRIMARY HEALTH CARE CLINICS



**Figure 3.** Number of patients testing positive for respiratory syncytial virus\* by subgroup and detection rate by week, Influenza-like illness (ILI) surveillance primary health care clinics, 03/01/2022 – 03/04/2022

Inconclusive: insufficient viral load in sample and unable to characterise further  
RSV AB: Both RSV A and B subgroup identified

\*RSV was not detected from 19 patients who met suspected SARS-CoV-2 case definition but did not meet influenza-like illness (ILI) case definition. These are not included in the epidemiological curve.

**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 primary health care clinics, 03/01/2022 – 03/04/2022

Clinic (Province)	RSVA	RSVB	RSVAB	RSV subgroup inconclusive	RSV subgroup pending**	Total samples
Agincourt (MP)	12	0	0	0	1	75
Eastridge (WC)	1	1	0	0	0	69
Edendale Gateway (KZ)	25	0	0	2	1	127
Jouberton (NW)	0	0	0	0	0	119
Mitchell's Plain (WC)	0	0	0	0	0	20
<b>Total</b>	<b>37</b>	<b>1</b>	<b>0</b>	<b>2</b>	<b>2</b>	<b>410</b>

KZ: KwaZulu-Natal; NW: North West; WC: Western Cape; MP: Mpumalanga  
Inconclusive: insufficient viral load in sample and unable to characterise further  
RSV AB: Both RSV A and B subgroup identified

\*RSV was not detected from 19 patients who met suspected SARS-CoV-2 case definition but did not meet influenza-like illness (ILI) case definition. These are not included in the table.

\*\*RSV results for subgroups are pending

## INFLUENZA-LIKE ILLNESS (ILI) SURVEILLANCE PRIMARY HEALTH CARE CLINICS



**Figure 4.** Number of samples testing positive for *B. pertussis*\* and detection rate by month, Influenza-like illness (ILI) surveillance primary health care clinics, 03/01/2022 – 03/04/2022

\*No *B. pertussis* was detected in 19 specimens of patients who met the suspected *B. pertussis* case definition but did not meet Influenza-like illness case definition. These are not included in the epidemiological curve

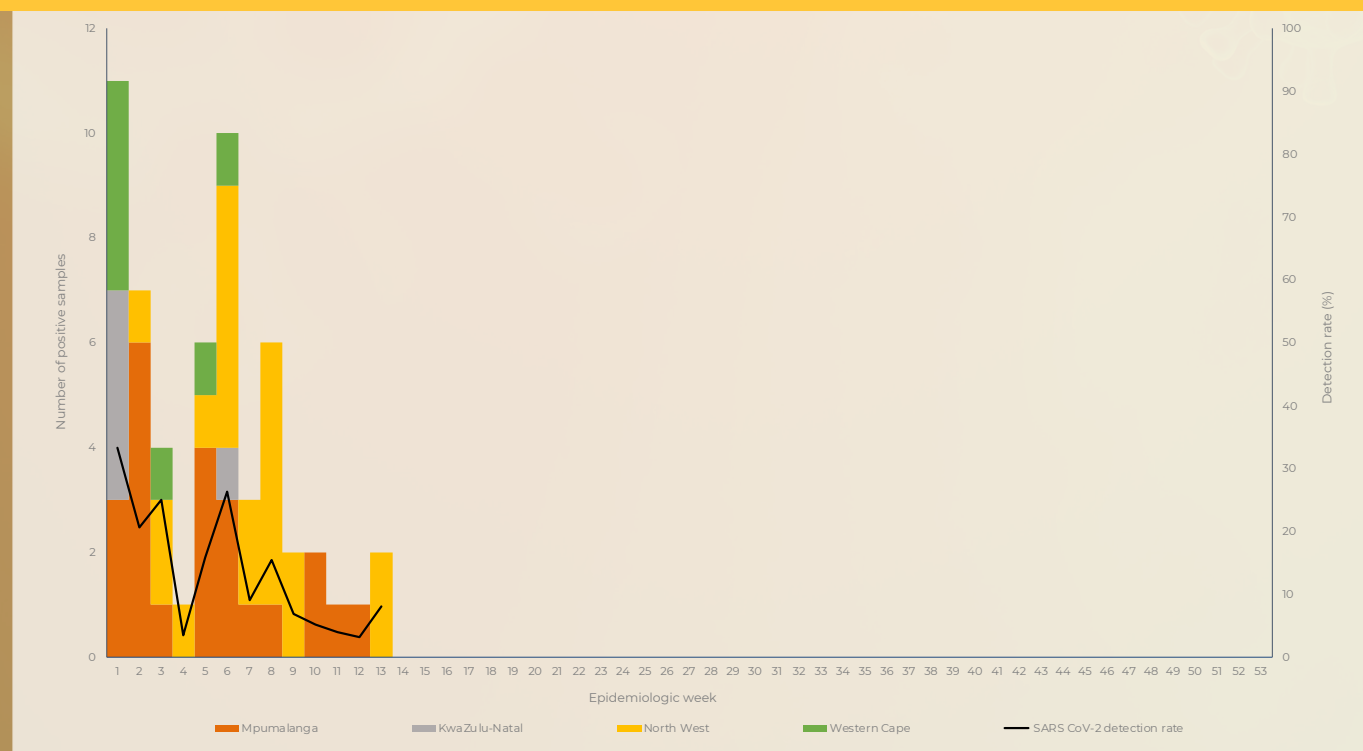
**Table 3.** Number of patients testing positive for *B. pertussis*\* identified and total number of samples tested by province, Influenza-like illness (ILI) surveillance primary health care clinics, 03/01/2022 – 03/04/2022

Clinic (Province)	<i>B. pertussis</i> Positive	Total samples tested
Agincourt (MP)	0	72
Eastridge (WC)	0	69
Edendale Gateway (KZ)	0	127
Jouberton (NW)	0	119
Mitchell's Plain (WC)	0	20
<b>Total:</b>	<b>0</b>	<b>406</b>

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

\*No *B. pertussis* was detected in 19 specimens of patients who met the suspected *B. pertussis* case definition but did not meet Influenza-like illness case definition. These are not included in the table.

## INFLUENZA-LIKE ILLNESS (ILI) SURVEILLANCE PRIMARY HEALTH CARE CLINICS



**Figure 5.** Number of patients testing positive for SARS-CoV-2\* by province and detection rate by week, Influenza-like illness (ILI) surveillance primary health care clinics, 03/01/2022 – 03/04/2022

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

\*SARS-CoV-2 was detected in five of 19 (26%) specimens from patients who met suspected SARS-CoV-2 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, 03/01/2022 – 03/04/2022

Clinic (Province)	SARS-CoV-2 positive	Total samples tested
Agincourt (MP)	23	75
Eastridge (WC)	3	69
Edendale Gateway (KZ)	5	127
Jouberton (NW)	21	119
Mitchell's Plain (WC)	4	20
<b>Total:</b>	<b>56</b>	<b>410</b>

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

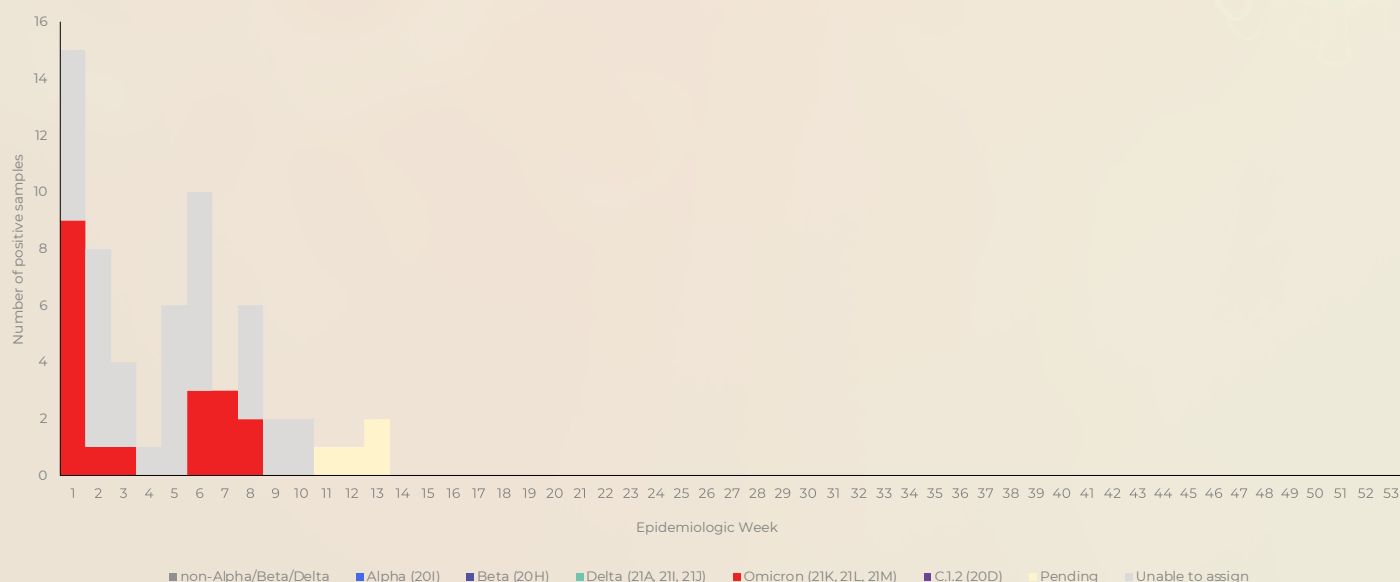
\*SARS-CoV-2 was detected in five of 19 (26%) specimens from patients who met suspected SARS-CoV-2 case definition but did not meet influenza-like illness (ILI) case definition. These are not included in the table.



# INFLUENZA, RSV, BORDETELLA PERTUSSIS AND SARS-COV-2 SURVEILLANCE REPORT

WEEK 13 2022

## INFLUENZA-LIKE ILLNESS (ILI) SURVEILLANCE PRIMARY HEALTH CARE CLINICS



**Figure 6.** Number and detection rate of laboratory confirmed SARS-CoV-2\* cases by variant type (variant PCR/sequencing) and week, Influenza-like illness (ILI) surveillance primary health care clinics, 03/01/2022 – 03/04/2022

\*Specimens are from patients with influenza-like illness at 5 sentinel sites in 4 provinces who met suspected SARS-CoV-2 case definition or met ILI 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 SARS-CoV-2\* positive cases 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, 03/01/2022 – 03/04/2022

Clinic (Province)	Non-Alpha/ Beta/Delta	Alpha (20I)	Beta (20H)	Delta (21A, 21I, 21J)	C.1.2 (20D)	Omicron (21K, 21L, 21M)	Pending	Unable to assign	Total SARS-CoV-2 positive
Agincourt (MP)	0	0	0	0	0	6	2	17	25
Eastridge (WC)	0	0	0	0	0	2	0	1	3
Edendale Gateway (KZ)	0	0	0	0	0	3	0	4	7
Jouberton (NW)	0	0	0	0	0	6	2	14	22
Mitchell's Plain (WC)	0	0	0	0	0	2	0	2	4
<b>Total:</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>19</b>	<b>4</b>	<b>38</b>	<b>61</b>

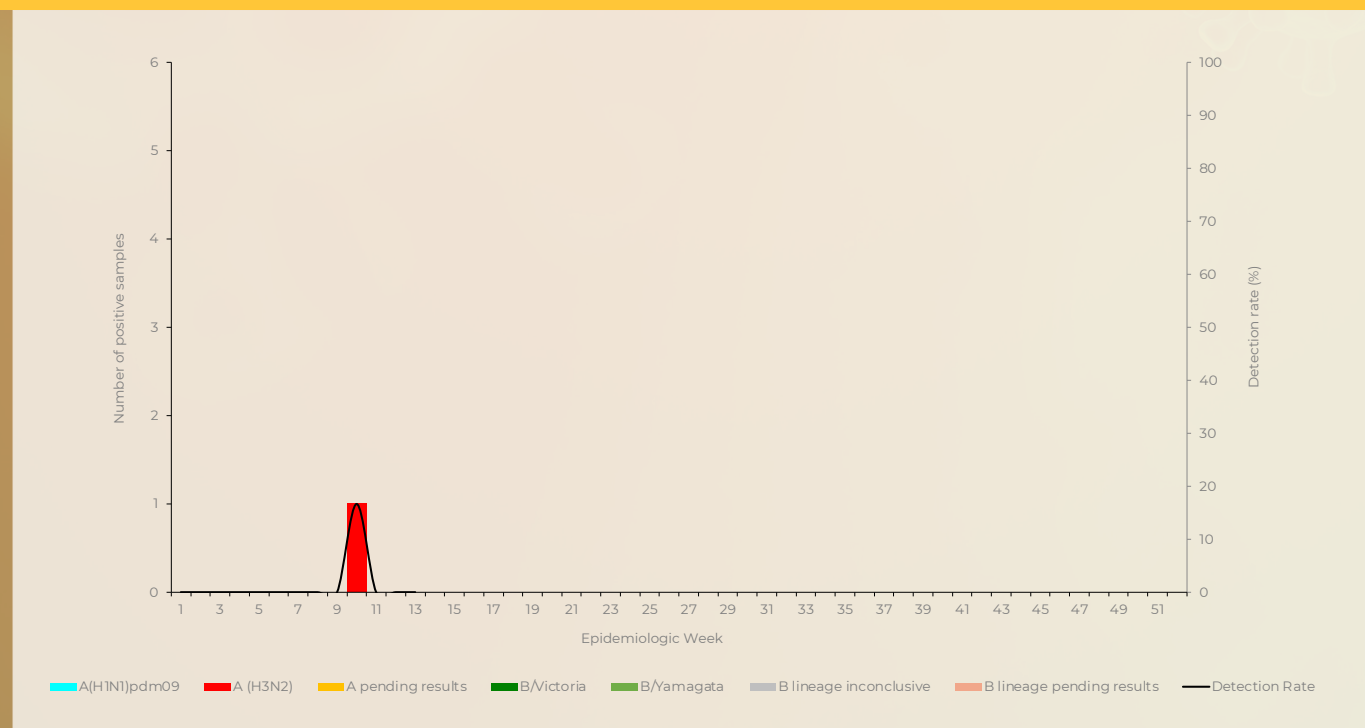
KZ: KwaZulu-Natal; NW: North West; WCP: Western Cape; MP: Mpumalanga

\*Specimens are from patients with influenza-like illness at 5 sentinel sites in 4 provinces who met suspected SARS-CoV-2 case definition or met ILI 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

# INFLUENZA-LIKE ILLNESS (ILI) SURVEILLANCE VIRAL WATCH



**Figure 7.** Number of positive patients\* by influenza subtype and lineage and detection rate\*\* by week, ILI surveillance - Viral Watch, 03/01/2022 – 03/04/2022

\*Specimens from patients with Influenza-like illnesses at 90 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, 03/01/2022 – 03/04/2022

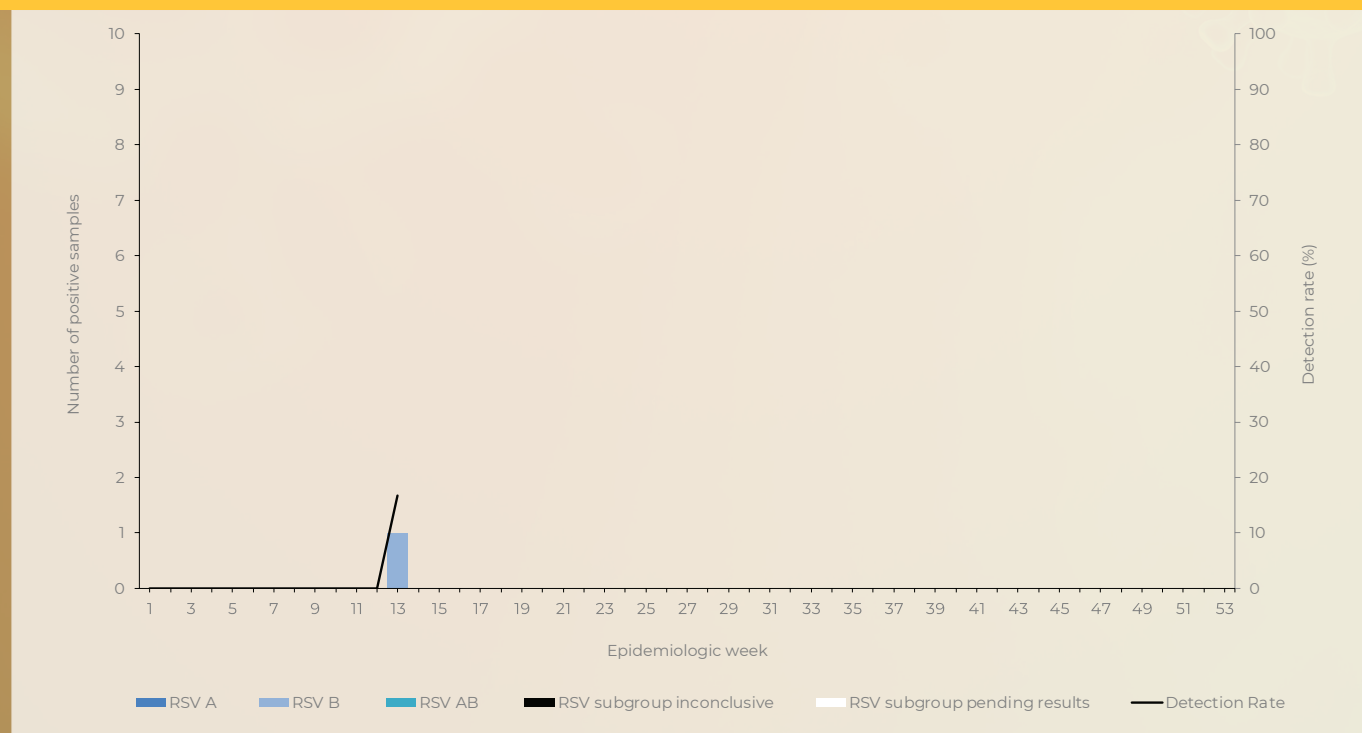
Province	A(H1N1)pdm09	A(H3N2)	A subtype inconclusive	A subtype pending results*	B/Victoria	B/Yamagata	B lineage inconclusive	B lineage pending results*	Total samples
Eastern Cape	0	0	0	0	0	0	0	0	2
Free State	0	0	0	0	0	0	0	0	0
Gauteng	0	1	0	0	0	0	0	0	34
Limpopo	0	0	0	0	0	0	0	0	0
Mpumalanga	0	0	0	0	0	0	0	0	0
North West	0	0	0	0	0	0	0	0	2
Northern Cape	0	0	0	0	0	0	0	0	0
Western Cape	0	0	0	0	0	0	0	0	16
<b>Total:</b>	<b>0</b>	<b>1</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>54</b>

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

\*Influenza A subtype or B lineage results are pending



## INFLUENZA-LIKE ILLNESS (ILI) SURVEILLANCE VIRAL WATCH



**Figure 8.** Number of RSV positive cases testing positive for respiratory syncytial virus (RSV)\* by subgroup and detection rate\*\* by week, ILI surveillance - Viral Watch, 03/01/2022 – 03/04/2022

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

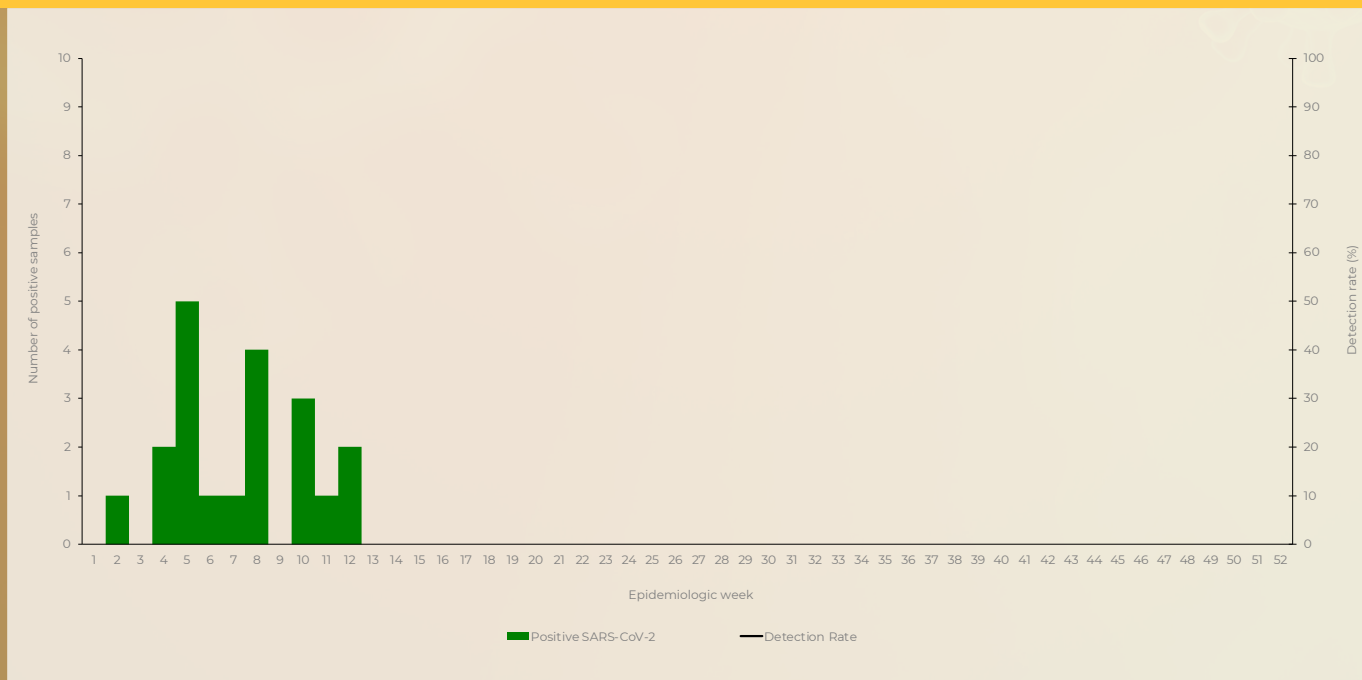
\*\* Only reported for weeks with >10 specimens submitted.

**Table 7.** Number of RSV positive cases identified and total number of samples tested by province, ILI surveillance - Viral Watch, 03/01/2022 – 03/04/2022

Province	RSV A	RSV B	RSV AB	RSV subgroup inconclusive	RSV subgroup pending results*	Total samples tested
Eastern Cape	0	0	0	0	0	2
Free State	0	0	0	0	0	0
Gauteng	0	0	0	0	0	34
Limpopo	0	0	0	0	0	0
Mpumalanga	0	0	0	0	0	0
North West	0	0	0	0	0	2
Northern Cape	0	0	0	0	0	0
Western Cape	0	1	0	0	0	16
<b>Total:</b>	<b>0</b>	<b>1</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>54</b>

\*RSV results for subgroups are pending

## INFLUENZA-LIKE ILLNESS (ILI) SURVEILLANCE VIRAL WATCH



**Figure 9.** Number of patients testing positive for SARS-CoV-2\*, by site and detection rate\*\* by week, ILI surveillance - Viral Watch, 03/01/2022 – 03/04/2022

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

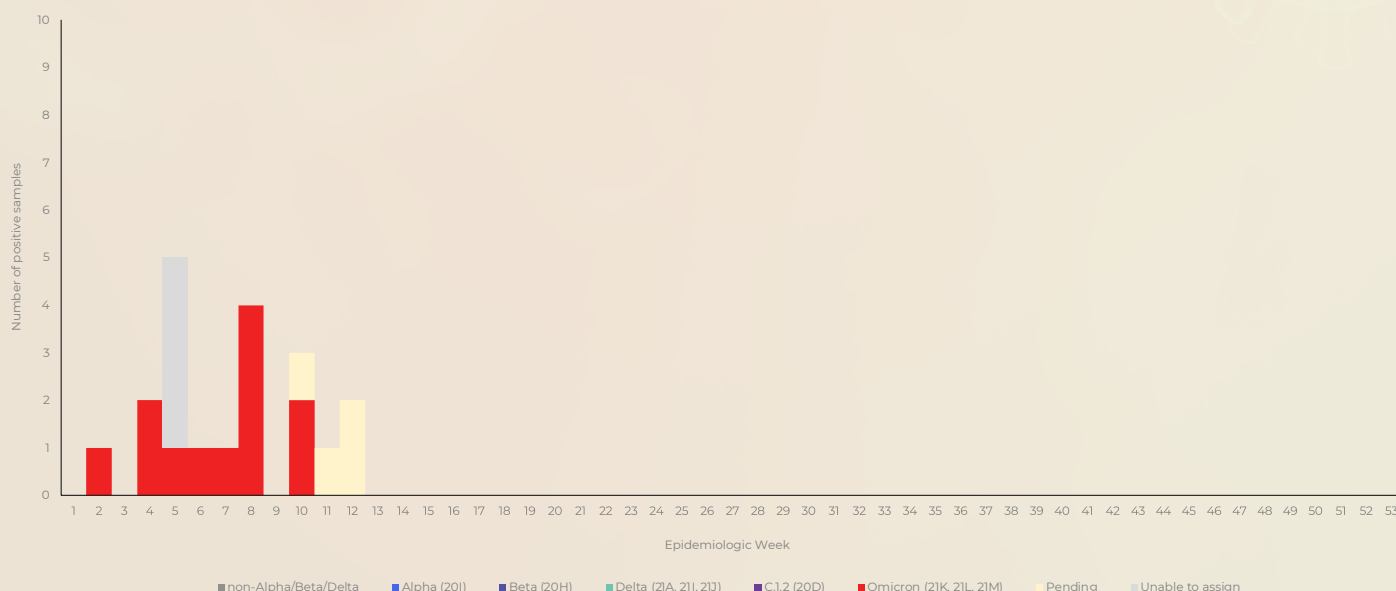
\*\* Only reported for weeks with >10 specimens submitted.

**Table 8.** Number of SARS-CoV-2 positive cases identified and total number tested by province, ILI surveillance - Viral Watch, 03/01/2022 – 03/04/2022

Province	SARS-CoV-2 positive	Total samples tested
Eastern Cape	1	2
Free State	0	0
Gauteng	14	34
Limpopo	0	0
Mpumalanga	0	0
North West	0	2
Northern Cape	0	0
Western Cape	5	16
<b>Total:</b>	<b>20</b>	<b>54</b>



# INFLUENZA-LIKE ILLNESS (ILI) SURVEILLANCE VIRAL WATCH



**Figure 10.** Number and detection rate of laboratory confirmed SARS-CoV-2\* cases by variant type (variant PCR/sequencing) and week, ILI surveillance - Viral Watch, 03/01/2022 – 03/04/2022

\*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 (ct=>35) OR variant PCR could not assign variant and no sequencing result  
**Pending:** outstanding variant results

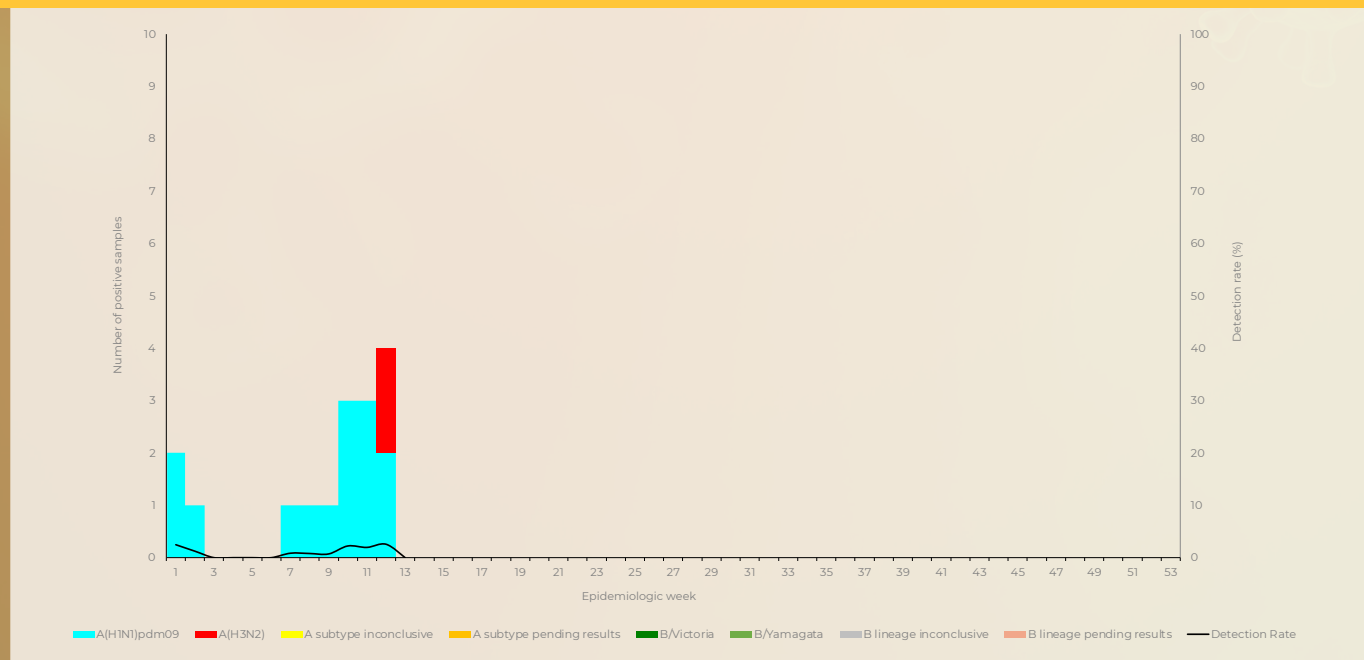
**Table 9.** Number of SARS-CoV-2\* positive cases by variant (variant PCR and/or sequencing) identified and total number of samples tested by province, ILI surveillance - Viral Watch, 03/01/2022 – 03/04/2022

Clinic (Province)	Non-Alpha/ Beta/Delta	Alpha (20I)	Beta (20H)	Delta (21A, 21I, 21J)	C.1.2 (20D)	Omicron (21K, 21L, 21M)	Pending	Unable to assign	Total SARS-CoV-2 positive
Eastern Cape	0	0	0	0	0	1	0	0	1
Free State	0	0	0	0	0	0	0	0	0
Gauteng	0	0	0	0	0	7	3	4	14
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	0	0	4	1	0	5
<b>Total:</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>12</b>	<b>4</b>	<b>4</b>	<b>20</b>

\*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 (ct=>35) OR variant PCR could not assign variant and no sequencing result  
**Pending:** outstanding variant results

# NATIONAL SYNDROMIC SURVEILLANCE FOR PNEUMONIA



**Figure 11.** Number of positive influenza positive cases\* by influenza subtype and lineage\*\* and detection rate\*\*\* by week, pneumonia surveillance public hospitals, 03/01/2022 – 03/04/2022

\*Specimens from patients hospitalized with pneumonia at 7 sentinel sites in 5 provinces

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

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

\*\* Influenza was not detected in 12 specimens from patients who met suspected SARS-CoV-2 case definition but did not meet pneumonia (SRI) case definition. These are not included in the epidemiological curve.

**Table 10.** Number of laboratory confirmed influenza cases by subtype and lineage\*\* and total number of samples tested by hospital, pneumonia surveillance public hospitals, 03/01/2022 – 03/04/2022

Hospital (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
Edendale (KZ)	9	0	0	0	0	0	0	0	313
Helen Joseph-Rahima Moosa (GP)	0	1	0	0	0	0	0	0	424
Klerksdorp-Tshepong (NW)	0	0	0	0	0	0	0	0	131
Mapulaneng-Matikwana (MP)	1	0	0	0	0	0	0	0	137
Red Cross (WC)	0	0	0	0	0	0	0	0	280
Mitchell's Plain (WC)	1	0	0	0	0	0	0	0	177
Tembisa (GP)	0	0	0	0	0	0	0	0	20
Tintswalo (MP)	3	1	0	0	0	0	0	0	81
<b>Total:</b>	<b>14</b>	<b>2</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>1563</b>

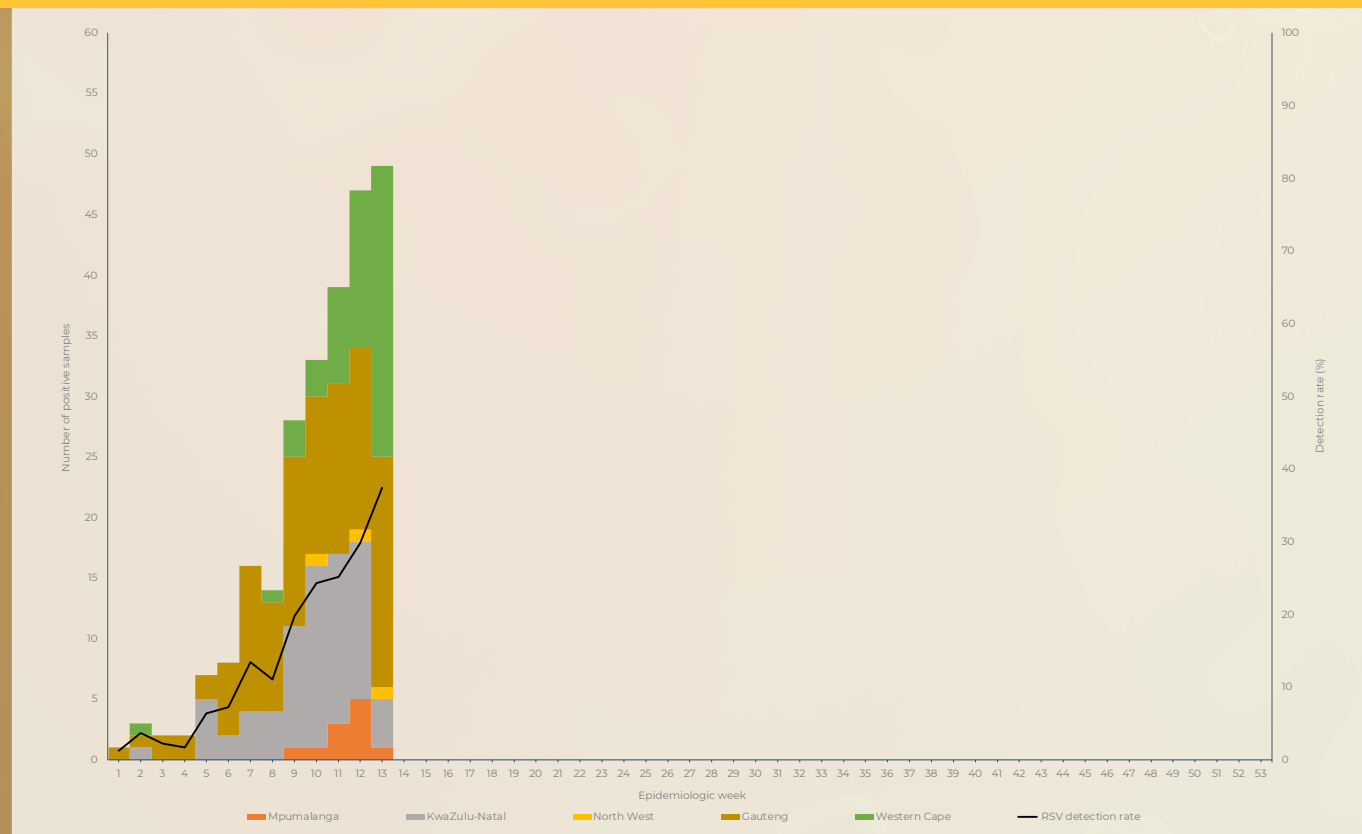
GP: Gauteng; KZ: KwaZulu-Natal; NW: North West; MP: Mpumalanga; WC: Western Cape  
Inconclusive: insufficient viral load in sample and unable to characterise further

\*Influenza A subtype or B lineage results are pending

\*\*Influenza was not detected in 12 specimens from patients who met suspected SARS-CoV-2 case definition but did not meet pneumonia (SRI) case definition. These are not included in the table.



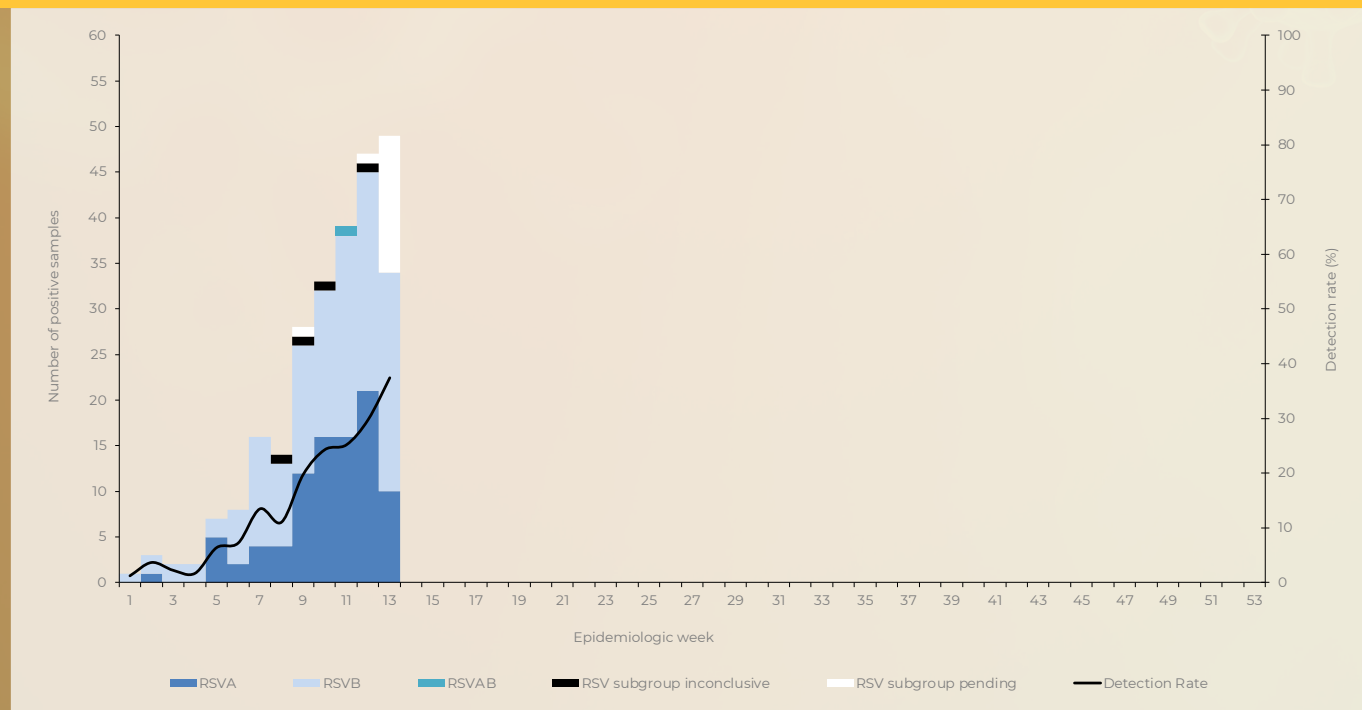
## NATIONAL SYNDROMIC SURVEILLANCE FOR PNEUMONIA



**Figure 12.** Number of patients testing positive for respiratory syncytial virus\* by province and detection rate by week, pneumonia surveillance public hospitals, 03/01/2022 – 03/04/2022

\*RSV was not detected in 12 specimens from patients who met suspected SARS-CoV-2 case definition but did not meet pneumonia (SRI) case definition. These are not included in the epidemiological curve.

# NATIONAL SYNDROMIC SURVEILLANCE FOR PNEUMONIA



**Figure 13.** Number of patients testing positive for respiratory syncytial virus\* by subgroup and detection rate by week, pneumonia surveillance public hospitals, 03/01/2022 – 03/04/2022

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

\*RSV was not detected in 12 specimens from patients who met suspected SARS-CoV-2 case definition but did not meet pneumonia (SRI) case definition. These are not included in the epidemiological curve.

**Table 11:** Number of patients positive for respiratory syncytial virus subgroups\* by subgroups identified and total number of samples tested by hospital, pneumonia surveillance public hospitals, 03/01/2022 – 03/04/2022

Hospital (Province)	RSVA	RSVB	RSVAB	RSV subgroup inconclusive	RSV subgroup pending*	Total samples
Edendale (KZ)	63	1	0	2	6	313
Helen Joseph-Rahima Moosa (GP)	12	88	1	0	8	424
Klerksdorp-Tshepong (NW)	0	2	0	0	1	131
Mapulaneng-Matikwana (MP)	4	2	0	0	0	137
Red Cross (WC)	11	26	0	0	2	280
Mitchell's Plain (WC)	1	13	0	0	0	177
Tembisa (GP)	0	1	0	0	0	20
Tintswalo (MP)	0	3	0	2	0	81
<b>Total:</b>	<b>91</b>	<b>136</b>	<b>1</b>	<b>4</b>	<b>17</b>	<b>1563</b>

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

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

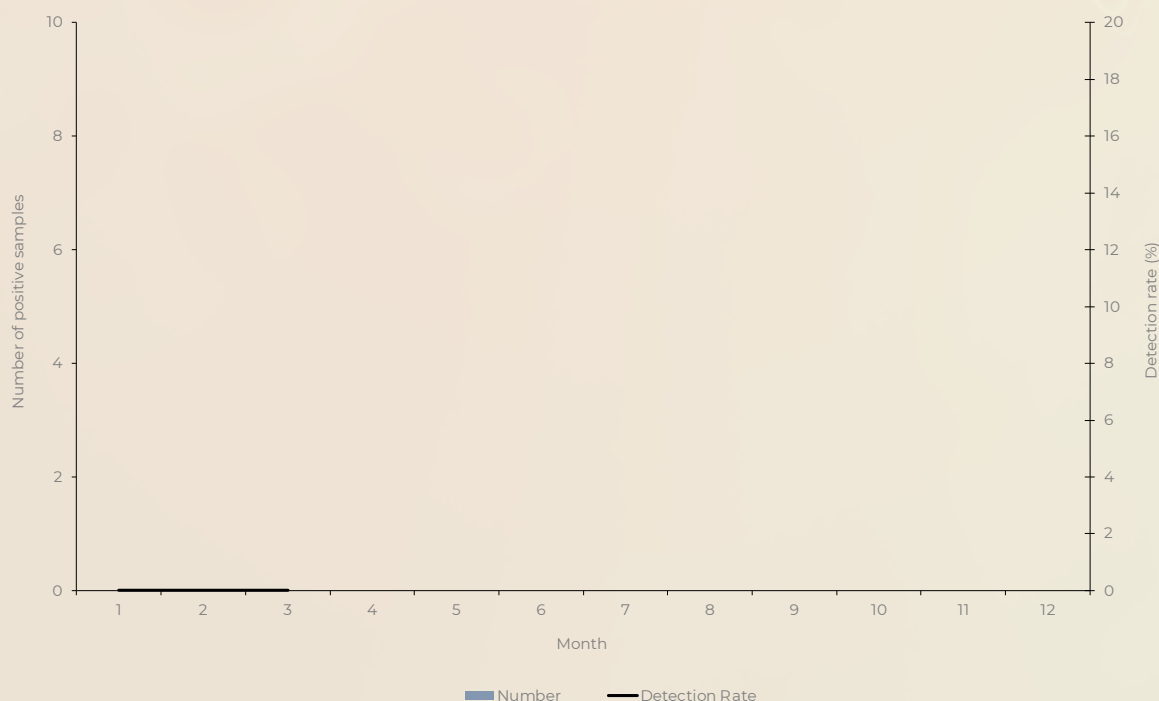
RSV AB: Both RSV A and B subgroup identified

\*\*RSV results for subgroups are pending

\*RSV was not detected in 12 specimens from patients who met suspected SARS-CoV-2 case definition but did not meet pneumonia (SRI) case definition. These are not included in the table



# NATIONAL SYNDROMIC SURVEILLANCE FOR PNEUMONIA



**Figure 14.** Number of patients testing positive for *B. pertussis*\* and detection rate by month, pneumonia surveillance public hospitals, 03/01/2022 – 03/04/2022

\*No *B. pertussis* was detected in 12 specimens of patients who met the suspected *B. pertussis* case definition but did not meet Pneumonia Surveillance case definition. These are not included in the epidemiologic curve.

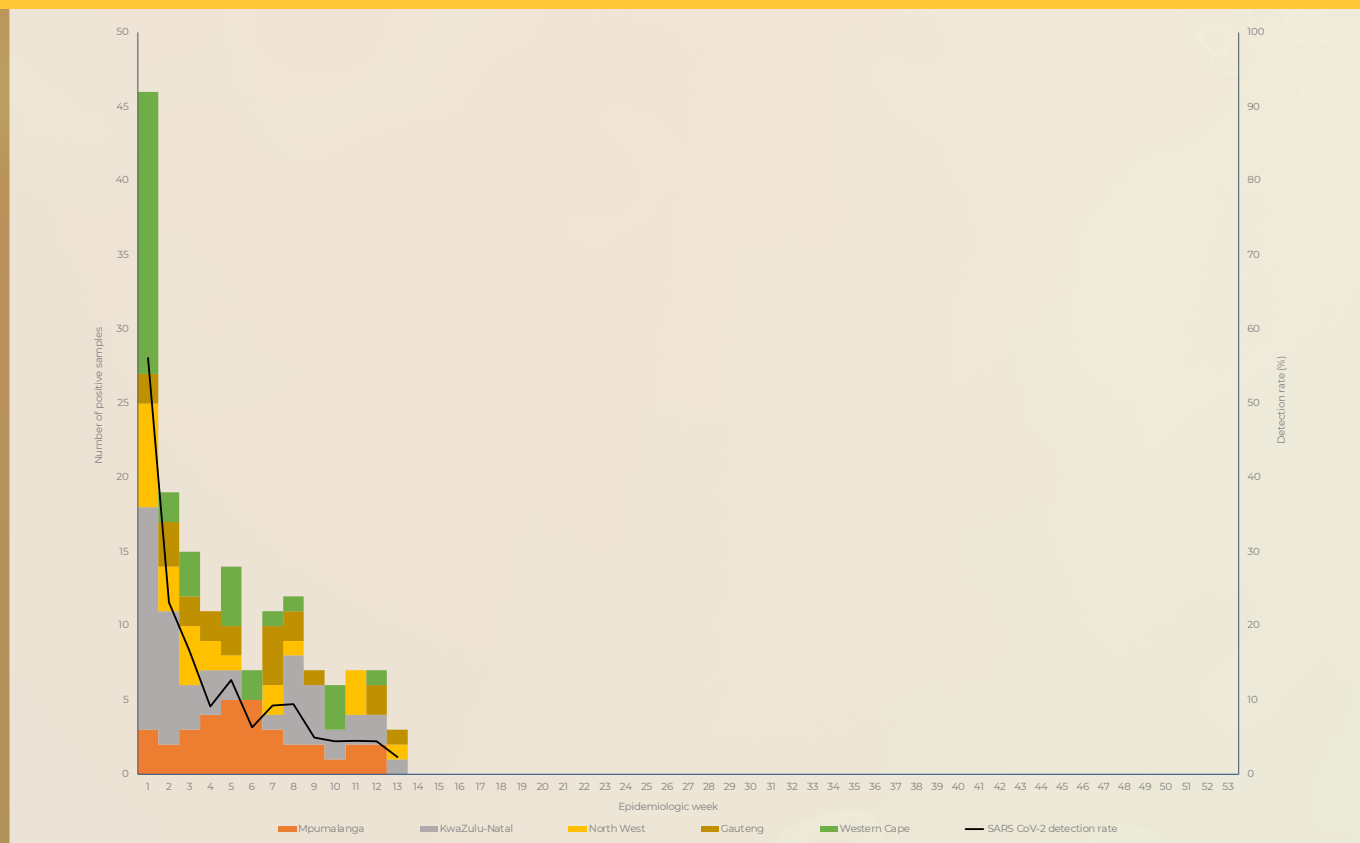
**Table 12.** Number of patients testing positive for *B. pertussis*\* identified and total number of samples tested by hospital and province, pneumonia surveillance public hospitals, 03/01/2022 – 03/04/2022

Hospital (Province)	<i>B. pertussis</i> Positive	Total samples tested
Edendale (KZ)	0	313
Helen Joseph-Rahima Moosa (GP)	0	424
Klerksdorp-Tshepong (NW)	0	131
Mapulaneng-Matikwana (MP)	0	135
Red Cross (WC)	0	274
Mitchell's Plain (WC)	0	174
Tembisa (GP)	0	20
Tintswalo (MP)	0	81
<b>Total:</b>	<b>0</b>	<b>1552</b>

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

\*No *B. pertussis* was detected in 12 specimens of patients who met the suspected *B. pertussis* case definition but did not meet Pneumonia Surveillance case definition. These are not included in the table

# NATIONAL SYNDROMIC SURVEILLANCE FOR PNEUMONIA



**Figure 15.** Number of patients testing positive for SARS-CoV-2\* by province and detection rate by week, pneumonia surveillance public hospitals, 03/01/2022 – 03/04/2022

\*Specimens from patients hospitalized with pneumonia at 6 sentinel sites in 5 provinces

\*SARS-CoV-2 was detected in seven of 12 (58%) specimens from patients who met suspected SARS-CoV-2 case definition but did not meet pneumonia (SRI) case definition. These are not included in the epidemiological curve.

**Table 13.** Number of patients positive for SARS-CoV-2\* and total number of samples tested by hospital, pneumonia surveillance public hospitals, 03/01/2022 – 03/04/2022

Hospital (Province)	SARS-CoV-2 positive	Total samples tested
Edendale (KZ)	50	313
Helen Joseph-Rahima Moosa (GP)	20	424
Klerksdorp-Tshepong (NW)	24	131
Mapulaneng-Matikwana (MP)	23	137
Red Cross (WC)	14	280
Mitchell's Plain (WC)	22	177
Tembisa (GP)	1	20
Tintswalo (MP)	11	81
<b>Total:</b>	<b>165</b>	<b>1563</b>

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

\*SARS-CoV-2 was detected in seven of 12 (58%) specimens from patients who met suspected SARS-CoV-2 case definition but did not meet pneumonia (SRI) case definition. These are not included in the table.

## NATIONAL SYNDROMIC SURVEILLANCE FOR PNEUMONIA



**Figure 16.** Number and detection rate of laboratory confirmed SARS-CoV-2 cases\* by variant type (variant PCR/sequencing), pneumonia surveillance public hospitals, 03/01/2022 – 03/04/2022

\*Specimens are from hospitalized patients at 7 sentinel sites in 5 provinces who met suspected SARS-CoV-2 case definition and met pneumonia (SRI) case definition as well as those that did not meet the SRI 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 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, 03/01/2022 – 03/04/2022

Hospital (Province)	Non-Alpha/ Beta/Delta	Alpha (20I)	Beta (20H)	Delta (21A, 21I, 21J)	C.1.2 (20D)	Omicron (21K, 21L, 21M)	Pending	Unable to assign	SARS-CoV-2 positive
Edendale (KZ)	0	0	0	0	0	33	6	16	55
Helen Joseph-Rahima Moosa (GP)	0	1	0	0	0	11	2	6	20
Klerksdorp-Tshepong (NW)	0	0	0	0	0	13	1	10	24
Mapulaneng- Matikwana (MP)	0	0	0	0	0	11	2	12	25
Red Cross (WC)	0	0	0	0	0	7	1	6	14
Mitchell's Plain (WC)	0	0	0	0	0	12	0	10	22
Tembisa (GP)	0	0	0	0	0	0	1	0	1
Tintswalo (MP)	0	0	0	0	0	6	1	4	11
<b>Total:</b>	<b>0</b>	<b>1</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>93</b>	<b>14</b>	<b>64</b>	<b>172</b>

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

\*Specimens are from hospitalized patients at 7 sentinel sites in 5 provinces who met suspected SARS-CoV-2 case definition and met pneumonia (SRI) case definition as well as those that did not meet the SRI 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



## SUMMARY OF LABORATORY CONFIRMED SARS-COV-2 CASES

**Table 15.** Characteristics of individuals with laboratory-confirmed SARS-CoV-2, enrolled in influenza-like illness (ILI) and pneumonia surveillance programmes, South Africa, 3 January 2022 - 03 April 2022

Characteristic	Influenza-like illness (ILI), public-sector, n=61 (%)	Pneumonia, n=172 (%)
<b>Age group</b>		
0-9	12/61 (20)	41/172 (24)
10-19	9/61 (15)	5/172 (3)
20-39	13/61 (21)	49/172 (28)
40-59	20/61 (33)	44/172 (26)
60-79	6/61 (10)	27/172 (16)
≥80	1/61 (2)	6/172 (3)
<b>Sex-female</b>	36/61 (59)	94/172 (55)
<b>Province*</b>		
Gauteng	N/A	21/172 (12)
KwaZulu-Natal	7/61 (11)	55/172 (32)
Mpumalanga	25/61 (41)	36/172 (21)
North West	22/61 (36)	24/172 (14)
Western Cape	7/61 (11)	36/172 (21)
<b>Race</b>		
Black	43/61 (70)	142/170 (84)
Coloured	8/61 (12)	25/170 (15)
Asian/Indian	0/61 (0)	0/170 (0)
White	9/61 (16)	1/170 (1)
Other	1/61 (2)	2/170 (1)
<b>Variant</b>		
Non-Alpha/Beta/Delta	0/61 (0)	0/172 (0)
Alpha(20I)	0/61 (0)	1/172 (1)
Beta(20H)	0/61 (0)	0/172 (0)
Delta(21A, 21I, 21J)	0/61 (0)	0/172 (0)
C.1.2(20D)	0/61 (0)	0/172 (0)
Omicron(21K,21L,21M)	19/61 (31)	93/172 (54)
Pending results <sup>\$</sup>	4/61 (7)	14/172 (8)
Unable to assign <sup>\$\$</sup>	38/61 (62)	64/172 (37)

# INFLUENZA, RSV, BORDETELLA PERTUSSIS AND SARS-COV-2 SURVEILLANCE REPORT

WEEK 13 2022

Characteristic	Influenza-like illness (ILI), public-sector, n=61 (%)	Pneumonia, n=172 (%)
<b>Presentation</b>		
Fever	71/170 (42)	56/137 (41)
Cough	152/170 (89)	122/137 (89)
Shortness of breath	101/170 (59)	82/137 (60)
Chest pain	70/170 (41)	56/137 (41)
Diarrhoea	20/170 (12)	16/137 (12)
<b>Underlying conditions</b>		
Hypertension	12/61 (20)	29/170 (17)
Cardiac	1/61 (2)	4/170 (2)
Lung disease	0/61 (0)	1/170 (1)
Diabetes	2/61 (3)	16/170 (9)
Cancer	0/61 (0)	3/170 (2)
Tuberculosis	0/61 (0)	17/170 (10)
HIV-infection	9/61 (15)	65/170 (38)
Other **	1/61 (2)	2/170 (1)
<b>SARS-CoV-2 Vaccine</b>		
Pfizer-BioNTech (1st dose)	10/57 (18)	16/166 (10)
Pfizer-BioNTech (2nd dose)	10/57 (18)	10/166 (6)
Johnson & Johnson	8/57 (14)	18/166 (11)
Booster	0/57 (0)	0/166 (0)
<b>Management</b>		
Oxygen therapy	0/61 (0)	88/170 (52)
ICU admission	N/A	0/170 (0)
Ventilation	N/A	2/170 (1)
<b>Outcome***</b>		
Died	0/58 (0)	11/158 (7)

\*ILI surveillance not conducted in Gauteng province

\*\*Chronic lung, liver and kidney disease, organ transplant, pregnancy, malnutrition, obesity, tracheostomy, prematurity, seizure, stroke, anaemia, asplenia, burns, Systemic lupus erythematosus, seizures

\*\*\*Outcome includes patients who are still hospitalised, have been discharged or referred, and those who died

<sup>†</sup>Pending results: outstanding variant results

<sup>§</sup>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

**Note:** Children may be over-represented amongst hospitalised patients due to the inclusion of a large paediatric hospital in Cape Town.

Of the 11 patients who died, four were in the 20-39 year age group, three were in 40-59 age group and four were ≥60 years; 6/11 (55%) were female.



## 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 (Ct) <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 (Ct) 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/hcov-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 fragmentation 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 fragmentation and adapter ligation using IDT for Illumina Nextera UD Indexes. Further enrichment and cleanup 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).