

WEEKLY RESPIRATORY PATHOGENS SURVEILLANCE REPORT

SOUTH AFRICA

WEEK 16 2022



NATIONAL INSTITUTE FOR
COMMUNICABLE DISEASES

Division of the National Health Laboratory Service

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CUMULATIVE DATA FROM



HIGHLIGHTS

- In 2022 to date, 46 sporadic influenza cases have been detected from Gauteng (n=2), Western Cape (n=1), Kwa-Zulu Natal (n=29) and Mpumalanga (n=14) sentinel surveillance sites.
- The 2022 respiratory syncytial virus (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 16, RSV activity among children aged < 5 years continues to be at moderate threshold.
- In 2022 to date, a total of 271 COVID-19 cases were detected from all surveillance programmes. In week 16, an increase in detection rate of COVID-19 cases has been noted in ILI whereas there is a decline in pneumonia surveillance. Of the 166 hospitalised COVID-19 cases reported with available data on outcome, 12 (7%) died.
- Of the 230/244 (96%) with variant data from ILI and pneumonia surveillance programmes, Omicron variant dominated, 53% (122/230), <1% (1/230) was Alpha variant, <1% (1/230) was Delta variant and for 46% (106/230) variant was not assigned.

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

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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>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 infection</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 BP 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 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</p> <ul style="list-style-type: none"> • positivity assigned if PCR cycle threshold is < 40 for ≥ 1 gene targets (N, S, OR RdRp) | <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 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</p> <ul style="list-style-type: none"> • positivity assigned if PCR cycle threshold is < 40 for ≥ 1 gene targets (N, S, OR RdRp) | <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 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</p> <ul style="list-style-type: none"> • positivity assigned if PCR cycle threshold is < 40 for ≥ 1 gene targets (N, S, OR RdRp) |

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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 46 influenza cases have been reported. In week 16, transmission and impact were below threshold.

ILI programme: In 2022 to date, specimens from 452 patients meeting ILI case definition were tested from 4 ILI sites. Influenza was detected in 17 (4%) patients. Of which, 14 (82%) were influenza A(H1N1)pdm09 and three (18%) were pending influenza A subtype results. (Fig1, Table1).

Viral Watch programme: In 2022 to date, specimens from 72 patients from four of the 8 provinces participating in Viral Watch surveillance were tested and influenza A(H3N2) was detected in one (1%). (Fig7, Table5)

Pneumonia surveillance: Since the beginning of 2022, specimens from 1 943 patients with severe respiratory illness (SRI) were tested from the 6 sentinel sites and influenza was detected in 27 (1%) patients. Of which, 20 (74%) were influenza A(H1N1)pdm09, two (7%) were influenza A(H3N2) and five (19%) had pending influenza A subtype results. (Fig12, Table9)

In addition, 32 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 for influenza, of which one (50%) was influenza A(H1N1)pdm09 and one (50%) was influenza B (Victoria).

Respiratory syncytial virus

The 2022 RSV season 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, 452 specimens from patients meeting the ILI case definition were tested and RSV was detected in 47 (10%) patients. Of which, 40 (85%) were RSV A, two (4%) RSV B, and RSV subgroup results were inconclusive for two (4%) and pending for three (6%). (Fig4, Table2)

Viral Watch programme: In 2022 to date, 72 specimens from Viral Watch patients were tested and RSV was detected in specimens of three (3). Of which, one (33.3%) was RSV A, one (33.3%) RSV B and results were pending for one (33.3%). (Fig9, Table6)

Pneumonia surveillance: Since the beginning of 2022, 1 943 specimens were tested and RSV was detected in specimens of 365 (19%) patients. Of which, 121 (33%) were RSV A, 185 (51%) RSV B, two (1%) RSV-AB, and RSV subgroup results were inconclusive for five (1%) and pending for 52 (14%) (Fig14, Table10)

In addition, 32 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.

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

ILI programme: From 3 January 2022 to date, 452 patients were tested and SARS-CoV-2 was detected in 60 (13%) patients. Of the 55 (55/60, 92%) with variant data, 31% (17/55) were Omicron and variant was not assigned for 69% (38/55). (Fig6, Table4)

Viral Watch programme: From 3 January 2022 to date, 72 patients presenting with ILI were tested and SARS-CoV-2 was detected in 27 (38%). Of the 20 (20/27, 74%) with variant data, majority were Omicron variant (15/20, 75%) and variant was not assigned for 25% (5/20). (Fig11, Table8)

Pneumonia surveillance: From 3 January 2022 to date, 1 943 patients with severe respiratory illness (SRI) were tested and SARS-CoV-2 was detected in 173 (9%) patients. Of the 164 (164/173, 95%) with variant data, majority were Omicron variant 60% (98/164), <1% (1/164) Alpha variant, <1% (1/164) Delta variant and the variant was not assigned for 39% (64/164). (Fig17, Table12)

In addition, SARS-CoV-2 was detected in 11 of 32 (34%) specimens from patients who met suspected SARS-CoV-2 case definition but did not meet the pneumonia/ILI surveillance case definitions. Of which 64% (7/11) were Omicron variant and variant was not assigned for 36% (4/11).

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

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

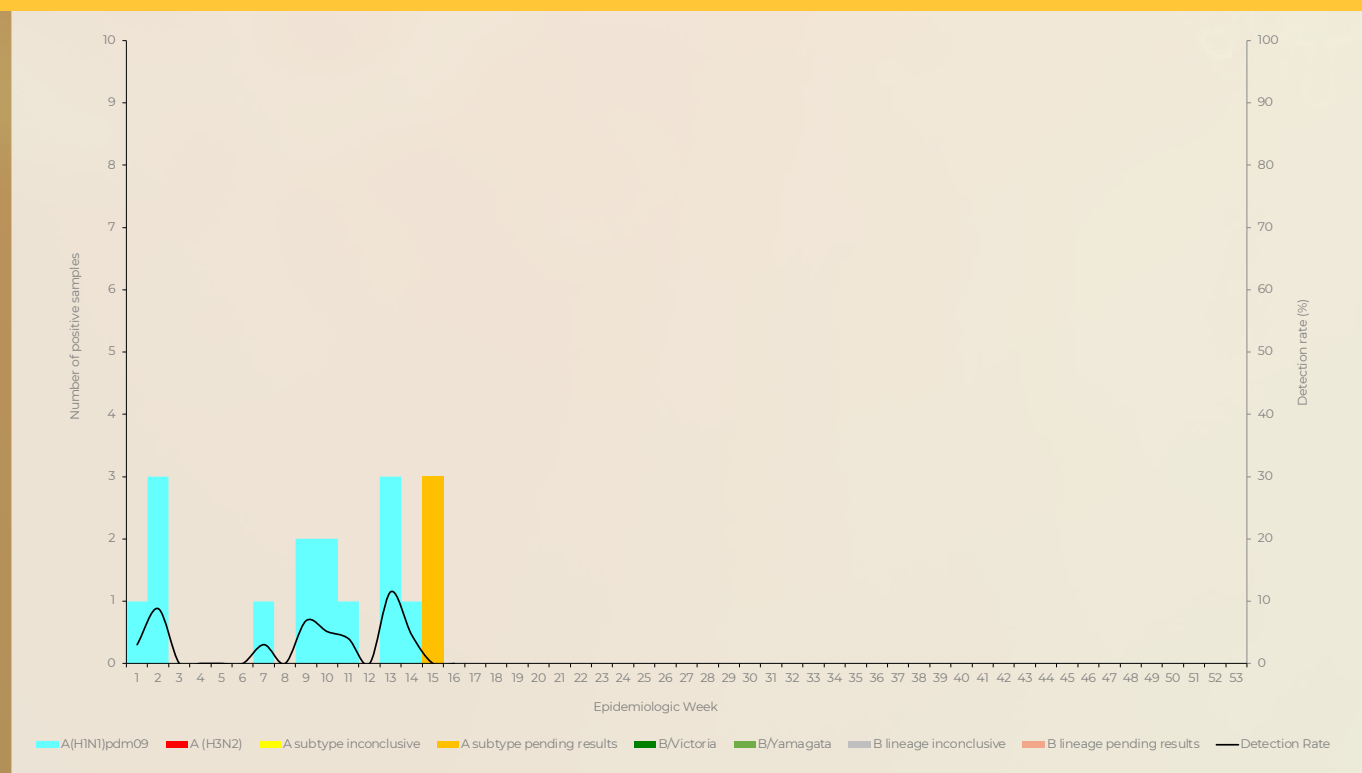


Figure 1. Number of influenza positive cases* by influenza subtype and lineage** and detection rate*** by week, Influenza-like illness (ILI) surveillance in primary health care clinics, 03/01/2022 – 24/04/2022

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

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

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

**Influenza A(H1N1)pdm09 and influenza B(Victoria) were detected in two (10%) of 21 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 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, 03/01/2022 – 24/04/2022

| 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) | 3 | 0 | 0 | 3 | 0 | 0 | 0 | 0 | 88 |
| Eastridge (WC) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 79 |
| Edendale Gateway (KZ) | 11 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 138 |
| Jouberton (NW) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 127 |
| Mitchell's Plain (WC) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 20 |
| Total: | 14 | 0 | 0 | 3 | 0 | 0 | 0 | 0 | 452 |

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

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

[§]Influenza A subtype or B lineage results are pending

**Influenza A(H1N1)pdm09 and influenza B(Victoria) were detected in two (10%) of 21 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, RESPIRATORY SYNCYTIAL VIRUS AND SARS-CoV-2 SURVEILLANCE REPORT

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



Figure 2. Influenza percentage detections and epidemic thresholds* among cases of all ages, Influenza-like illness (ILI) surveillance in primary health care clinics, 03/01/2022 – 24/04/2022

*Thresholds based on 2012-2019 data

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

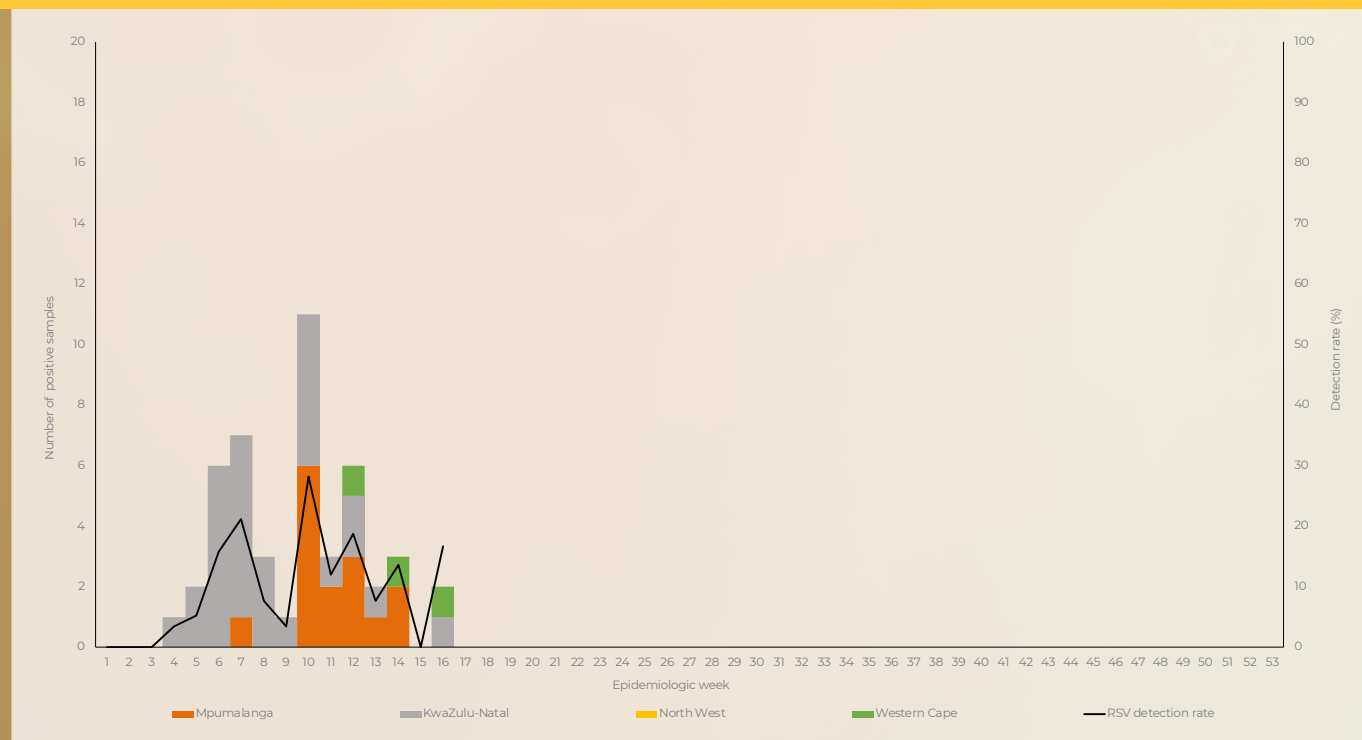


Figure 3. Number of patients testing positive for respiratory syncytial virus* by province and detection rate by week, Influenza-like illness (ILI) surveillance in primary health care clinics, 03/01/2022 – 24/04/2022

*RSV was not detected from 21 specimens of patients who met suspected SARS-CoV-2 case definition but did not meet influenza-like illness (ILI) case definition.

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

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

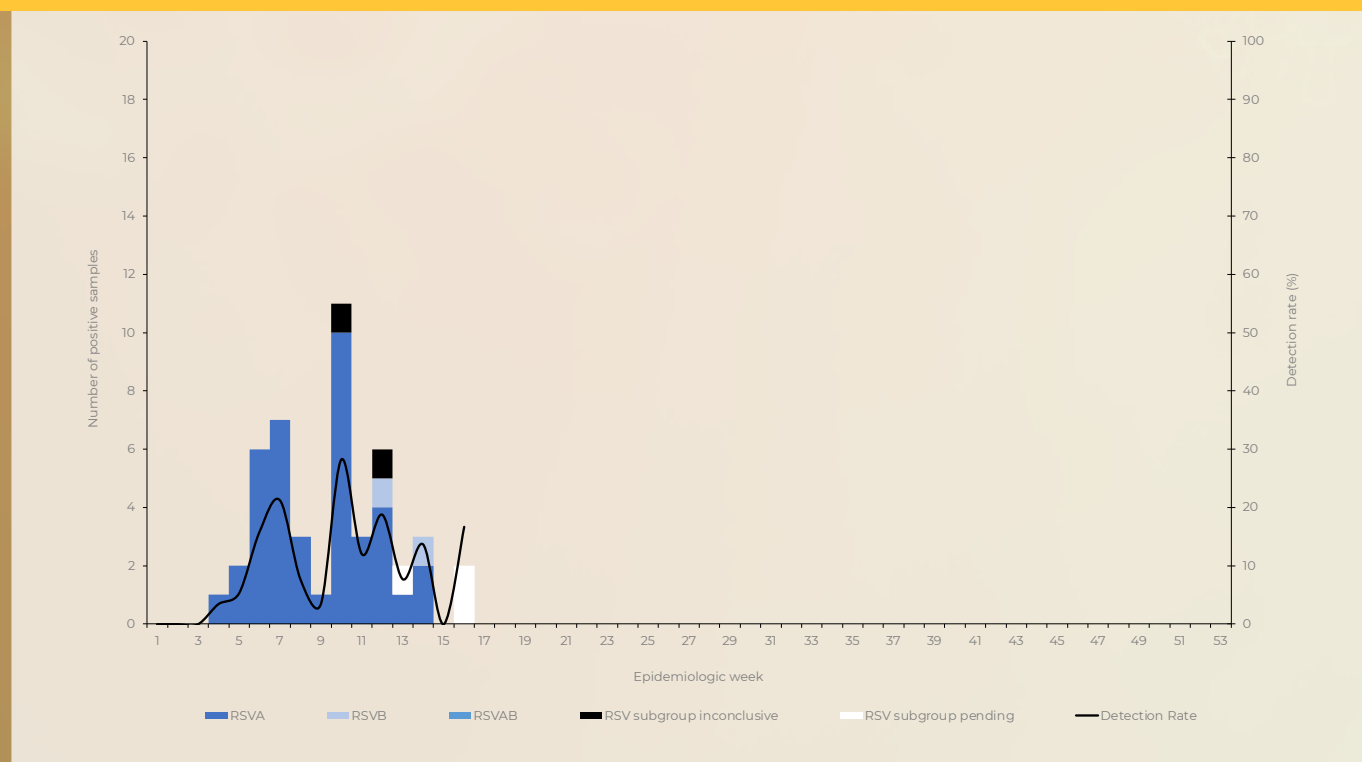


Figure 4. Number of patients testing positive for respiratory syncytial virus* by subgroup and detection rate by week, Influenza-like illness (ILI) surveillance in primary health care clinics, 03/01/2022 – 24/04/2022

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 21 specimens of 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 in primary health care clinics, 03/01/2022 – 24/04/2022

| Clinic (Province) | RSVA | RSVB | RSVAB | RSV subgroup inconclusive | RSV subgroup pending* | Total samples |
|-----------------------|-----------|----------|----------|---------------------------|-----------------------|---------------|
| Agincourt (MP) | 15 | 0 | 0 | 0 | 0 | 88 |
| Eastridge (WC) | 0 | 2 | 0 | 0 | 1 | 79 |
| Edendale Gateway (KZ) | 25 | 0 | 0 | 2 | 2 | 138 |
| Jouberton (NW) | 0 | 0 | 0 | 0 | 0 | 127 |
| Mitchell's Plain (WC) | 0 | 0 | 0 | 0 | 0 | 20 |
| Total | 40 | 2 | 0 | 2 | 3 | 452 |

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 results for subgroups are pending

**RSV was not detected from 21 specimens of 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.

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

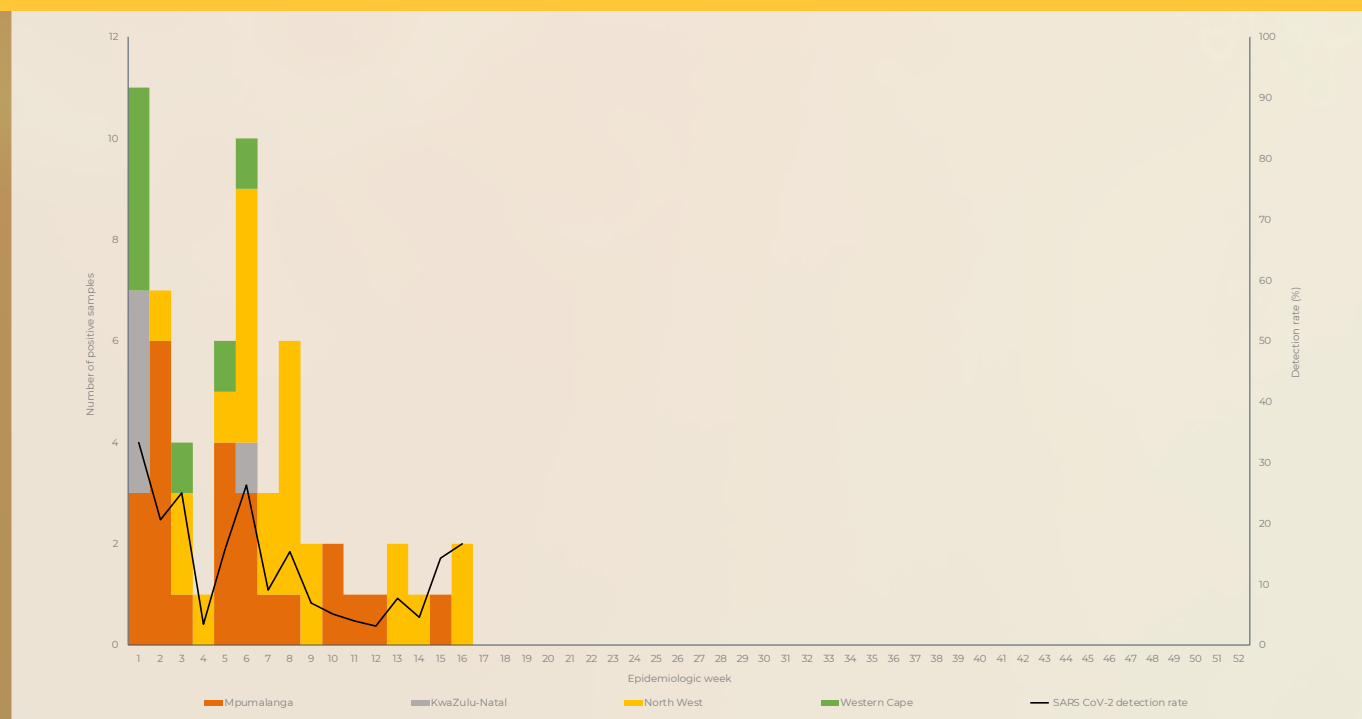


Figure 5. Number of patients testing positive for SARS-CoV-2* by province and detection rate by week, Influenza-like illness (ILI) surveillance in primary health care clinics, 03/01/2022 – 24/04/2022

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

*SARS-CoV-2 was detected in 5 of 21 (24%) 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 3. 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 – 24/04/2022

| Clinic (Province) | SARS-CoV-2 positive | Total samples tested |
|-----------------------|---------------------|----------------------|
| Agincourt (MP) | 24 | 88 |
| Eastridge (WC) | 3 | 79 |
| Edendale Gateway (KZ) | 5 | 138 |
| Jouberton (NW) | 24 | 127 |
| Mitchell's Plain (WC) | 4 | 20 |
| Total: | 60 | 452 |

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

*SARS-CoV-2 was detected in 5 of 21 (24%) 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, RESPIRATORY SYNCYTIAL VIRUS AND SARS-CoV-2 SURVEILLANCE REPORT

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

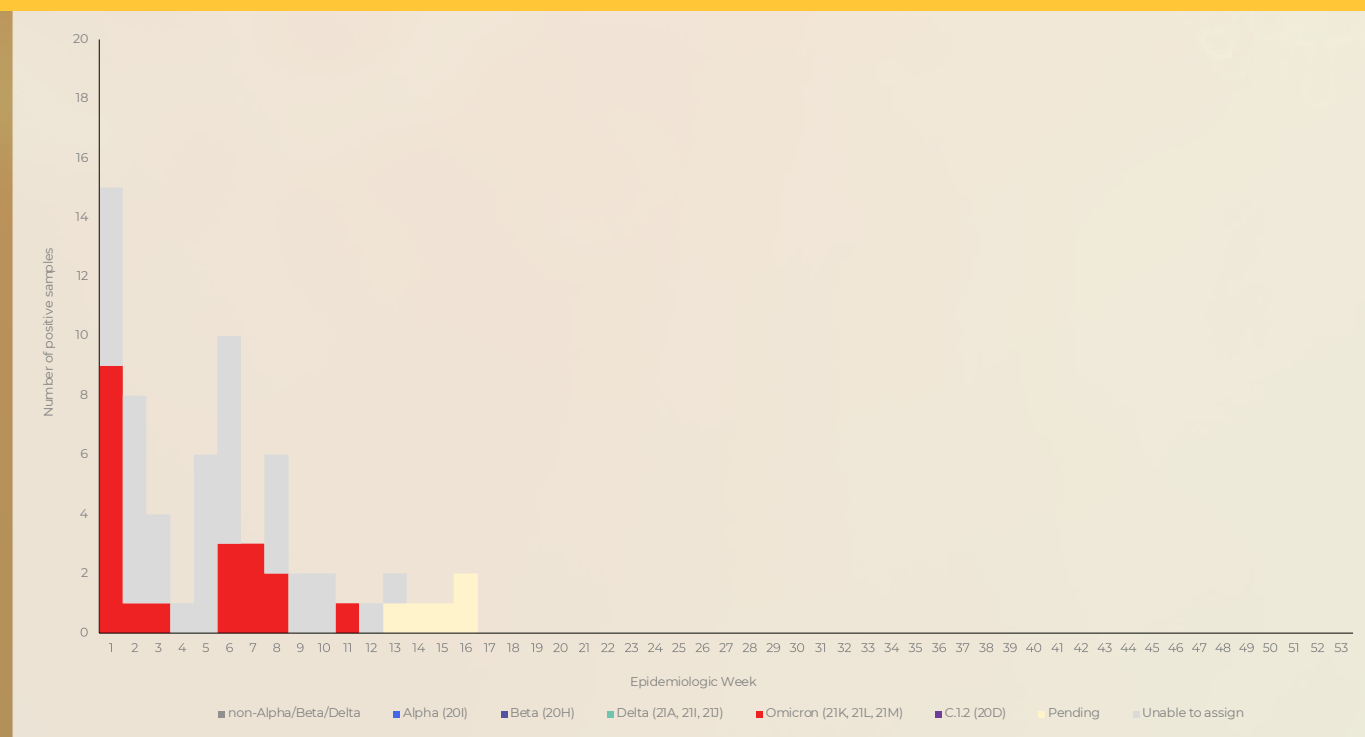


Figure 6. Number and detection rate of laboratory confirmed SARS-CoV-2* cases by variant type (variant PCR/sequencing) and week, Influenza-like illness (ILI) surveillance in primary health care clinics, 03/01/2022 – 24/04/2022

*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 4. 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 – 24/04/2022

| 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 | 7 | 1 | 18 | 26 |
| 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 | 4 | 15 | 25 |
| Mitchell's Plain (WC) | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 2 | 4 |
| Total: | 0 | 0 | 0 | 0 | 0 | 20 | 5 | 40 | 65 |

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

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INFLUENZA-LIKE ILLNESS (ILI) SURVEILLANCE VIRAL WATCH

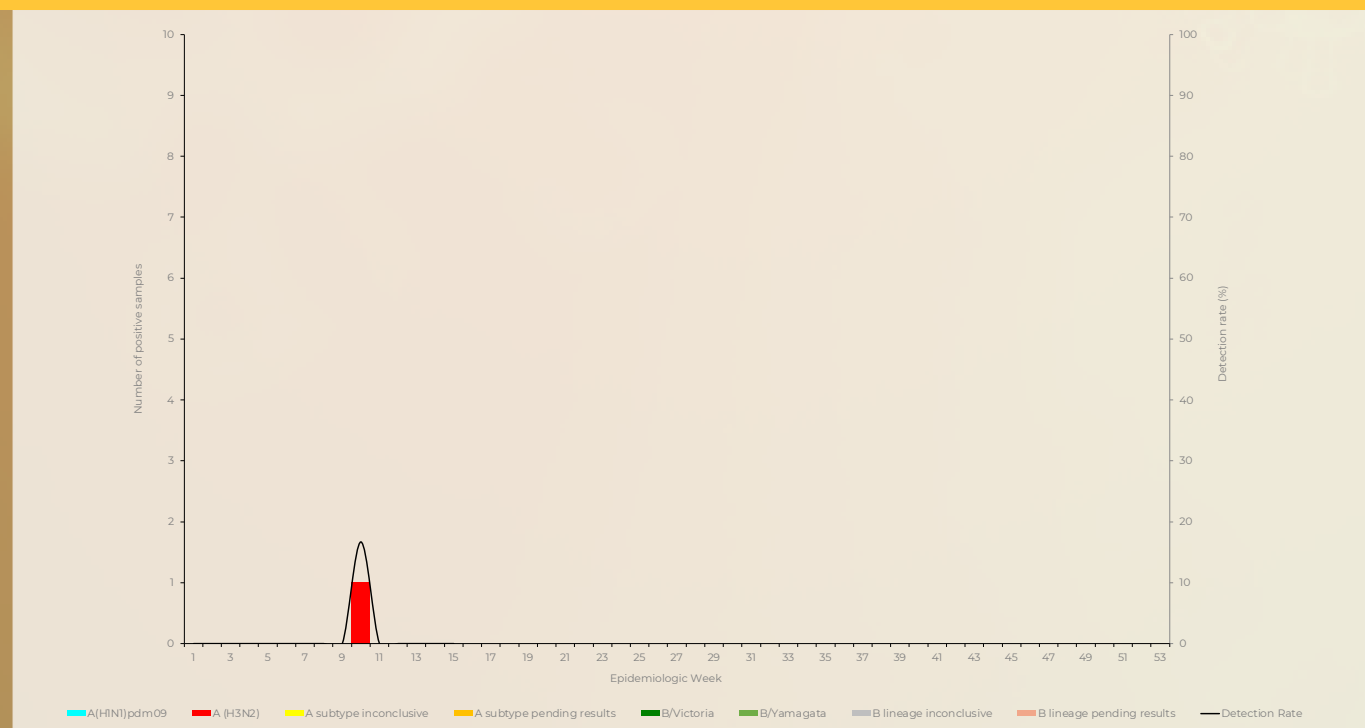


Figure 7. Number of positive patients* by influenza subtype and lineage and detection rate** by week, ILI surveillance - Viral Watch, 03/01/2022 – 24/04/2022

*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 5. 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 – 24/04/2022

| 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 | 46 |
| 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 | 22 |
| Total: | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 72 |

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

*Influenza A subtype or B lineage results are pending

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

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INFLUENZA-LIKE ILLNESS (ILI) SURVEILLANCE VIRAL WATCH

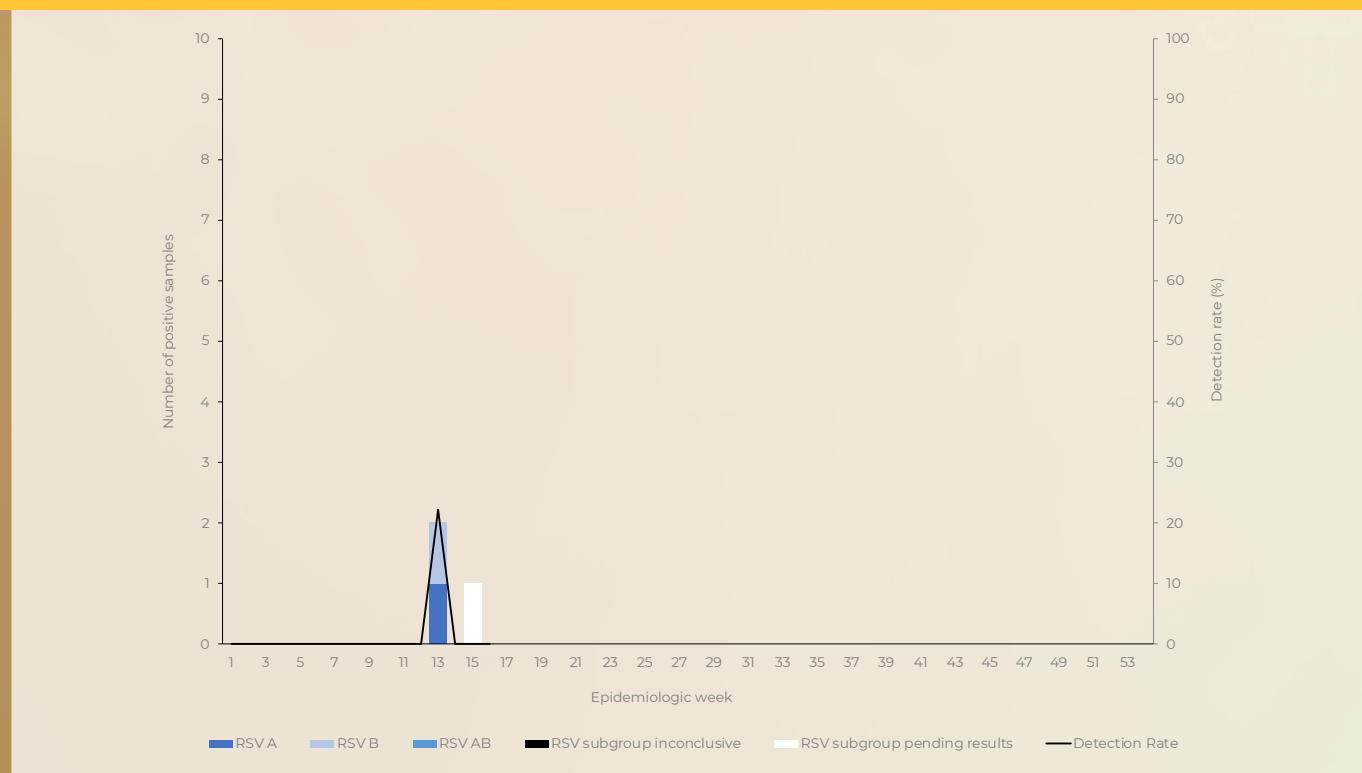


Figure 8. Number of RSV positive cases testing positive for respiratory syncytial virus (RSV)* by subgroup and detection rate** by week, ILI surveillance - Viral Watch, 03/01/2022 – 24/04/2022

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

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

Table 6. Number of RSV positive cases identified and total number of samples tested by province, ILI surveillance - Viral Watch, 03/01/2022 – 24/04/2022

| 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 | 1 | 0 | 0 | 0 | 0 | 46 |
| 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 | 1 | 22 |
| Total: | 1 | 1 | 0 | 0 | 1 | 72 |

*RSV results for subgroups are pending

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

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

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INFLUENZA-LIKE ILLNESS (ILI) SURVEILLANCE VIRAL WATCH

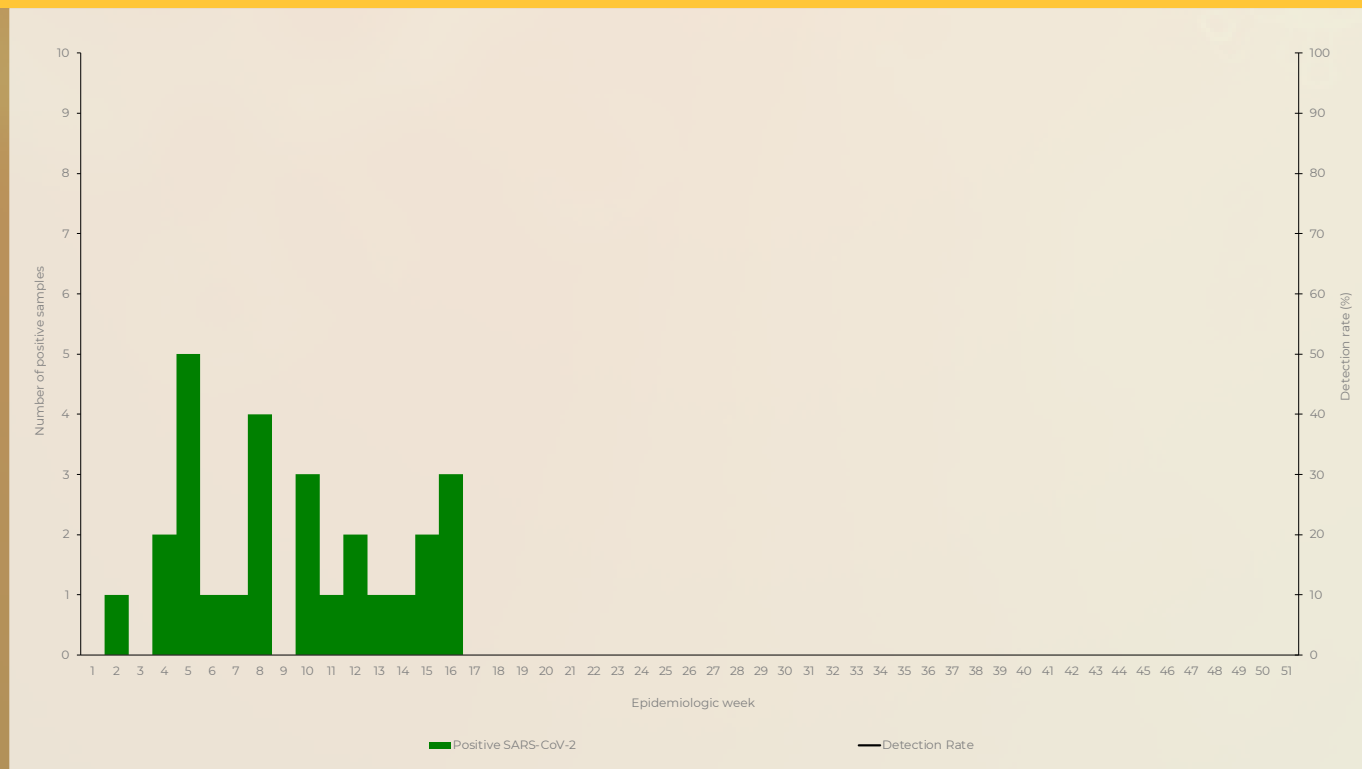


Figure 9. Number of patients testing positive for SARS-CoV-2*, by site and detection rate** by week, ILI surveillance - Viral Watch, 03/01/2022 – 24/04/2022

*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 SARS-CoV-2 positive cases identified and total number tested by province, ILI surveillance - Viral Watch, 03/01/2022 – 24/04/2022

| Province | SARS-CoV-2 positive | Total samples tested |
|---------------|---------------------|----------------------|
| Eastern Cape | 1 | 2 |
| Free State | 0 | 0 |
| Gauteng | 18 | 46 |
| Limpopo | 0 | 0 |
| Mpumalanga | 0 | 0 |
| North West | 0 | 2 |
| Northern Cape | 0 | 0 |
| Western Cape | 8 | 22 |
| Total: | 27 | 72 |

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

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INFLUENZA-LIKE ILLNESS (ILI) SURVEILLANCE VIRAL WATCH

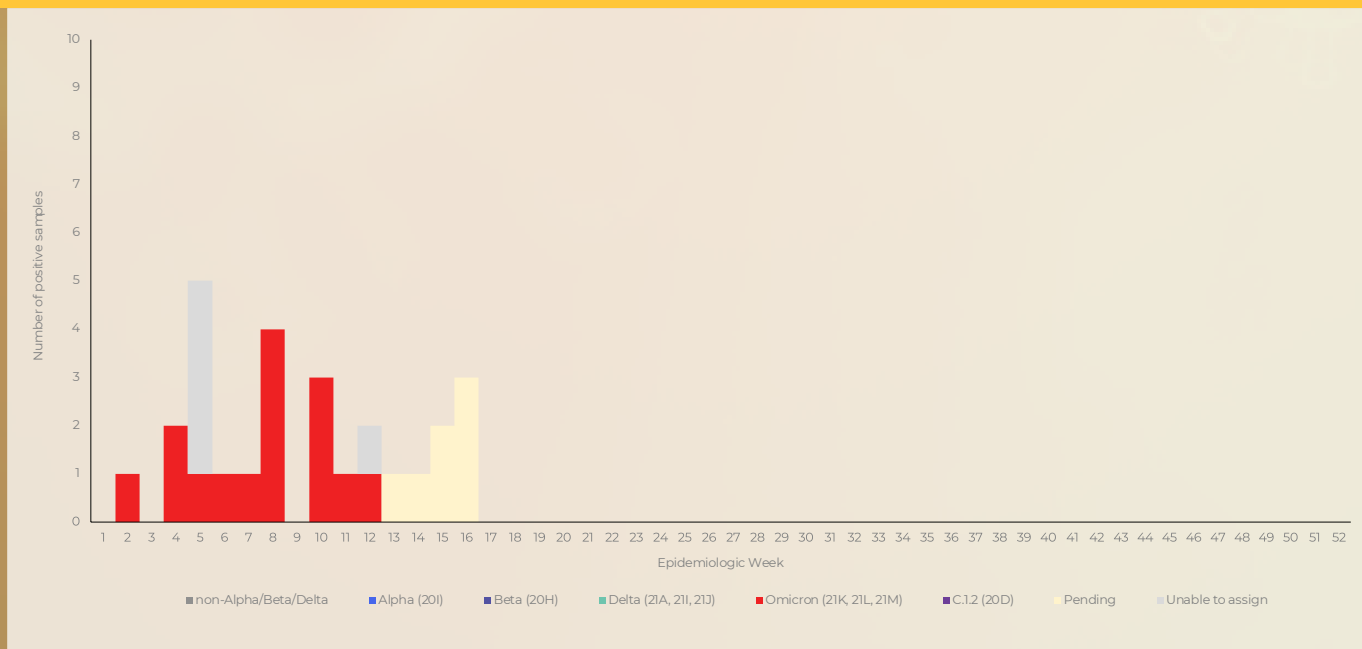


Figure 10. Number and detection rate of laboratory confirmed SARS-CoV-2* cases by variant type (variant PCR/sequencing) and week, ILI surveillance - Viral Watch, 03/01/2022 – 24/04/2022

*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 8. 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 – 24/04/2022

| 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 | 9 | 4 | 5 | 18 |
| 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 | 5 | 3 | 0 | 8 |
| Total: | 0 | 0 | 0 | 0 | 0 | 15 | 7 | 5 | 27 |

*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

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NATIONAL SYNDROMIC SURVEILLANCE FOR PNEUMONIA

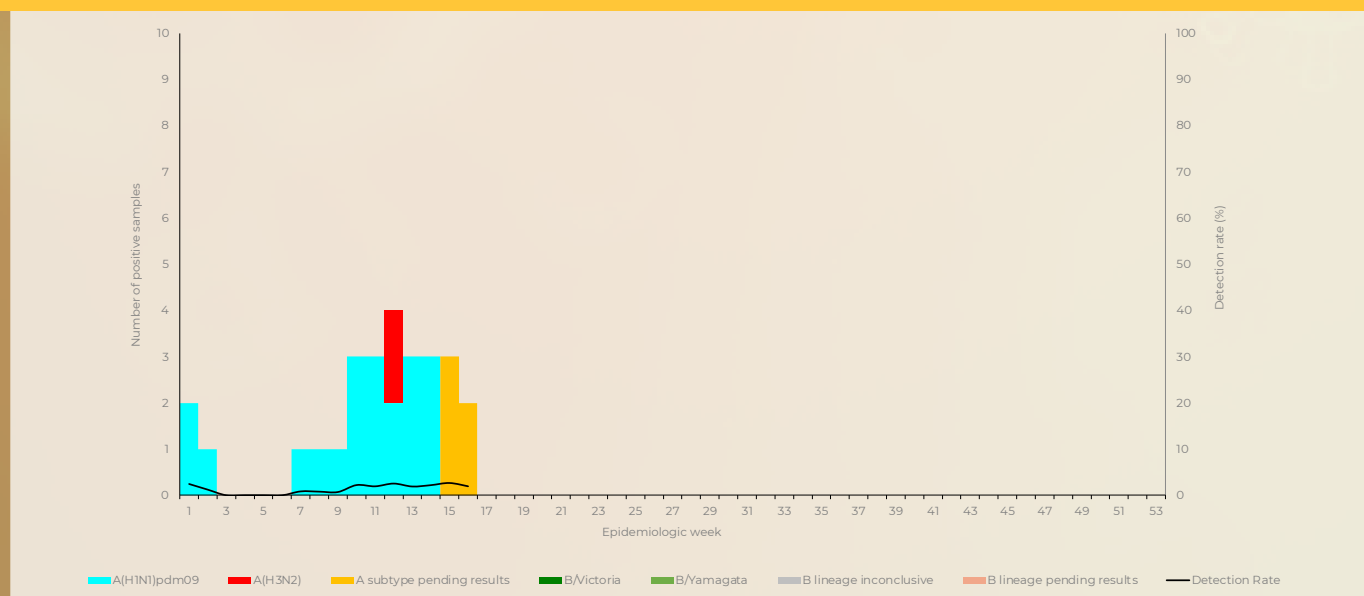


Figure 11. Number of positive influenza positive cases* by influenza subtype and lineage** and detection rate*** by week, pneumonia surveillance public hospitals, 03/01/2022 – 24/04/2022

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

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

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

**Influenza was not detected in 11 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 9. 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 – 24/04/2022

| 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) | 14 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 377 |
| Helen Joseph-Rahima Moosa (GP) | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 516 |
| Klerksdorp-Tshepong (NW) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 159 |
| Mapulaneng-Matikwana (MP) | 2 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 168 |
| Red Cross (WC) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 357 |
| Mitchell's Plain (WC) | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 221 |
| Tembisa (GP) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 35 |
| Tintswalo (MP) | 3 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 107 |
| Tygerberg (WC) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3 |
| Total: | 20 | 2 | 0 | 5 | 0 | 0 | 0 | 0 | 1 943 |

GP: Gauteng (Tembisa started enrolling on the 10th March 2022); 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 11 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.

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NATIONAL SYNDROMIC SURVEILLANCE FOR PNEUMONIA

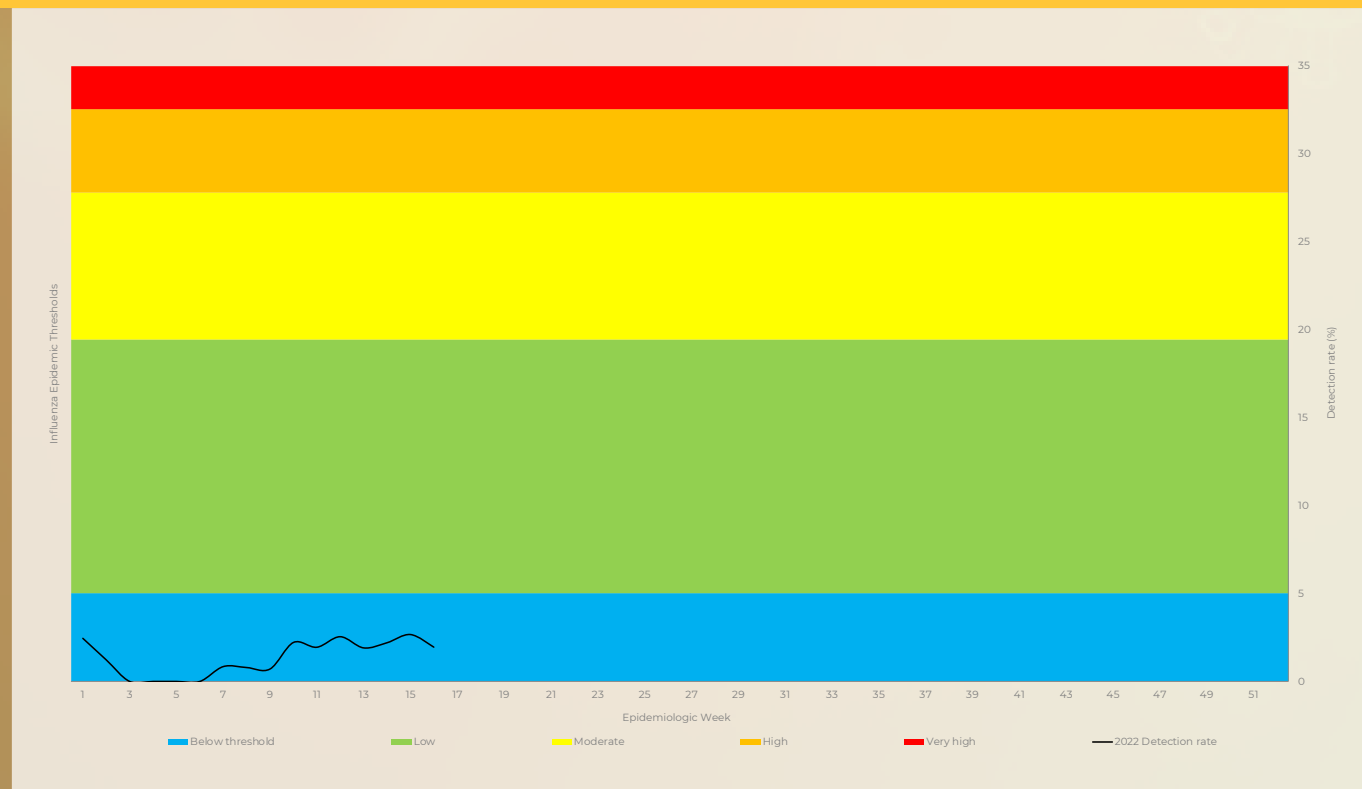


Figure 12. Influenza percentage detections and epidemic thresholds* among cases of all ages, pneumonia surveillance public hospitals, 03/01/2022 – 24/04/2022

*Thresholds based on 2010-2019 data

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

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NATIONAL SYNDROMIC SURVEILLANCE FOR PNEUMONIA

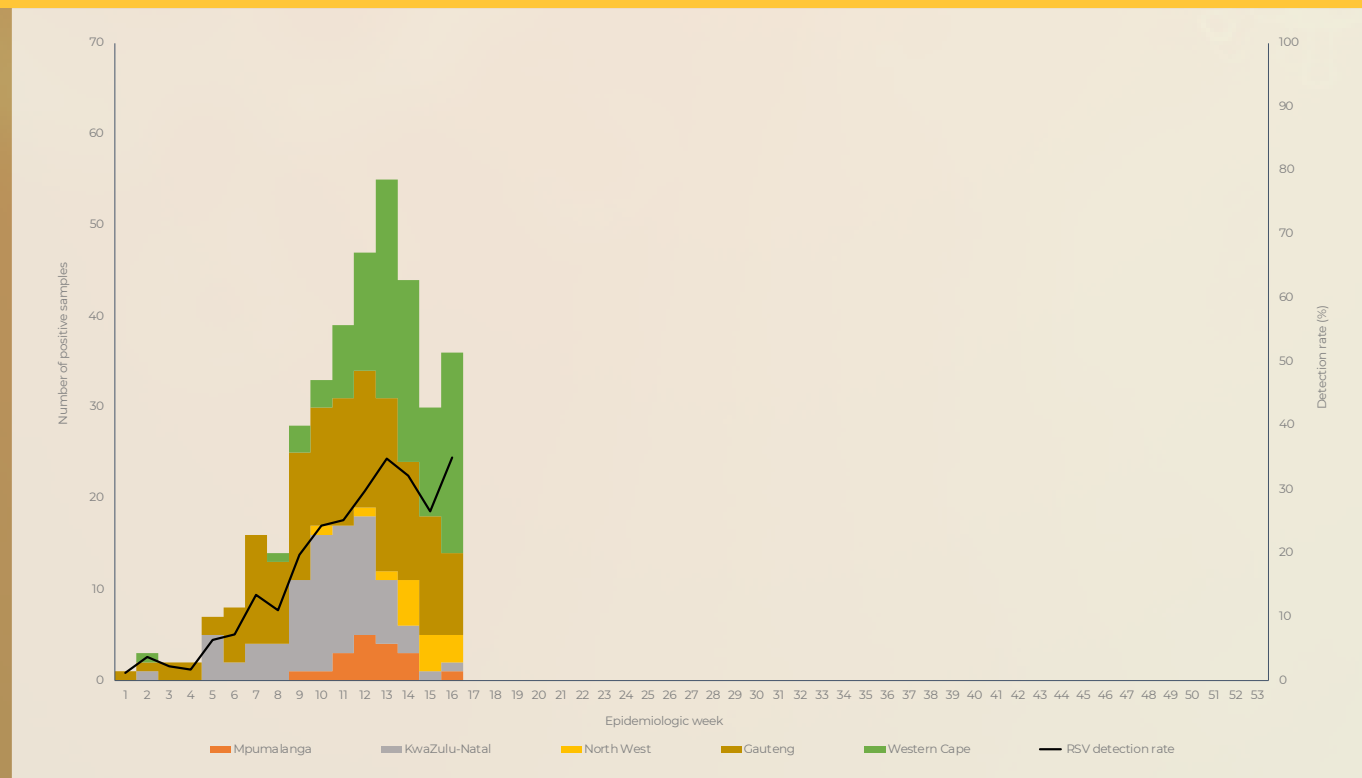


Figure 13. Number of patients (all ages) testing positive for respiratory syncytial virus* by province and detection rate by week, pneumonia surveillance public hospitals, 03/01/2022 – 24/04/2022

*RSV was not detected in 11 specimens from patients who met suspected SARS-CoV-2 case definition but did not meet pneumonia (SRI) case definition.

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NATIONAL SYNDROMIC SURVEILLANCE FOR PNEUMONIA

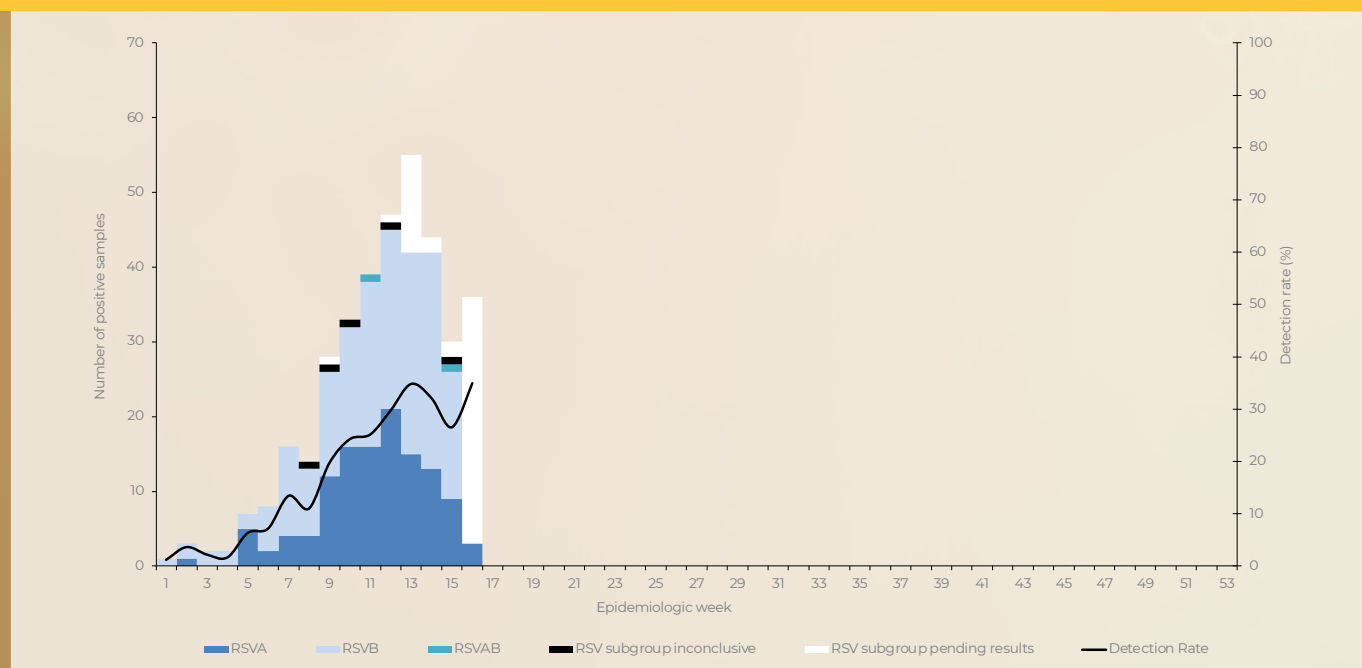


Figure 14. Number of patients (all ages) testing positive for respiratory syncytial virus* by subgroup and detection rate by week, pneumonia surveillance public hospitals, 03/01/2022 – 24/04/2022

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 11 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 patients (all ages) positive for respiratory syncytial virus subgroups** by subgroups identified and total number of samples tested by hospital, pneumonia surveillance public hospitals, 03/01/2022 – 24/04/2022

| Hospital (Province) | RSVA | RSVB | RSVAB | RSV subgroup inconclusive | RSV subgroup pending* | Total samples |
|--------------------------------|------------|------------|----------|---------------------------|-----------------------|---------------|
| Edendale (KZ) | 70 | 1 | 0 | 2 | 7 | 377 |
| Helen Joseph-Rahima Moosa (GP) | 21 | 104 | 2 | 1 | 16 | 516 |
| Klerksdorp-Tshepong (NW) | 5 | 8 | 0 | 0 | 2 | 159 |
| Mapulaneng-Matikwana (MP) | 4 | 2 | 0 | 0 | 2 | 168 |
| Red Cross (WC) | 16 | 48 | 0 | 0 | 18 | 357 |
| Mitchell's Plain (WC) | 2 | 17 | 0 | 0 | 6 | 221 |
| Tembisa (GP) | 0 | 1 | 0 | 0 | 0 | 35 |
| Tintswalo (MP) | 3 | 4 | 0 | 2 | 1 | 107 |
| Tygerberg (WC) | 0 | 0 | 0 | 0 | 0 | 3 |
| Total: | 121 | 185 | 2 | 5 | 52 | 1 943 |

GP: Gauteng (Tembisa started enrolling on the 10th March 2022); 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 11 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.

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NATIONAL SYNDROMIC SURVEILLANCE FOR PNEUMONIA



Figure 15. RSV percentage detections and epidemic thresholds* among children aged < 5 years, pneumonia surveillance public hospitals, 03/01/2022 – 24/04/2022

*Thresholds based on 2010-2019 data

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NATIONAL SYNDROMIC SURVEILLANCE FOR PNEUMONIA

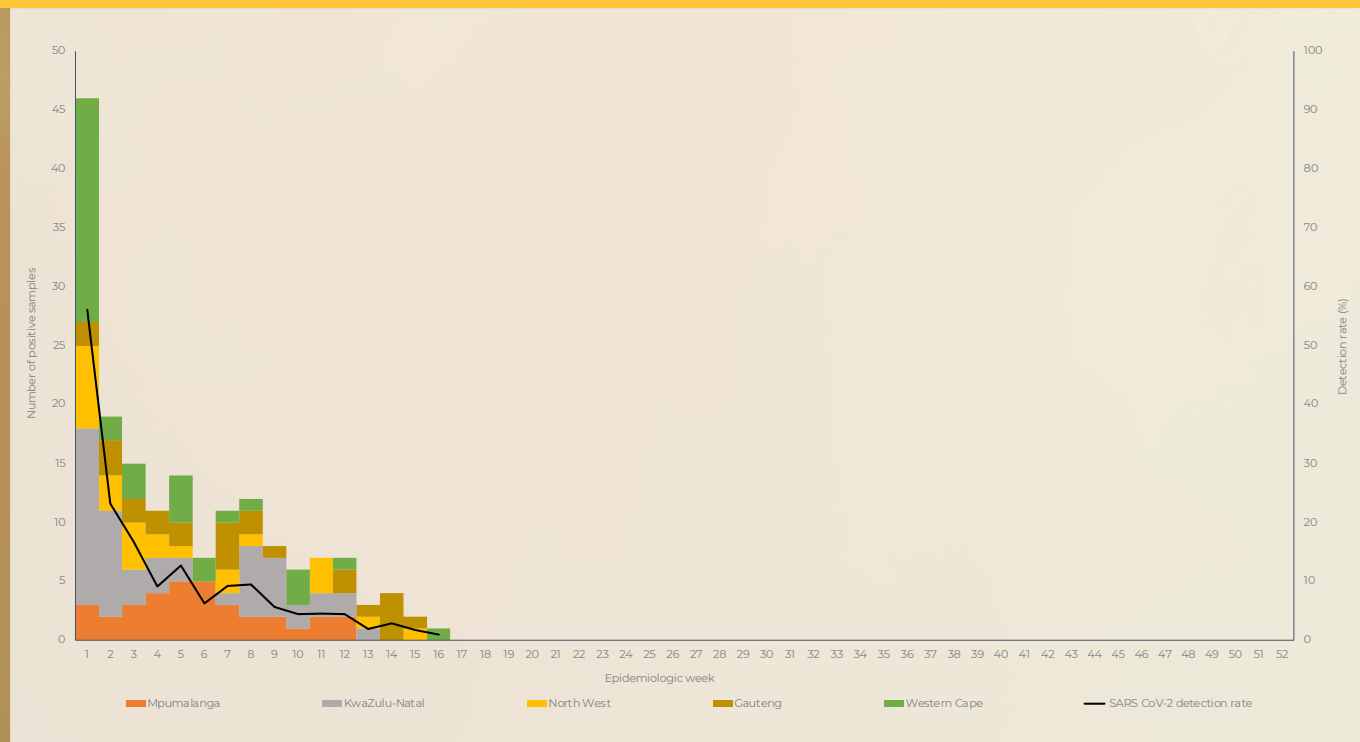


Figure 16. Number of patients testing positive for SARS-CoV-2* by province and detection rate by week, pneumonia surveillance public hospitals, 03/01/2022 – 24/04/2022

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

*SARS-CoV-2 was detected in 6 of 11 (55%) 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 SARS-CoV-2* and total number of samples tested by hospital, pneumonia surveillance public hospitals, 03/01/2022 – 24/04/2022

| Hospital (Province) | SARS-CoV-2 positive | Total samples tested |
|--------------------------------|---------------------|----------------------|
| Edendale (KZ) | 51 | 377 |
| Helen Joseph-Rahima Moosa (GP) | 25 | 516 |
| Klerksdorp-Tshepong (NW) | 25 | 159 |
| Mapulaneng-Matikwana (MP) | 23 | 168 |
| Red Cross (WC) | 15 | 357 |
| Mitchell's Plain (WC) | 22 | 221 |
| Tembisa (GP) | 1 | 35 |
| Tintswalo (MP) | 11 | 107 |
| Tintswalo (MP) | 0 | 3 |
| Total: | 173 | 1 943 |

GP: Gauteng (Tembisa started enrolling on the 10th March 2022); KZ: KwaZulu-Natal; NW: North West; MP: Mpumalanga; WC: Western Cape

*SARS-CoV-2 was detected in 6 of 11 (55%) 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.

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NATIONAL SYNDROMIC SURVEILLANCE FOR PNEUMONIA

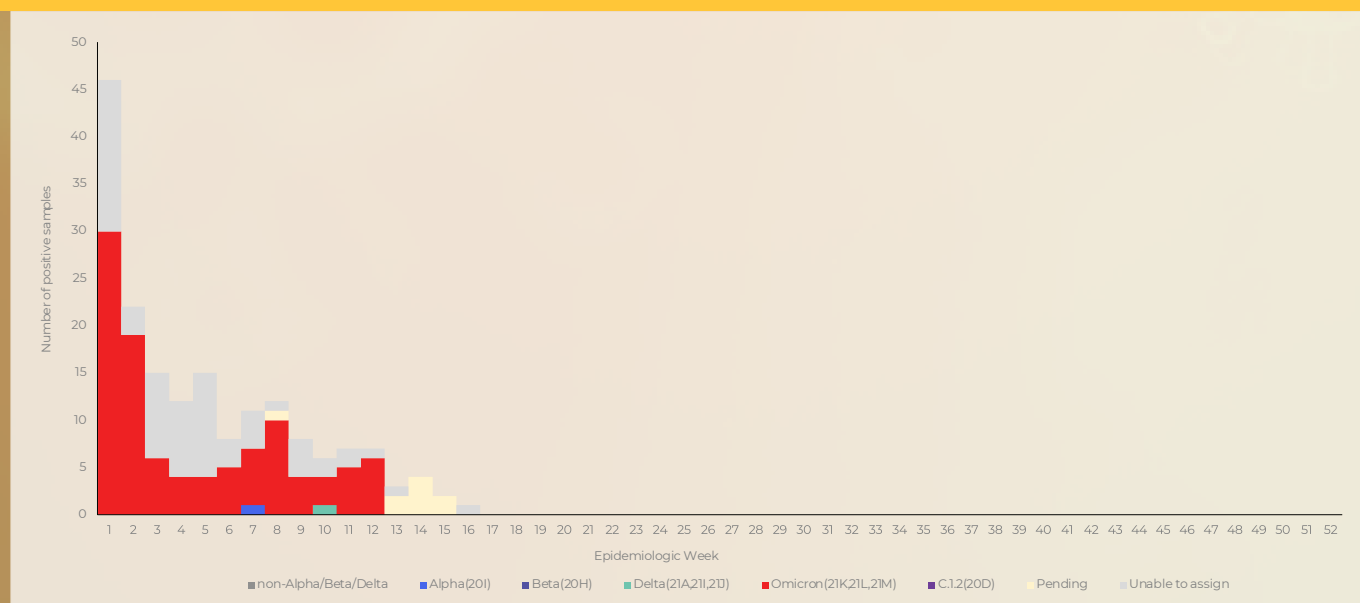


Figure 17. Number and detection rate of laboratory confirmed SARS-CoV-2 cases* by variant type (variant PCR/sequencing), pneumonia surveillance public hospitals, 03/01/2022 – 24/04/2022

*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

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 12. 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 – 24/04/2022

| Hospital (Province) | Non-Alpha/ Beta/Delta | 20I (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 | 1 | 0 | 37 | 1 | 16 | 55 |
| Helen Joseph-Rahima Moosa (GP) | 0 | 1 | 0 | 0 | 0 | 12 | 6 | 6 | 25 |
| Klerksdorp-Tshepong (NW) | 0 | 0 | 0 | 0 | 0 | 13 | 1 | 11 | 25 |
| Mapulaneng- Matikwana (MP) | 0 | 0 | 0 | 0 | 0 | 12 | 1 | 12 | 25 |
| Red Cross (WC) | 0 | 0 | 0 | 0 | 0 | 8 | 0 | 7 | 15 |
| Mitchell's Plain (WC) | 0 | 0 | 0 | 0 | 0 | 12 | 0 | 10 | 22 |
| Tembisa (GP) | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 |
| Tintswalo (MP) | 0 | 0 | 0 | 0 | 0 | 7 | 0 | 4 | 11 |
| Tygerberg (WC) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Total: | 0 | 1 | 0 | 1 | 0 | 102 | 9 | 66 | 179 |

GP: Gauteng (Tembisa started enrolling on the 10th March 2022); 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

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

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SUMMARY OF LABORATORY CONFIRMED SARS-COV-2 CASES

Table 13. Characteristics of individuals with laboratory-confirmed SARS-CoV-2, enrolled in influenza-like illness (ILI) and pneumonia surveillance programmes, South Africa, 3 January 2022 – 24 April 2022

| Characteristic | Influenza-like illness (ILI), public-sector, n=65 (%) | Pneumonia, n=179 (%) |
|----------------------------------|---|----------------------|
| Age group (years) | | |
| 0-9 | 13/65 (20) | 42/179 (23) |
| 10-19 | 9/65 (14) | 5/179 (3) |
| 20-39 | 15/65 (23) | 51/179 (28) |
| 40-59 | 21/65 (32) | 45/179 (25) |
| 60-79 | 6/65 (9) | 30/179 (17) |
| ≥80 | 1/65 (2) | 6/179 (3) |
| Sex-female | 39/65 (60) | 97/179 (54) |
| Province* | | |
| Gauteng | N/A | 26/179 (15) |
| KwaZulu-Natal | 7/65 (11) | 55/179 (31) |
| Mpumalanga | 26/65 (40) | 36/179 (20) |
| North West | 25/65 (38) | 25/179 (14) |
| Western Cape | 7/65 (11) | 37/179 (21) |
| Race | | |
| Black | 48/65 (74) | 149/178 (83) |
| Coloured | 8/65 (12) | 27/178 (15) |
| Asian/Indian | 0/65 (0) | 0/178 (0) |
| White | 9/65 (14) | 1/178 (1) |
| Other | 0/65 (0) | 1/178 (1) |
| Variant | | |
| Non-Alpha/Beta/Delta | 0/65 (0) | 0/179 (0) |
| Alpha(20I) | 0/65 (0) | 1/179 (1) |
| Beta(20H) | 0/65 (0) | 0/179 (0) |
| Delta(21A, 21I, 21J) | 0/65 (0) | 1/179 (1) |
| C.1.2(20D) | 0/65 (0) | 0/179 (0) |
| Omicron(21K,21L,21M) | 20/65 (31) | 102/179 (57) |
| Pending results ^{\$} | 5/65 (8) | 9/179 (5) |
| Unable to assign ^{\$\$} | 40/65 (62) | 66/179 (37) |

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

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| Characteristic | Influenza-like illness (ILI), public-sector, n=65 (%) | Pneumonia, n=179 (%) |
|------------------------------|---|----------------------|
| Presentation | | |
| Fever | 48/65 (74) | 73/178 (41) |
| Cough | 64/65 (98) | 158/178 (89) |
| Shortness of breath | 28/65 (43) | 108/178 (61) |
| Chest pain | 27/65 (42) | 72/178 (40) |
| Diarrhoea | 7/65 (11) | 21/178 (12) |
| Underlying conditions | | |
| Hypertension | 12/65 (18) | 31/178 (17) |
| Cardiac | 1/65 (2) | 4/178 (2) |
| Lung disease | 0/65 (0) | 1/178 (1) |
| Diabetes | 2/65 (3) | 18/178 (10) |
| Cancer | 0/65 (0) | 3/178 (2) |
| Tuberculosis | 0/65 (0) | 17/178 (10) |
| HIV-infection | 9/65 (14) | 67/178 (38) |
| Other ** | 1/65 (2) | 2/178 (1) |
| SARS-CoV-2 Vaccine | | |
| Pfizer-BioNTech (1st dose) | 10/61 (16) | 18/178 (10) |
| Pfizer-BioNTech (2nd dose) | 10/61 (16) | 12/178 (7) |
| Johnson & Johnson | 9/61 (15) | 18/178 (10) |
| Booster | 1/61 (2) | 0/178 (0) |
| Unknown | 4/61 (7) | 2/178 (1) |
| No vaccine | 37/61 (61) | 138/178 (78) |
| Management | | |
| Oxygen therapy | 0/65 (0) | 91/178 (51) |
| ICU admission | N/A | 0/178 (0) |
| Ventilation | N/A | 2/178 (1) |
| Outcome*** | | |
| Died | 0/64 (0) | 12/166 (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

[†]Pending results: outstanding variant results

[§]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 12 patients who died, four were in the 20-39 year age group, four were in 40-59 age group and four were ≥60 years; 7/12 (58%) were female.

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

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METHODS

SARS-CoV-2 Testing

March 2020 – March 2021: SARS-CoV-2 was detected using the Roche E gene real-time PCR assay (Corman et al. Euro Surveillance 2020) with cycle threshold (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/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 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).