



Weekly respiratory pathogens report

Week 49 of 2022

Highlights

- The 2022 influenza season started in week 17 (week starting 25 April 2022), and ended in week 42 (week starting 16 October) when the influenza detection rate in the pneumonia surveillance programme dropped below 10% and remained below the seasonal threshold for at least two weeks.
- In 2022 to date, 1 158 influenza cases have been detected from all surveillance programmes. The beginning of the 2022 season was dominated by influenza A(H1N1)pdm09, whereas influenza B Victoria was most commonly detected towards the end of the season. Influenza A(H3N2) circulated throughout the season.
- The 2022 respiratory syncytial virus (RSV) season started in week 7 (week starting 14 February 2022) when the RSV detection rate among children under five years of age, in the pneumonia surveillance programme, rose above the seasonal threshold. The season ended in week 26. In 2022 to date, 908 respiratory syncytial virus (RSV) cases have been detected. The detection rate in children under 5 years has been increasing in the past few weeks in pneumonia surveillance. Both RSV A and B have been detected throughout the year.
- In 2022 to date, we detected 121 cases of *Bordetella pertussis*; 76% (92) from Western Cape, 13% (16) from Mpumalanga, 6% (7) from Gauteng, 3% (4) from KwaZulu-Natal and 2% (2) from North West provinces. Detection rate for *B. pertussis* cases in influenza-like illness surveillance (ILI) and in pneumonia surveillance is slightly higher in December compared to November at 5% (December, ILI 0/4 and pneumonia surveillance 4/73) vs 4% (November, ILI 8/142 and pneumonia surveillance 25/618) and remain elevated compared to June detection levels at 0.1% (ILI 0/189, pneumonia surveillance 1/800).
- In 2022 to date, a total of 864 COVID-19 cases were detected from all surveillance programmes. Of the 416 hospitalised cases, with available data on outcome, 7% (31) died. Since week 32 of 2022, COVID-19 cases have remained relatively stable in ILI and pneumonia surveillance programmes. Although the detection rate in the viral watch surveillance programme remains high (19-37%) in weeks 43 through 46, the number of samples submitted is low.
- Of the 864 SARS-CoV-2 specimens sequenced, 578 were assigned a variant. Omicron was the dominant variant 99%, (573/578) of sequences; of which 16% (94/573) was Omicron 21K/BA.1, 14% (78/573) was Omicron 21L/BA.2, 0.3% (2/573) was Omicron 21M/BA.3, 28% (159/573) was Omicron 22A/BA.4, 41% (236/573) was Omicron 22B/BA.5, 0.2% (1/573) was Omicron 22C/BA.2.12.1, and 0.2% (1/573) Omicron (22E/BQ.1.1). Alpha, Delta, C.1.2 (20D), and XAY variants contributed <1% each. The remaining 248/864 (29%) could not be assigned a variant, due to a low viral load or insufficient sample.
- A lower number of specimens was submitted in week 30 (31 July – 6 August 2022) due to staff training this likely affected numbers and proportions of viruses detected during this week, therefore trends should be regarded with caution.

Programme Descriptions

| Programme | Influenza-like illness (ILI) | Viral Watch | National syndromic surveillance for pneumonia |
|--------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Start year | 2012 | 1984 | 2009 |
| Provinces* | KZ NW WC MP | EC FS GP LP MP NC NW WC | EC GP KZ MP NW WC |
| Type of site | Primary health care clinics | General practitioners | Public hospitals |
| Case definition | <p>ILI: An acute respiratory illness with a temperature ($\geq 38^{\circ}\text{C}$) and cough, & onset ≤ 10 days</p> <p>Suspected pertussis Any person with an acute cough illness lasting ≥ 14 days (or cough illness of any duration for children < 1 year), without a more likely diagnosis AND one or more of the following signs or symptoms:</p> <ul style="list-style-type: none"> • paroxysms of coughing, • or inspiratory "whoop", • or post-tussive vomiting • or apnoea in children < 1 year; <p>OR</p> <p>Any person in whom a clinician suspects pertussis</p> <p>Suspected SARS-CoV-2 Any person presenting with an acute (≤ 14 days) respiratory tract infection or other clinical illness compatible with COVID-19**</p> | <p>ILI: An acute respiratory illness with a temperature ($\geq 38^{\circ}\text{C}$) and cough, & onset ≤ 10 days</p> <p>Suspected SARS-CoV-2 Any person presenting with an acute (≤ 14 days) respiratory tract infection or other clinical illness compatible with COVID-19**</p> | <p>SRI: Acute (symptom onset ≤ 10 days) or chronic (symptom onset > 10) lower respiratory tract 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 assay (Corman <i>et al.</i>, <i>Euro Surv</i> 2020) 1 April 2021 to date: Allplex™ SARS-CoV-2/FluA/FluB/RSV PCR kit</p> <p>- positivity assigned if PCR cycle threshold is < 40 for ≥ 1 gene targets (N, S, OR RdRp)</p> | <p>INF and RSV - Fast-Track Diagnostics multiplex real-time reverse transcription polymerase chain reaction (until 31 March 2021)</p> <p>B. pertussis Multiplex real-time PCR (Tatti <i>et al.</i>, <i>J Clin Microbiol</i> 2011) and culture (if PCR cycle threshold ≤ 25)</p> <p>SARS-CoV-2 1 April 2020 – 31 March 2021: Roche E gene real-time PCR assay Corman <i>et al.</i>, <i>Euro Surv</i> 2020) 1 April 2021 to date: Allplex™ SARS-CoV-2/FluA/FluB/RSV PCR kit</p> <p>- positivity assigned if PCR cycle threshold is < 40 for ≥ 1 gene targets (N, S, OR RdRp)</p> | <p>INF and RSV - Fast Track Diagnostics multiplex real-time reverse transcription polymerase chain reaction (until 31 March 2021)</p> <p>B. pertussis Multiplex real-time PCR (Tatti <i>et al.</i>, <i>J Clin Microbiol</i> 2011) and culture (if PCR cycle threshold ≤ 25)</p> <p>SARS-CoV-2 1 April 2020 – 31 March 2021: Roche E gene real-time PCR assay (Corman <i>et al.</i>, <i>Euro Surv</i> 2020) 1 April 2021 to date: Allplex™ SARS-CoV-2/FluA/FluB/RSV PCR kit</p> <p>- positivity assigned if PCR cycle threshold is < 40 for ≥ 1 gene targets (N, S, OR RdRp)</p> |

Epidemic Threshold

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

* EC: Eastern Cape; FS: Free State; GP: Gauteng; KZ: KwaZulu-Natal; LP: Limpopo; MP: Mpumalanga; NC: Northern Cape; NW: North West; WC: Western Cape

Symptoms include ANY of the following respiratory symptoms: cough, sore throat, shortness of breath, anosmia (loss of sense of smell) or dysgeusia (alteration of the sense of taste), with or without other symptoms (which may include fever, weakness, myalgia, or diarrhoea). Testing for SARS-CoV-2 was initiated in all three surveillance programmes in week 10 of 2020 (week starting 2 March 2020).*INF: influenza virus; RSV: respiratory syncytial virus; BP: *Bordetella pertussis*; SARS-CoV-2: severe acute respiratory syndrome coronavirus 2

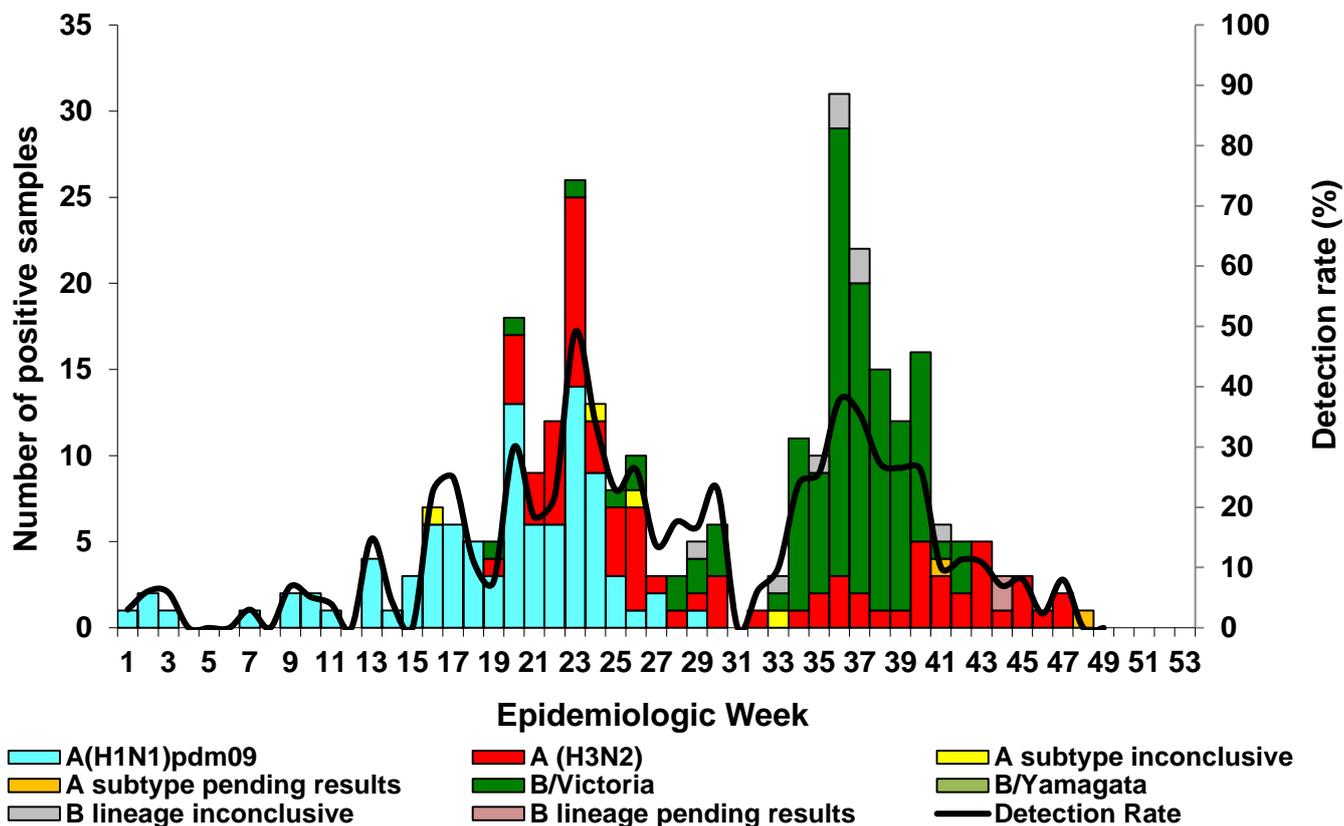


Figure 1. Number of influenza positive cases* by influenza subtype and lineage and detection rate*** by week, influenza-like illness (ILI) surveillance in primary health care clinics, 03/01/2022 – 10/12/2022**

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

**Influenza was detected in two (8%) of 28 specimens from patients who met suspected SARS-CoV-2 or B. pertussis case definition but did not meet influenza-like illness (ILI) case definition. Of which one (50%) was influenza A(H3N2) and one (50%) was influenza B(Victoria). 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

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

Table 1. Number of laboratory-confirmed influenza* cases by subtype and lineage and total number of samples tested by clinic and province, influenza-like illness (ILI) surveillance in primary health care clinics, 03/01/2022 – 10/12/2022

| 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) | 21 | 14 | 0 | 1 | 15 | 0 | 2 | 0 | 275 |
| Eastridge (WC) | 11 | 14 | 0 | 0 | 31 | 0 | 1 | 2 | 320 |
| Edendale Gateway (KZ) | 23 | 31 | 0 | 0 | 34 | 0 | 3 | 0 | 557 |
| Jouberton (NW) | 24 | 10 | 1 | 1 | 26 | 0 | 1 | 0 | 378 |
| Mitchell's Plain (WC) | 15 | 9 | 3 | 0 | 11 | 0 | 1 | 0 | 270 |
| Total: | 94 | 78 | 4 | 2 | 117 | 0 | 8 | 2 | 1800 |

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

*Influenza was detected in two (8%) of 28 specimens from patients who met suspected SARS-CoV-2 or B. pertussis case definition but did not meet influenza-like illness (ILI) case definition. Of which one (50%) was influenza A(H3N2) and one (50%) was influenza B(Victoria). These are not included in the table.

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

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

Two dual infections of influenza B(Victoria) + influenza A(H1N1)pdm09 from Eastridge (WC) and influenza B(Victoria) + influenza A(H3N2) from Agincourt (MP) indicated in both columns.

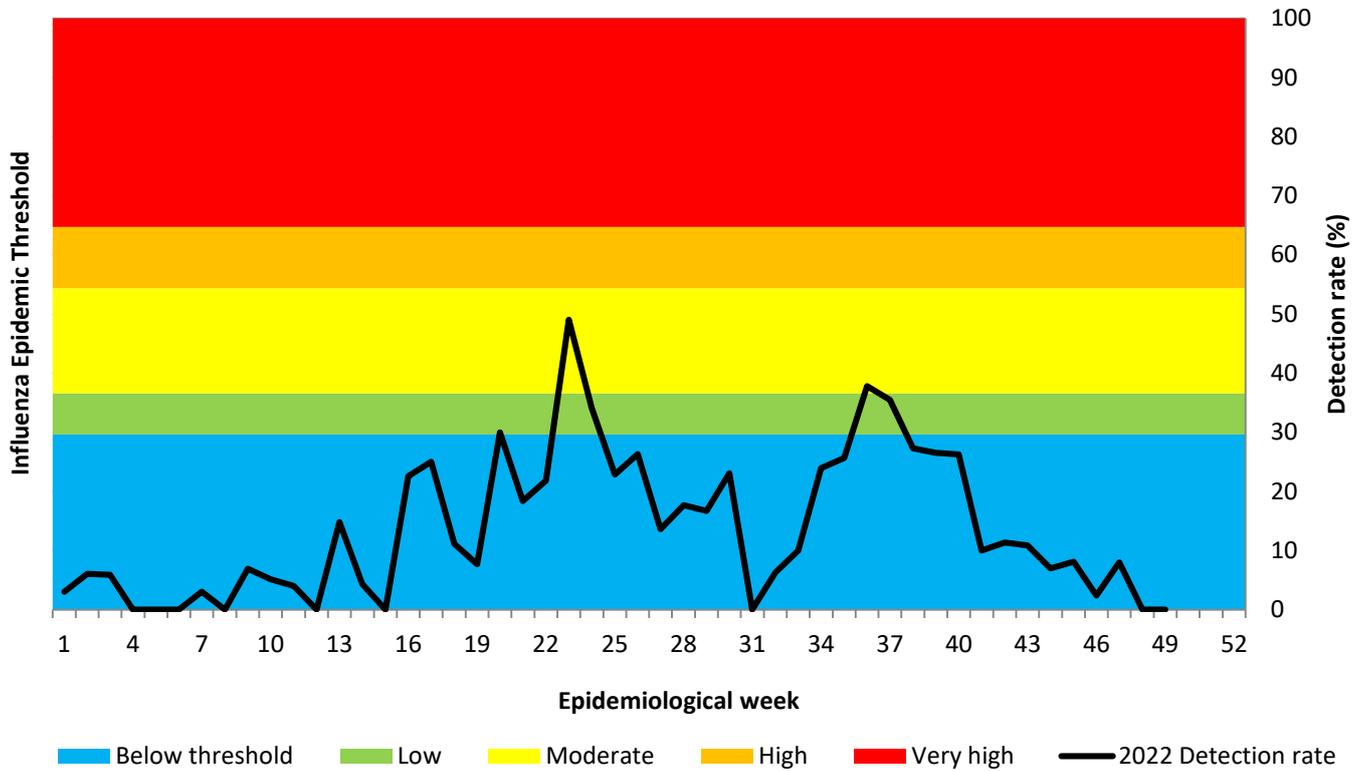


Figure 2. Influenza percentage detections and epidemic thresholds* among cases of all ages, influenza-like illness (ILI) surveillance in primary health care clinics, 03/01/2022 – 10/12/2022

*Thresholds based on 2012-2019 data

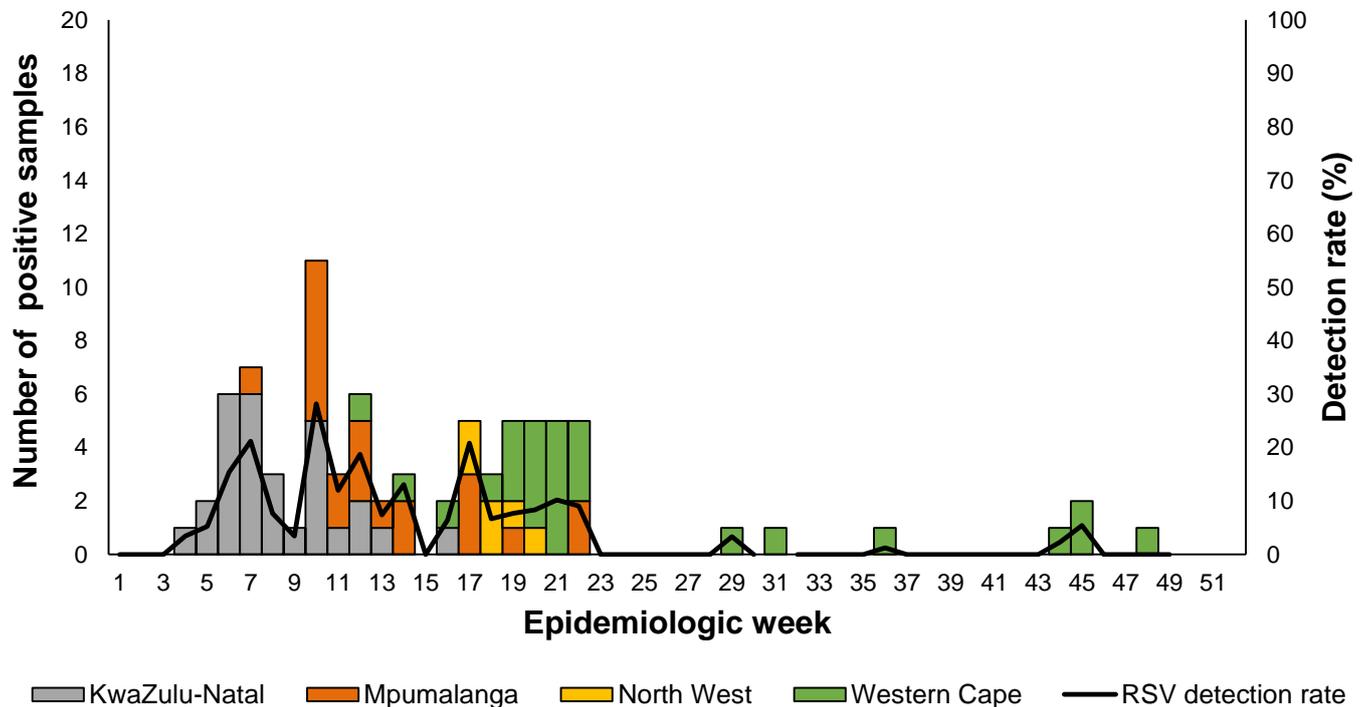


Figure 3. Number of patients testing positive for respiratory syncytial virus* by province and detection rate by week, influenza-like illness (ILI) surveillance in primary health care clinics, 03/01/2022 – 10/12/2022

*RSV was not detected from 28 specimens of patients who met suspected SARS-CoV-2 or *B. pertussis* case definition but did not meet influenza-like illness (ILI) case definition.

**Only reported for weeks with >10 specimens submitted

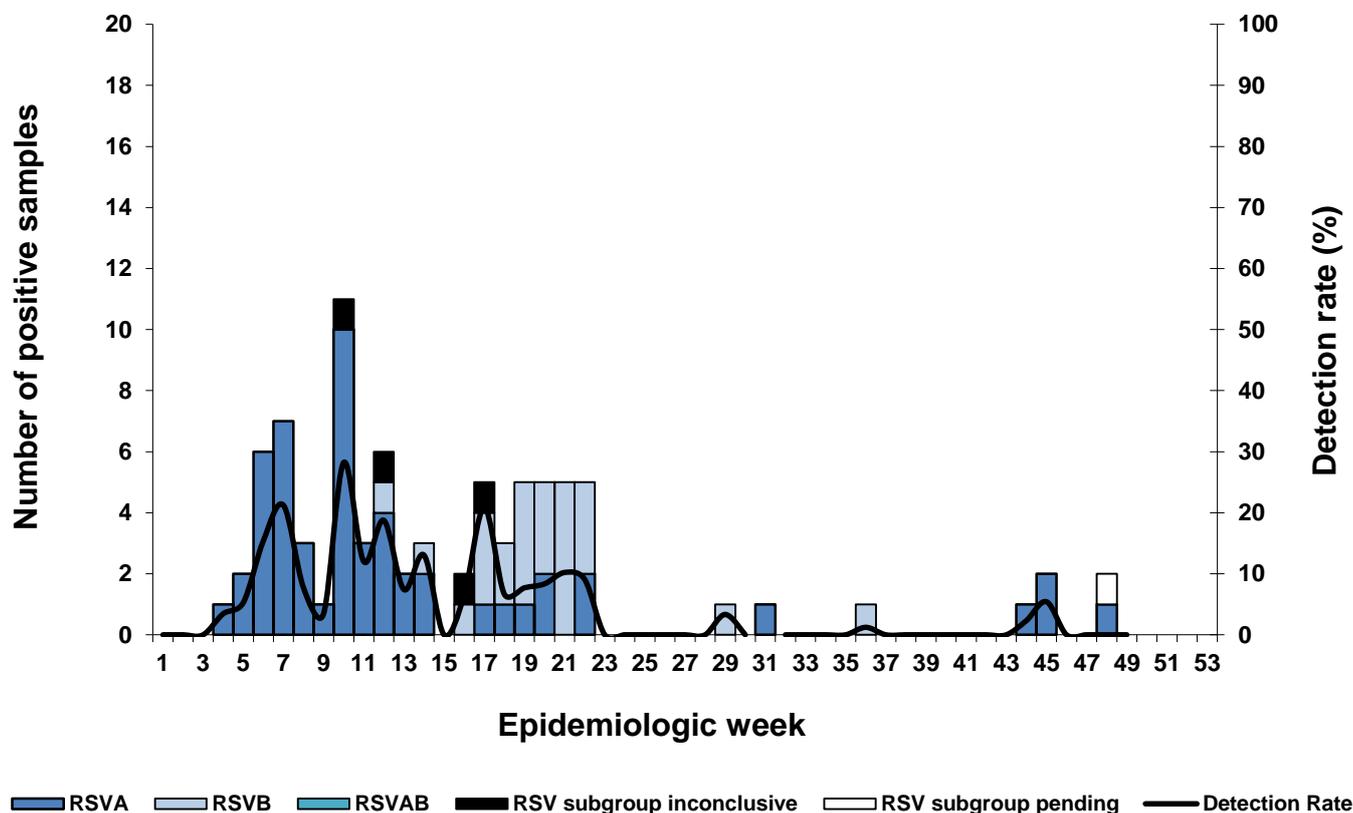


Figure 4. Number of patients testing positive for respiratory syncytial virus* by subgroup and detection rate by week, influenza-like illness (ILI) surveillance in primary health care clinics, 03/01/2022 – 10/12/2022

*RSV was not detected from 28 specimens of patients who met suspected SARS-CoV-2 or *B. pertussis* case definition but did not meet influenza-like illness (ILI) case definition. These are not included in the epidemiological curve.

**Only reported for weeks with >10 specimens submitted

RSV AB: Both RSV A and B subgroups identified.

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

Table 2. Number of patients testing positive for respiratory syncytial virus (RSV)* by subgroups identified and total number of samples tested by clinic and province, influenza-like illness (ILI) surveillance in primary health care clinics, 03/01/2022 – 10/12/2022

| Clinic (Province) | RSVA | RSVB | RSVAB** | RSV subgroup inconclusive* ** | RSV subgroup pending** ** | Total samples |
|-----------------------|-----------|-----------|----------|-------------------------------|---------------------------|---------------|
| Agincourt (MP) | 18 | 2 | 0 | 1 | 0 | 275 |
| Eastridge (WC) | 5 | 10 | 0 | 0 | 1 | 320 |
| Edendale Gateway (KZ) | 26 | 0 | 0 | 3 | 0 | 557 |
| Jouberton (NW) | 3 | 3 | 0 | 0 | 0 | 378 |
| Mitchell's Plain (WC) | 1 | 10 | 0 | 0 | 0 | 270 |
| Total | 53 | 25 | 0 | 4 | 1 | 1800 |

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

*RSV was not detected from 28 specimens of patients who met suspected SARS-CoV-2 or *B. pertussis* case definition but did not meet influenza-like illness (ILI) case definition. These are not included in the table.

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

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

****RSV results for subgroups are pending

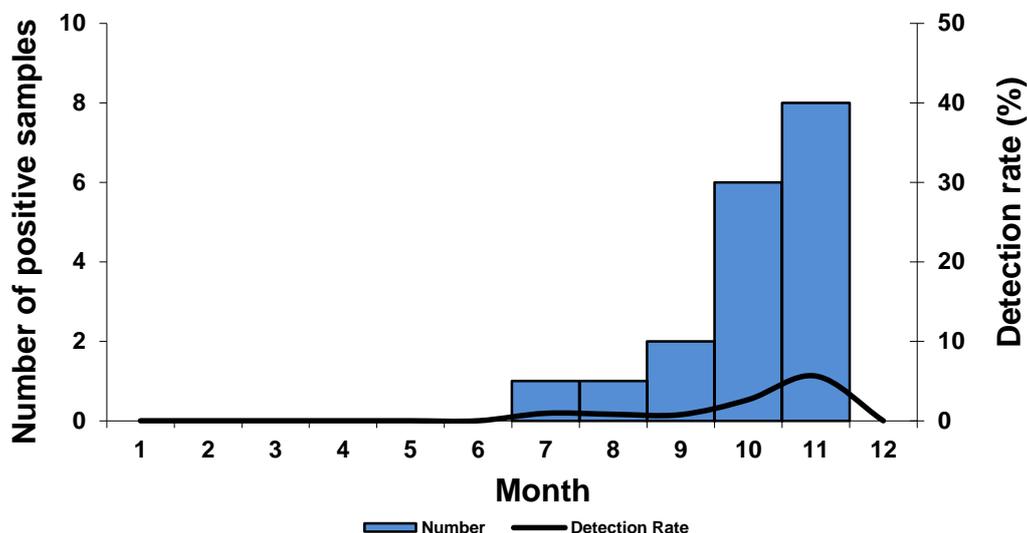


Figure 5. Number of patients testing positive for *B. pertussis and detection rate by month, influenza-like illness (ILI) surveillance primary health care clinics**, 03/01/2022 – 10/12/2022**

*No *B. pertussis* was detected in 28 specimens of patients who met the suspected SARS-CoV-2 or *B. pertussis* case definition but did not meet influenza-like illness (ILI) case definition. These are not included in the epidemiological curve.

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

Table 3. Number of patients testing positive for *B. pertussis identified and total number of samples tested by province, influenza-like illness (ILI) surveillance primary health care clinics, 03/01/2022 – 10/12/2022**

| Clinic (Province) | <i>B. pertussis</i> Positive | Total samples |
|-----------------------|------------------------------|---------------|
| Agincourt (MP) | 5 | 275 |
| Eastridge (WC) | 8 | 310 |
| Edendale Gateway (KZ) | 2 | 556 |
| Jouberton (NW) | 1 | 378 |
| Mitchell's Plain (WC) | 2 | 269 |
| Total: | 18 | 1788 |

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

*No *B. pertussis* was detected in 28 specimens of patients who met the suspected SARS-CoV-2 or *B. pertussis* case definition but did not meet influenza-like illness (ILI) case definition. These are not included in the table.

NB: Results pending for 12 samples.

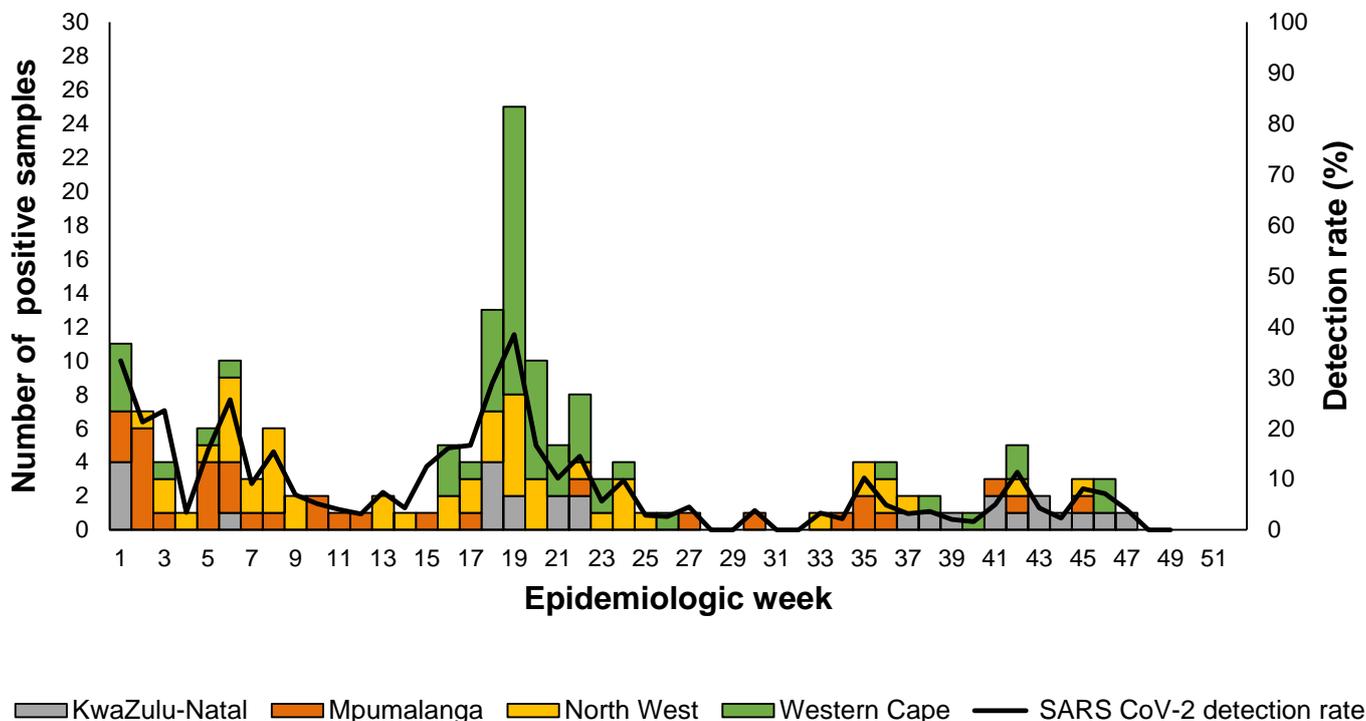


Figure 6. Number of patients* testing positive for SARS-CoV-2 by province and detection rate*** by week, influenza-like illness (ILI) surveillance in primary health care clinics, 03/01/2022 – 10/12/2022**

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

**SARS-CoV-2 was detected in (18%) 5 of 28 specimens from patients who met suspected SARS-CoV-2 or *B. pertussis* case definition but did not meet influenza-like illness (ILI) case definition. These are not included in the epidemiological curve.

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

Table 4. Number of patients positive for SARS-CoV-2* identified and total number of samples tested by clinic and province, influenza-like illness (ILI) surveillance primary health care clinics, 03/01/2022 – 10/12/2022

| Clinic (Province) | SARS-CoV-2 positive | Total samples tested |
|-----------------------|---------------------|----------------------|
| Agincourt (MP) | 35 | 275 |
| Eastridge (WC) | 12 | 320 |
| Edendale Gateway (KZ) | 27 | 557 |
| Jouberton (NW) | 52 | 378 |
| Mitchell's Plain (WC) | 47 | 270 |
| Total: | 173 | 1800 |

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

*SARS-CoV-2 was detected in (18%) 5 of 28 specimens from patients who met suspected SARS-CoV-2 or *B. pertussis* case definition but did not meet influenza-like illness (ILI) case definition. These are not included in the table.

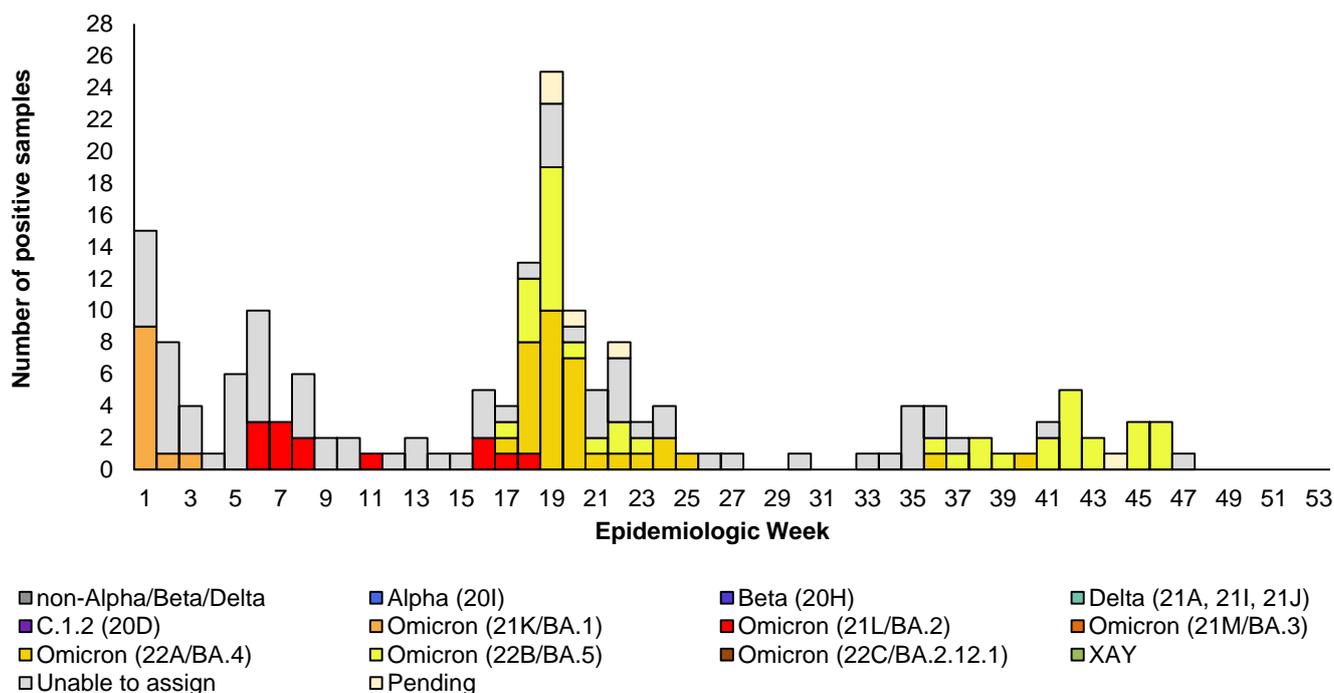


Figure 7. Number and detection rate of laboratory-confirmed SARS-CoV-2* cases by variant (variant PCR/sequencing) and week, influenza-like illness (ILI) surveillance in primary health care clinics, 03/01/2022 – 10/12/2022

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

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

Table 5. Number of cases positive for SARS-CoV-2* by variant (variant PCR and/or sequencing) identified and total number of samples tested by clinic and province, influenza-like illness (ILI) surveillance primary health care clinics, 03/01/2022 – 10/12/2022**

| Clinic (Province) | Delta (21A, 21I, 21J) | Omicron (21K/BA.1) | Omicron (21L/BA.2) | Omicron (21M/BA.3) | Omicron (22A/BA.4) | Omicron (22B/BA.5) | Omicron (22C/BA.2.12.1) | XAY | Unable to assign | Pending | Total SARS-CoV-2 positive | Total samples tested |
|-------------------|-----------------------|--------------------|--------------------|--------------------|--------------------|--------------------|-------------------------|----------|------------------|----------|---------------------------|----------------------|
| Agincourt (MP) | 0 | 4 | 3 | 0 | 0 | 2 | 0 | 0 | 28 | 0 | 37 | 280 |
| Eastridge (WC) | 0 | 2 | 0 | 0 | 3 | 1 | 0 | 0 | 3 | 3 | 12 | 321 |
| Edendale | 0 | 2 | 1 | 0 | 0 | 18 | 0 | 0 | 7 | 1 | 29 | 572 |
| Gateway (KZ) | 0 | 1 | 5 | 0 | 9 | 9 | 0 | 0 | 28 | 1 | 53 | 385 |
| Jouberton (NW) | 0 | 2 | 4 | 0 | 21 | 9 | 0 | 0 | 11 | 0 | 47 | 270 |
| Total: | 0 | 11 | 13 | 0 | 33 | 39 | 0 | 0 | 77 | 5 | 178 | 1828 |

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

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

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

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

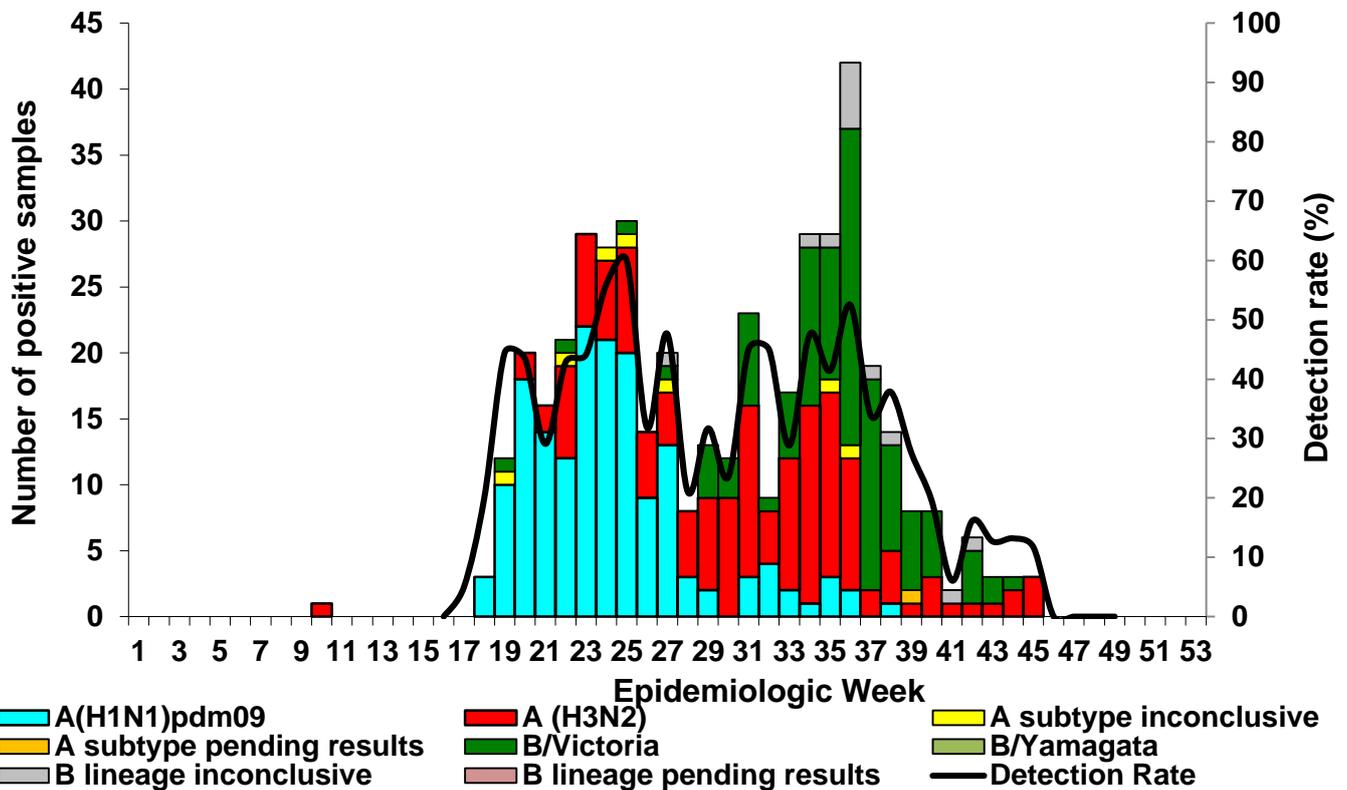


Figure 8. Number of positive patients* by influenza subtype and lineage and detection rate** by week, ILI surveillance - Viral Watch, 03/01/2022 – 10/12/2022

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

**Only reported for weeks with >10 specimens submitted. Three-week rolling average of detection rate (current week and two weeks prior) used from week 42-week 45 due to low number of specimens collected.

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

Three dual infections from GP (one influenza A(H3N2) + influenza A(H1N1)pdm09 in week 17, one influenza B(lineage inconclusive) + influenza A(H1N1)pdm09 in week 23, and one influenza A(H3N2) + influenza B(Victoria) in week 37) not included in the epidemiological curve.

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 – 10/12/2022

| 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 | 20 | 8 | 0 | 0 | 10 | 0 | 1 | 0 | 60 |
| Free State | 7 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 8 |
| Gauteng | 85 | 50 | 4 | 1 | 62 | 0 | 7 | 0 | 852 |
| Limpopo | 2 | 2 | 1 | 0 | 1 | 0 | 0 | 0 | 8 |
| Mpumalanga | 7 | 2 | 0 | 0 | 4 | 0 | 3 | 0 | 41 |
| North West | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 6 |
| Northern Cape | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Western Cape | 41 | 87 | 2 | 0 | 36 | 0 | 2 | 0 | 366 |
| Total: | 165 | 149 | 7 | 1 | 113 | 0 | 13 | 0 | 1341 |

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

**Influenza A subtype or B lineage results are pending

Three dual infections from GP (one influenza A(H3N2) + influenza A(H1N1)pdm09 in week 17, one influenza B(lineage inconclusive) + influenza A(H1N1)pdm09 in week 23, and one influenza A(H3N2) + influenza B(Victoria) in week 37) indicated in both columns.

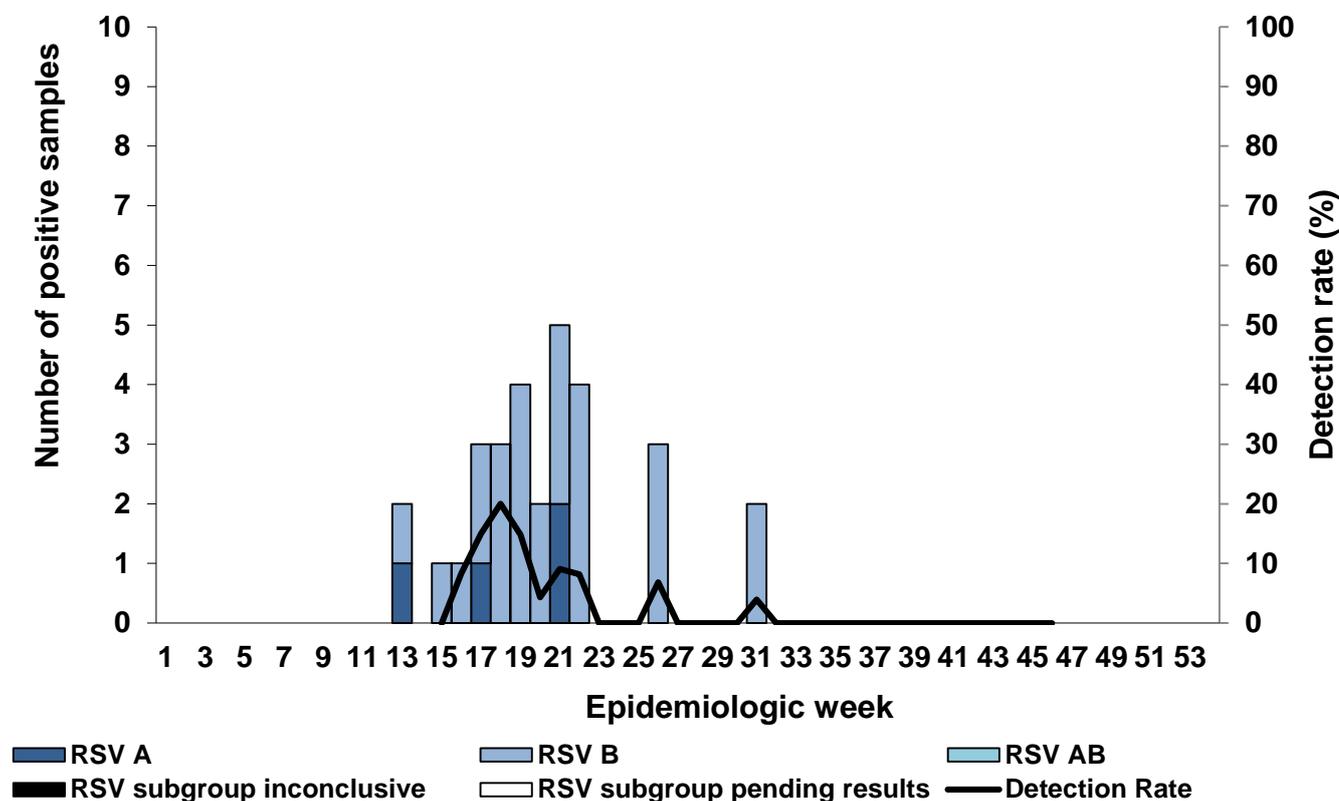


Figure 9. Number of RSV positive cases testing positive for respiratory syncytial virus (RSV)* by subgroup and detection rate** by week, ILI surveillance - Viral Watch, 03/01/2022 – 10/12/2022

*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 – 10/12/2022

| Province | RSV A | RSV B | RSV AB* | RSV subgroup inconclusive** | RSV subgroup pending results*** | Total samples tested |
|---------------|----------|-----------|----------|-----------------------------|---------------------------------|----------------------|
| Eastern Cape | 0 | 1 | 0 | 0 | 0 | 60 |
| Free State | 0 | 0 | 0 | 0 | 0 | 8 |
| Gauteng | 4 | 13 | 0 | 0 | 0 | 852 |
| Limpopo | 0 | 0 | 0 | 0 | 0 | 8 |
| Mpumalanga | 0 | 0 | 0 | 0 | 0 | 41 |
| North West | 0 | 0 | 0 | 0 | 0 | 6 |
| Northern Cape | 0 | 0 | 0 | 0 | 0 | 0 |
| Western Cape | 0 | 12 | 0 | 0 | 0 | 366 |
| Total: | 4 | 26 | 0 | 0 | 0 | 1341 |

*RSV AB: Both RSV A and B subgroup identified

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

***RSV results for subgroups are pending

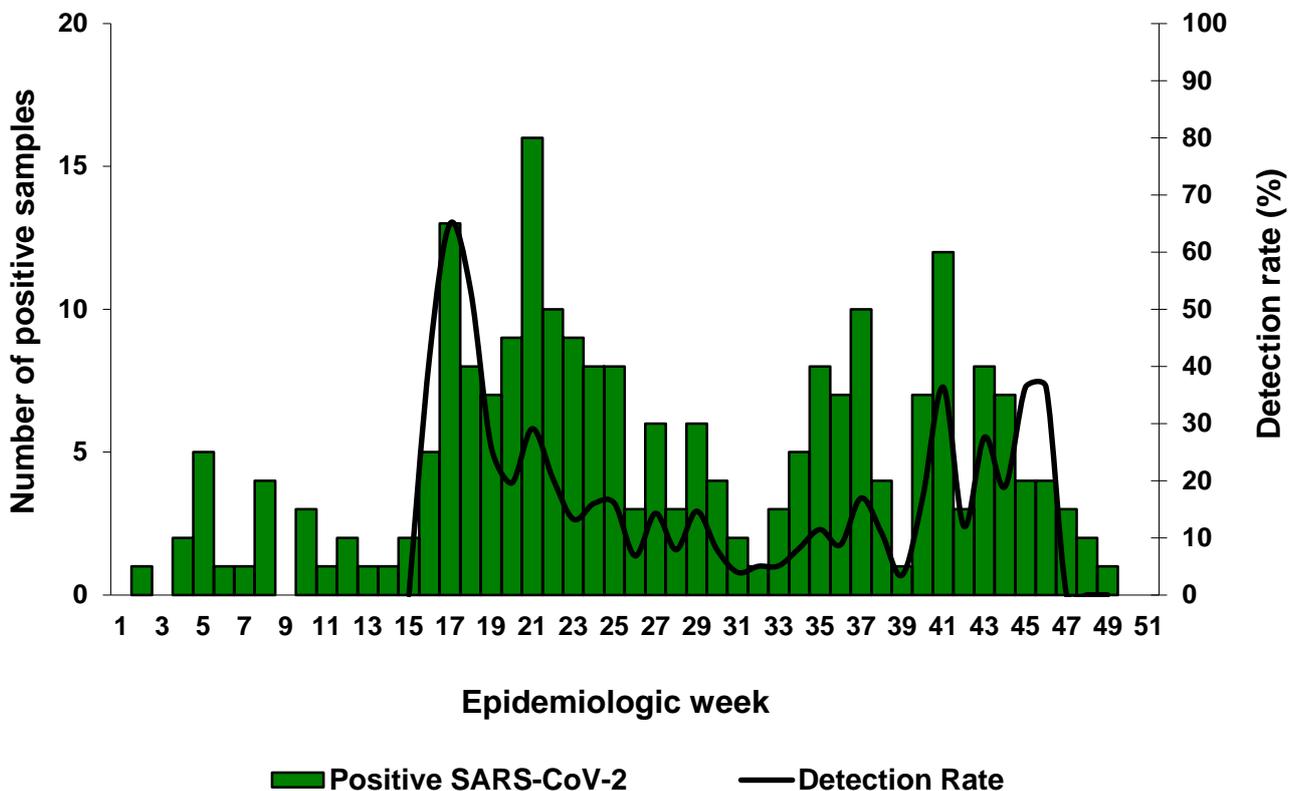


Figure 10. Number of patients testing positive for SARS-CoV-2*, by site and detection rate** by week, ILI surveillance - Viral Watch, 03/01/2022 – 10/12/2022

*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 – 10/12/2022

| Province | SARS-CoV-2 positive | Total samples tested |
|---------------|---------------------|----------------------|
| Eastern Cape | 4 | 60 |
| Free State | 0 | 8 |
| Gauteng | 158 | 852 |
| Limpopo | 1 | 8 |
| Mpumalanga | 3 | 41 |
| North West | 0 | 6 |
| Northern Cape | 0 | 0 |
| Western Cape | 65 | 366 |
| Total: | 231 | 1341 |

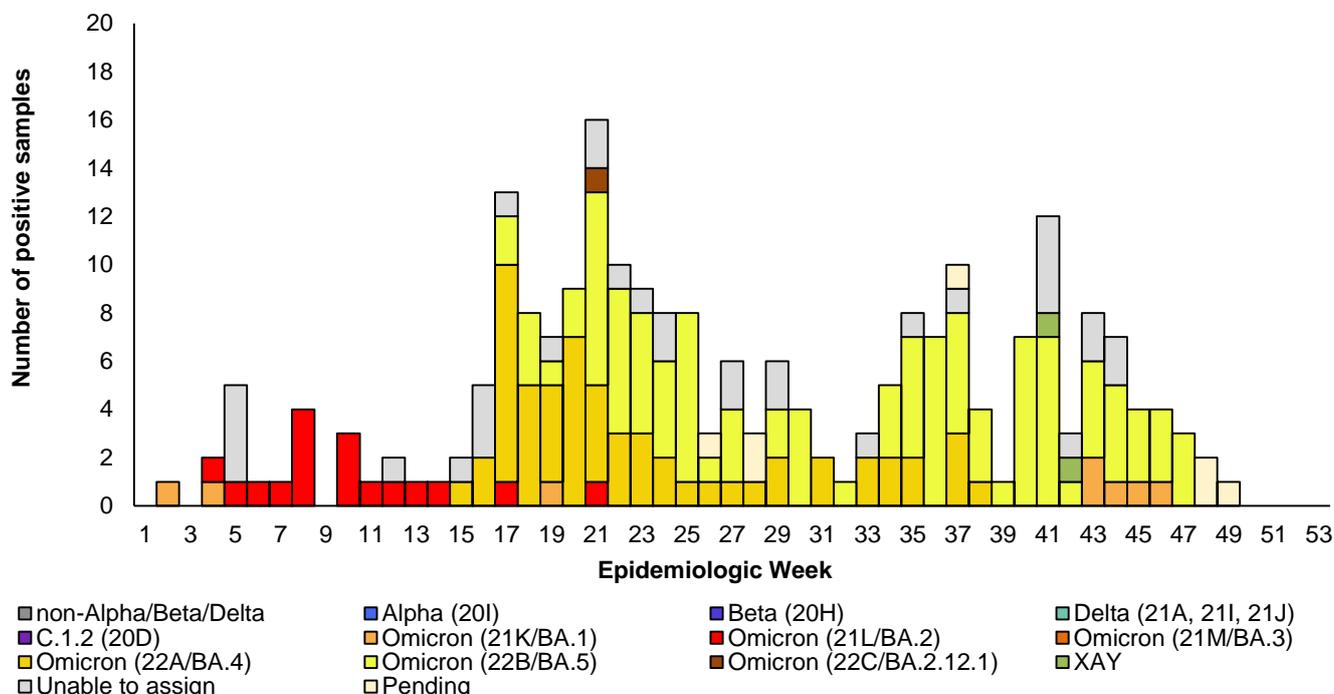


Figure 11. Number and detection rate of laboratory confirmed SARS-CoV-2* cases by variant (variant PCR/sequencing) and week, ILI surveillance - Viral Watch, 03/01/2022 – 10/12/2022

*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 ($C_t \geq 35$) **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 – 10/12/2022

| Clinic (Province) | Omicron (21K/BA.1) | Omicron (21L/BA.2) | Omicron (21M/BA.3) | Omicron (22A/BA.4) | Omicron (22B/BA.5) | Omicron (22C/BA.2.12.1) | XAY | Unable to assign | Pending | Total SARS-CoV-2 positive | Total samples tested |
|-------------------|--------------------|--------------------|--------------------|--------------------|--------------------|-------------------------|----------|------------------|----------|---------------------------|----------------------|
| Eastern Cape | 0 | 1 | 0 | 2 | 1 | 0 | 0 | 0 | 0 | 4 | 60 |
| Free State | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 8 |
| Gauteng | 7 | 8 | 0 | 46 | 65 | 1 | 2 | 24 | 5 | 158 | 852 |
| Limpopo | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 8 |
| Mpumalanga | 0 | 0 | 0 | 1 | 2 | 0 | 0 | 0 | 0 | 3 | 41 |
| North West | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 6 |
| Northern Cape | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Western Cape | 1 | 8 | 0 | 9 | 37 | 0 | 0 | 8 | 2 | 65 | 366 |
| Total: | 8 | 17 | 0 | 58 | 105 | 1 | 2 | 33 | 7 | 231 | 1341 |

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

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

Unable to assign: no lineage assigned due to poor- sequence quality **OR** low viral load ($C_t \geq 35$) **OR** variant PCR could not assign variant and no sequencing result

Pending: outstanding variant results

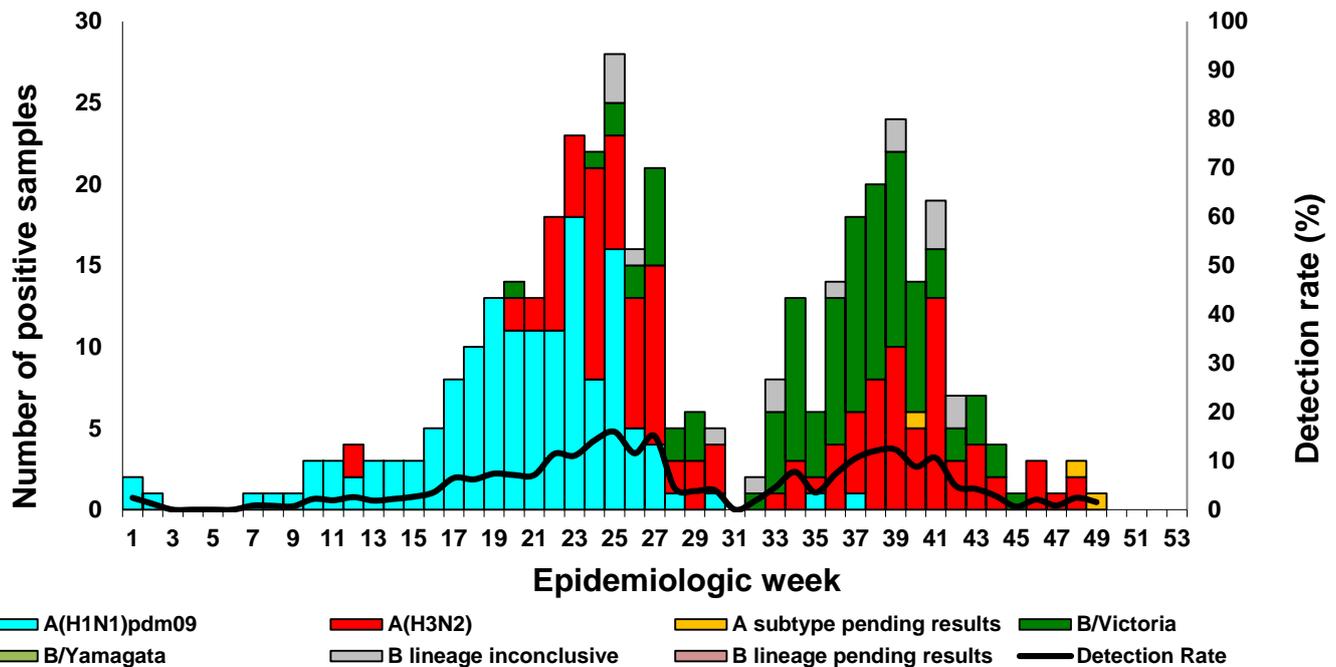


Figure 12. Number of positive influenza positive cases* by influenza subtype and lineage and detection rate*** by week, pneumonia surveillance public hospitals, 03/01/2022 – 10/12/2022**

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

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

**Influenza was not detected in 16 specimens from patients who met suspected the SARS-CoV-2 or *B. pertussis* case definition but did not meet pneumonia (SRI) case definition. These are not included in the epidemiological curve.

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

One dual infection of influenza B(Victoria) + influenza A(H3N2) in week 24 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 – 10/12/2022

| 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) | 26 | 16 | 1 | 0 | 11 | 0 | 2 | 0 | 922 |
| Helen Joseph-Rahima Moosa (GP) | 30 | 11 | 1 | 1 | 14 | 0 | 2 | 0 | 1365 |
| Klerksdorp-Tshepong (NW) | 28 | 5 | 0 | 1 | 16 | 0 | 3 | 0 | 580 |
| Livingstone (EC) | 9 | 11 | 2 | 0 | 7 | 0 | 2 | 0 | 563 |
| Mapulaneng-Matikwana (MP) | 11 | 21 | 1 | 0 | 13 | 0 | 1 | 0 | 592 |
| Mitchell's Plain (WC) | 5 | 13 | 1 | 0 | 5 | 0 | 3 | 0 | 747 |
| Red Cross (WC) | 9 | 20 | 2 | 0 | 14 | 0 | 1 | 0 | 1320 |
| Tambo Memorial (GP) | 0 | 7 | 0 | 0 | 3 | 0 | 1 | 0 | 76 |
| Tembisa (GP) | 7 | 3 | 0 | 1 | 12 | 0 | 1 | 0 | 358 |
| Tintswalo (MP) | 18 | 20 | 1 | 0 | 4 | 0 | 0 | 0 | 367 |
| Tygerberg (WC) | 4 | 4 | 1 | 0 | 3 | 0 | 0 | 0 | 178 |
| Total: | 147 | 131 | 10 | 3 | 102 | 0 | 16 | 0 | 7068 |

EC: Eastern Cape (Livingstone started enrolling on the 3rd of May 2022); GP: Gauteng (Tembisa started enrolling on the 10th of March 2022 and Tambo Memorial on the 21st of September 2022); KZ: KwaZulu-Natal; NW: North West; MP: Mpumalanga; WC: Western Cape (Tygerberg started enrolling on the 20th April 2022)

*Influenza was not detected in 16 specimens from patients who met suspected SARS-CoV-2 or *B. pertussis* case definition but did not meet pneumonia (SRI) case definition. These are not included in the table.

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

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

One dual infection of influenza B(Victoria) + influenza A(H3N2) in week 24 from Tintswalo (MP) indicated in both columns.

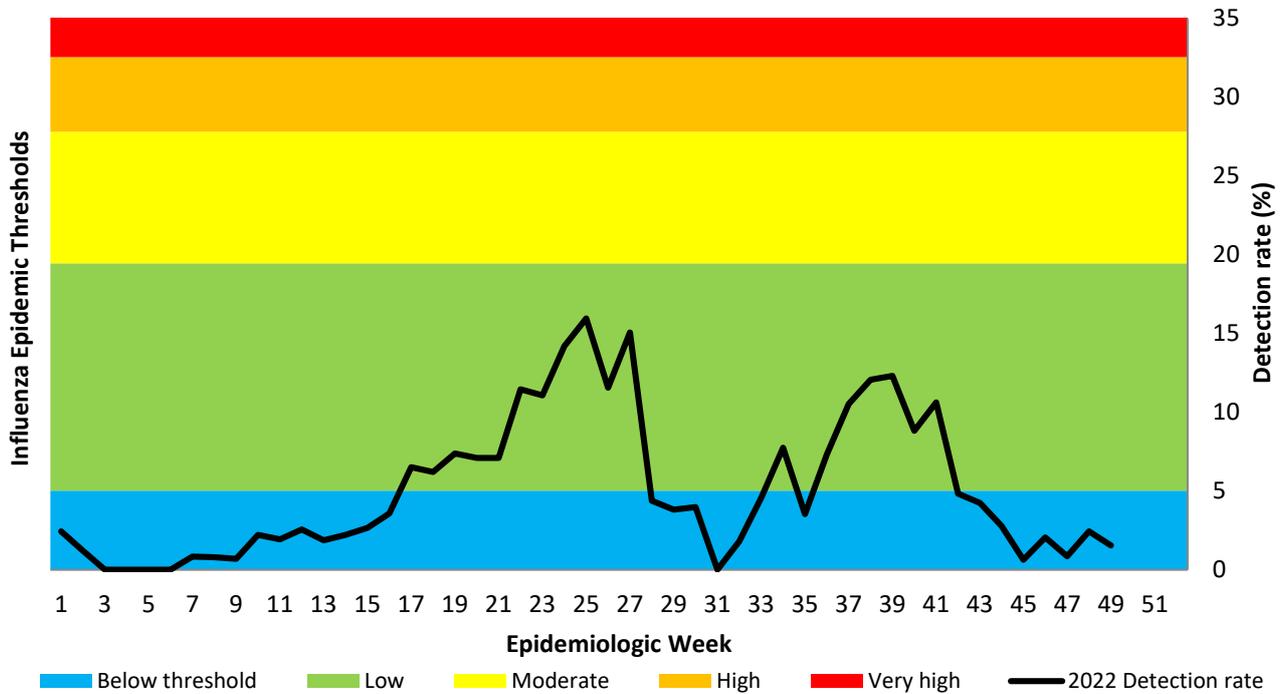


Figure 13. Influenza percentage detections and epidemic thresholds* among cases of all ages, pneumonia surveillance public hospitals, 03/01/2022 – 10/12/2022

*Thresholds based on 2010-2019 data

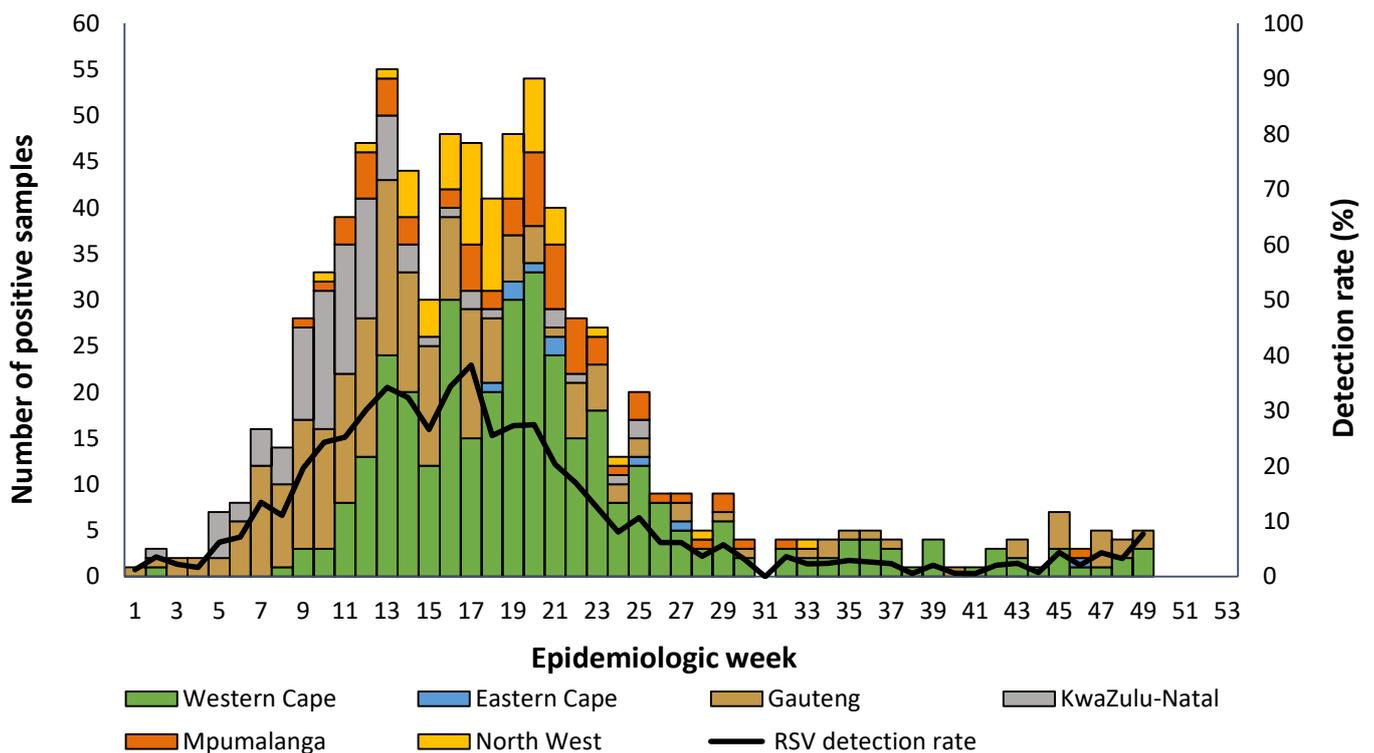


Figure 14. Number of patients (all ages) testing positive for respiratory syncytial virus* by province and detection rate by week, pneumonia surveillance public hospitals, 03/01/2022 – 10/12/2022

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

*RSV was not detected in 16 specimens from patients who met suspected SARS-CoV-2 or *B. pertussis* case definition but did not meet pneumonia (SRI) case definition.

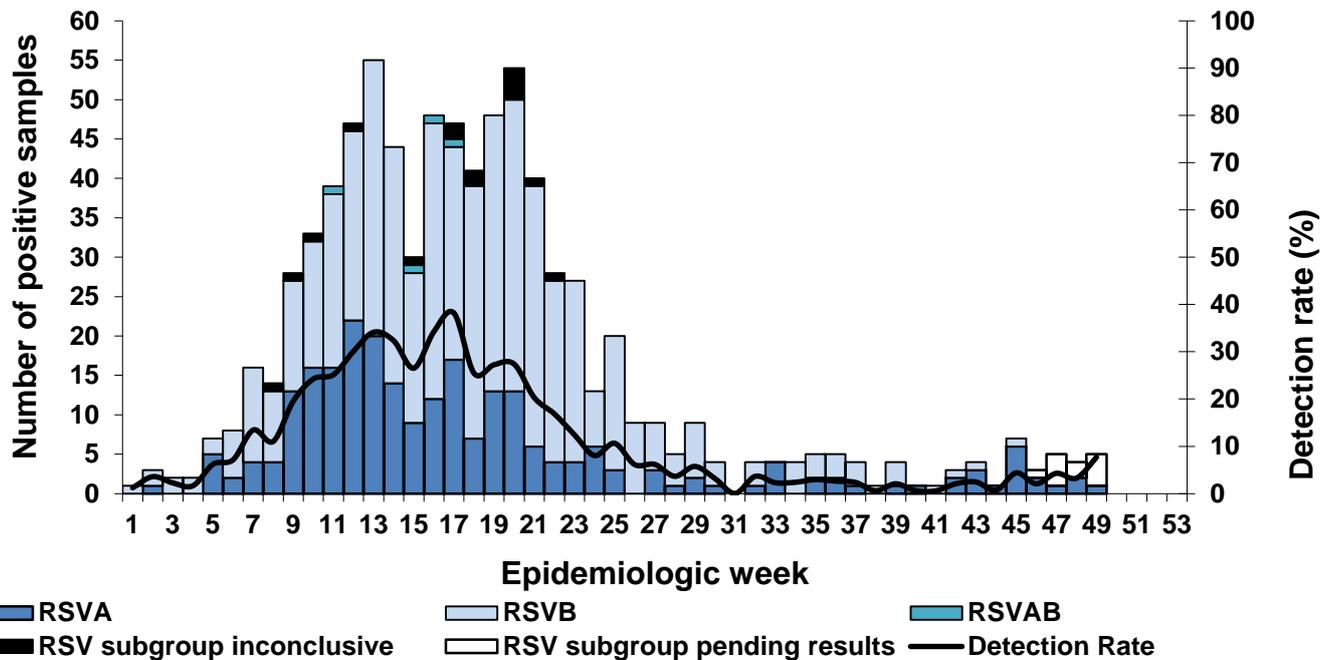


Figure 15. Number of patients (all ages) testing positive for respiratory syncytial virus* by subgroup and detection rate by week, pneumonia surveillance public hospitals, 03/01/2022 – 10/12/2022

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

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

RSV AB: Both RSV A and B subgroup identified

RSV subgroup pending: RSV results for subgroups are pending

*RSV was not detected in 16 specimens from patients who met suspected SARS-CoV-2 or *B. pertussis* case definition but did not meet pneumonia (SRI) case definition. These are not included in the epidemiological curve.

Table 11. Number of patients (all ages) positive for respiratory syncytial virus subgroups* by subgroups identified and total number of samples tested by hospital, pneumonia surveillance public hospitals, 03/01/2022 – 10/12/2022

| Hospital (Province) | RSVA | RSVB | RSVAB** | RSV subgroup inconclusive** * | RSV subgroup pending** ** | Total samples |
|--------------------------------|------------|------------|----------|----------------------------------|------------------------------|---------------|
| Edendale (KZ) | 86 | 1 | 0 | 2 | 0 | 922 |
| Helen Joseph-Rahima Moosa (GP) | 51 | 155 | 3 | 1 | 4 | 1365 |
| Klerksdorp-Tshepong (NW) | 30 | 31 | 1 | 0 | 0 | 580 |
| Livingstone (EC) | 2 | 6 | 0 | 1 | 0 | 563 |
| Mapulaneng-Matikwana (MP) | 19 | 25 | 0 | 0 | 0 | 592 |
| Mitchell's Plain (WC) | 10 | 67 | 0 | 0 | 1 | 747 |
| Red Cross (WC) | 46 | 212 | 0 | 8 | 6 | 1320 |
| Tambo Memorial (GP) | 0 | 0 | 0 | 0 | 0 | 76 |
| Tembisa (GP) | 0 | 2 | 0 | 0 | 0 | 358 |
| Tintswalo (MP) | 4 | 15 | 0 | 3 | 0 | 367 |
| Tygerberg (WC) | 0 | 4 | 0 | 0 | 0 | 178 |
| Total: | 248 | 518 | 4 | 15 | 11 | 7068 |

EC: Eastern Cape (Livingstone started enrolling on the 3rd of May 2022); GP: Gauteng (Tembisa started enrolling on the 10th of March 2022 and Tambo Memorial on the 21st of September 2022); KZ: KwaZulu-Natal; NW: North West; MP: Mpumalanga; WC: Western Cape (Tygerberg started enrolling on the 20th April 2022)

*RSV was not detected in 16 specimens from patients who met suspected SARS-CoV-2 or *B. pertussis* case definition but did not meet pneumonia (SRI) case definition. These are not included in the table.

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

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

****RSV results for subgroups are pending

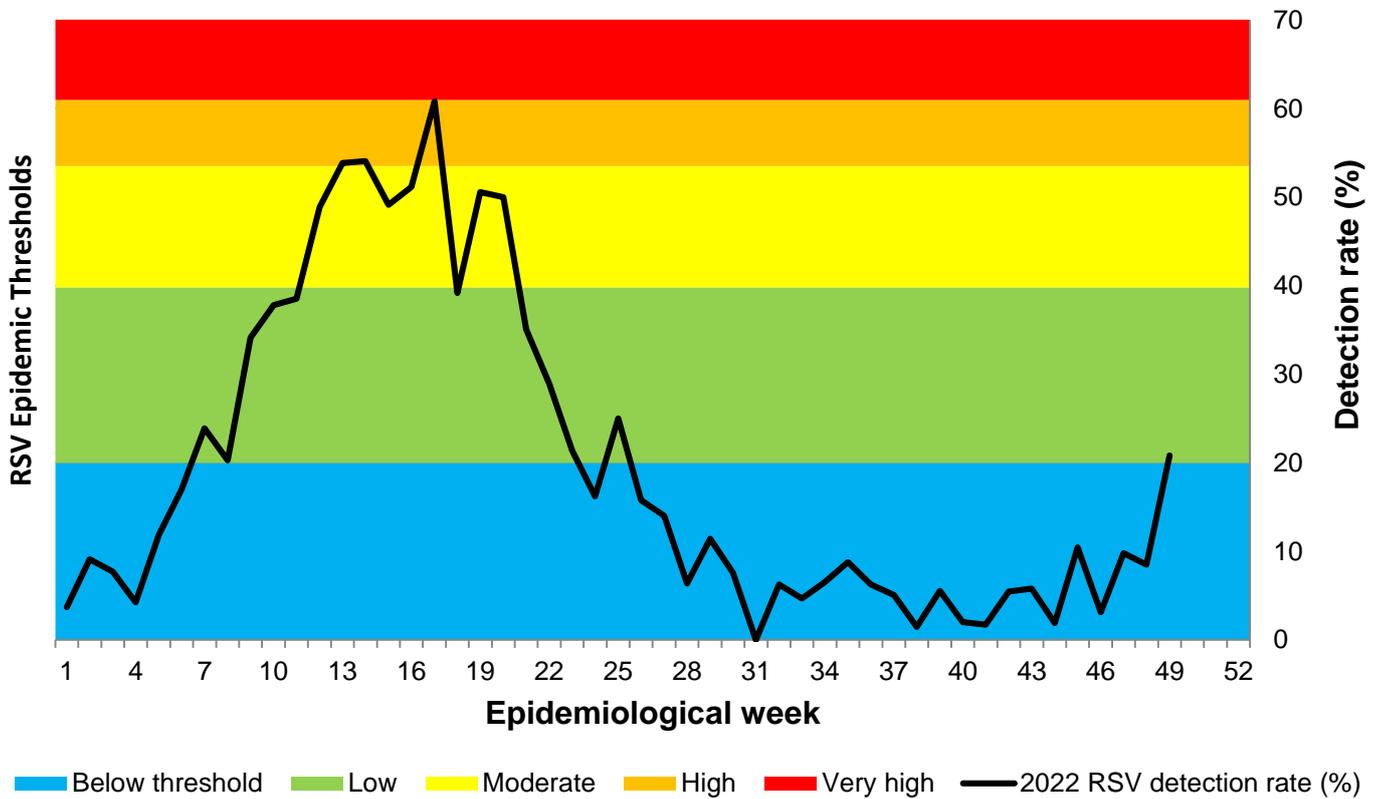


Figure 16. RSV percentage detections and epidemic thresholds* among children aged < 5 years, pneumonia surveillance public hospitals, 03/01/2022 – 10/12/2022

*Thresholds based on 2010-2019 data

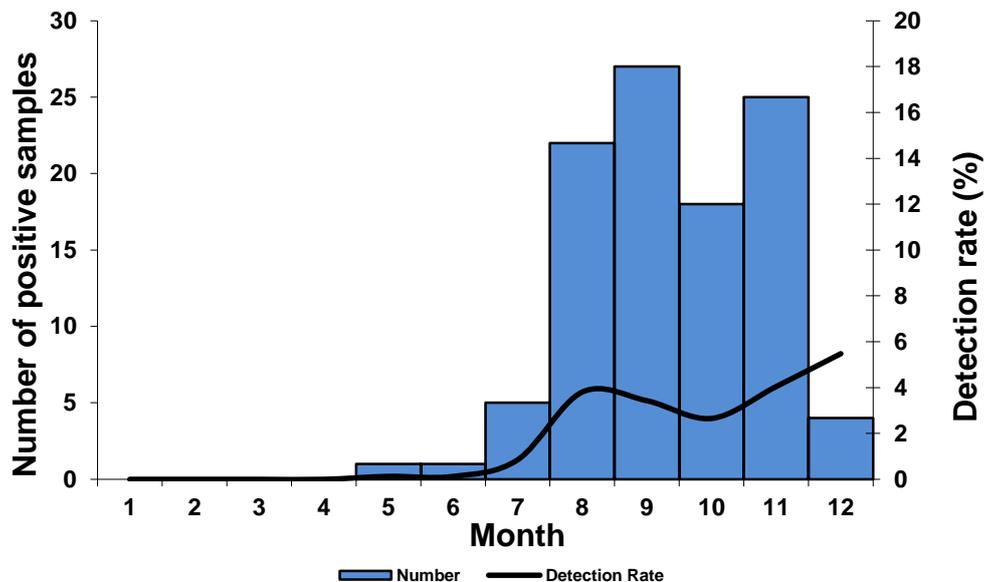


Figure 17. Number of patients testing positive for *B. pertussis and detection rate by month, pneumonia surveillance public hospitals**, 03/01/2022 – 10/12/2022**

*No *B. pertussis* was detected in 16 specimens of patients who met the suspected SARS-CoV-2 or *B. pertussis* case definition but did not meet Pneumonia Surveillance case definition. These are not included in the epidemiologic curve.

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

Table 12. Number of patients testing positive for *B. pertussis identified and total number of samples tested by hospital and province, pneumonia surveillance public hospitals, 03/01/2022 – 10/12/2022**

| Hospital (Province) | <i>B. pertussis</i> Positive | Total samples |
|--------------------------------|------------------------------|---------------|
| Edendale (KZ) | 2 | 922 |
| Helen Joseph-Rahima Moosa (GP) | 1 | 1362 |
| Klerksdorp-Tshepong(NW) | 1 | 580 |
| Livingstone (EC) | 0 | 546 |
| Mapulaneng-Matikwana (MP) | 9 | 592 |
| Mitchell's Plain (WC) | 15 | 740 |
| Red Cross (WC) | 65 | 1303 |
| Tambo Memorial (GP) | 3 | 76 |
| Tembisa (GP) | 3 | 358 |
| Tintswalo (MP) | 2 | 366 |
| Tygerberg (WC) | 2 | 176 |
| Total: | 103 | 7021 |

EC: Eastern Cape (Livingstone started enrolling on the 3rd of May 2022); GP: Gauteng (Tembisa started enrolling on the 10th of March 2022 and Tambo Memorial on the 21st of September 2022); KZ: KwaZulu-Natal; NW: North West; MP: Mpumalanga; WC: Western Cape (Tygerberg started enrolling on the 20th April 2022)

*No *B. pertussis* was detected in 16 specimens of patients who met the suspected SARS-CoV-2 or *B. pertussis* case definition but did not meet the pneumonia (SRI) case definition. These are not included in the table.

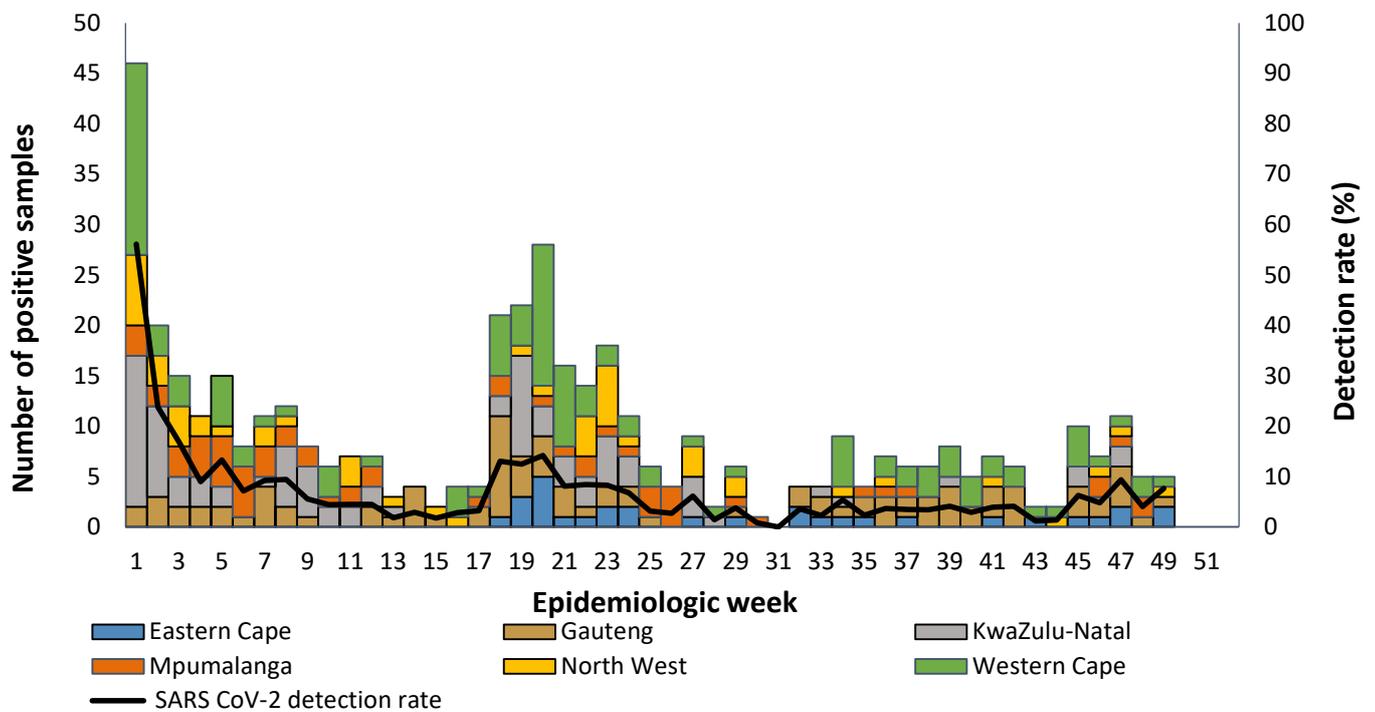


Figure 18. Number of patients testing positive for SARS-CoV-2 by province and detection rate by week, pneumonia surveillance public hospitals, 03/01/2022 – 10/12/2022**

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

**SARS-CoV-2 was detected in 6 of 16 (38%) specimens from patients who met suspected SARS-CoV-2 or *B. pertussis* 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 – 10/12/2022

| Hospital (Province) | SARS-CoV-2 positive | Total samples tested |
|--------------------------------|---------------------|----------------------|
| Edendale (KZ) | 92 | 922 |
| Helen Joseph-Rahima Moosa (GP) | 70 | 1365 |
| Klerksdorp-Tshepong (NW) | 51 | 580 |
| Livingstone (EC) | 31 | 563 |
| Mapulaneng-Matikwana (MP) | 39 | 592 |
| Mitchell's Plain (WC) | 59 | 747 |
| Red Cross (WC) | 54 | 1320 |
| Tambo Memorial (GP) | 5 | 76 |
| Tembisa (GP) | 21 | 358 |
| Tintswalo (MP) | 21 | 367 |
| Tygerberg (WC) | 6 | 178 |
| Total: | 449 | 7068 |

EC: Eastern Cape (Livingstone started enrolling on the 3rd of May 2022); GP: Gauteng (Tembisa started enrolling on the 10th of March 2022 and Tambo Memorial on the 21st of September 2022); KZ: KwaZulu-Natal; NW: North West; MP: Mpumalanga; WC: Western Cape (Tygerberg started enrolling on the 20th April 2022)

*SARS-CoV-2 was detected in 6 of 16 (38%) specimens from patients who met suspected SARS-CoV-2 or *B. pertussis* case definition but did not meet pneumonia (SRI) case definition. These are not included in the table.

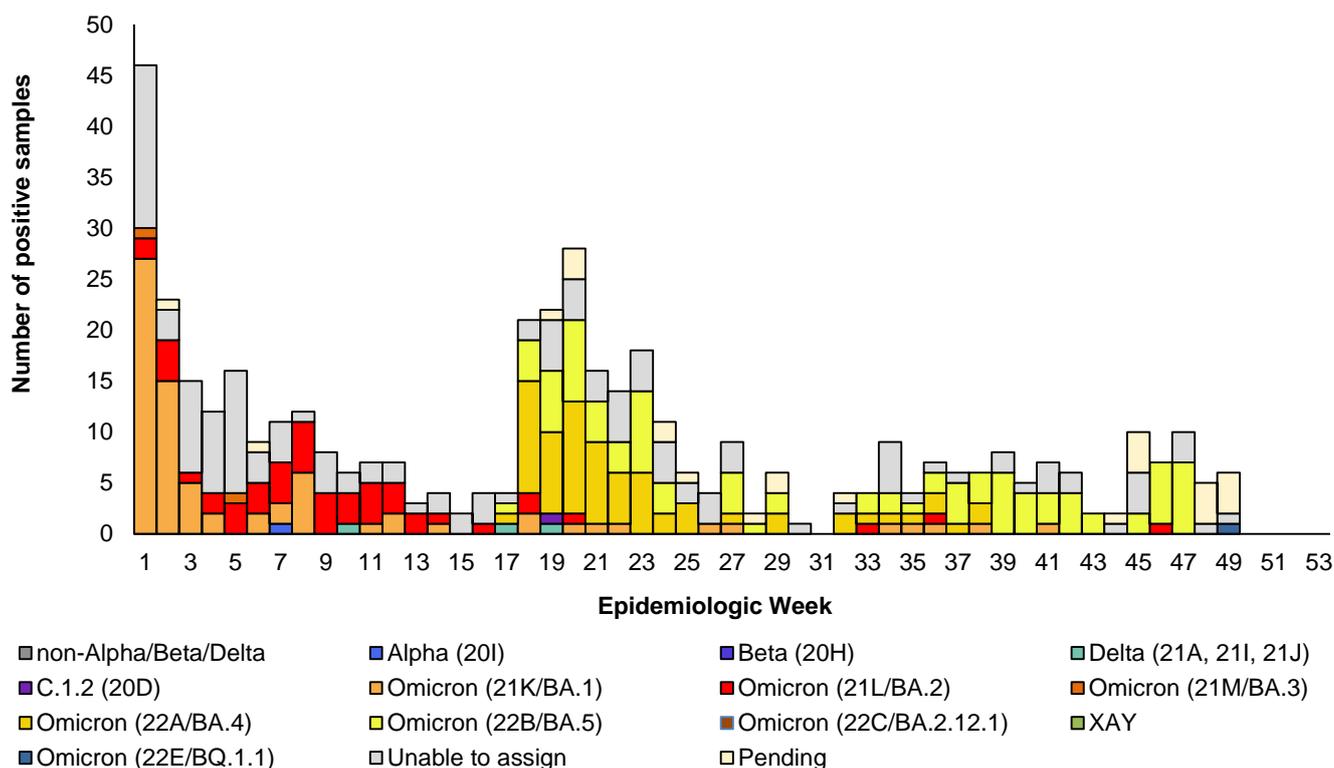


Figure 19. Number and detection rate of laboratory-confirmed SARS-CoV-2 cases* by variant type (variant PCR/sequencing), pneumonia surveillance public hospitals, 03/01/2022 – 10/12/2022

*Specimens are from hospitalized patients at 11 sentinel sites in 6 provinces who met the pneumonia (SRI), suspected SARS-CoV-2 or *B. pertussis* case definition
Unable to assign: no lineage assigned due to poor- sequence quality **OR** low viral load ($C_t \geq 35$) **OR** variant PCR could not assign variant and no sequencing result
Pending: outstanding variant results

Table 14. Number of SARS-CoV-2 positive cases* by variant (variant PCR and/or sequencing) identified and total number of samples tested by hospital, pneumonia surveillance public hospitals, 03/01/2022 – 10/12/2022

| Hospital (Province) | Delta (21A, 21I, 21J) | Omicron n (21K/B A.1) | Omicron n (21L/BA .2) | Omicron n (21M/B A.3) | Omicron n (22A/B A.4) | Omicron n (22B/B A.5) | Omicron n (22C/ BA.2.12 .1) | Omicron n (22E/B Q.1.1) | Unable to assign | Pending | Total SARS-CoV-2 positive | Total samples tested |
|--------------------------------|-----------------------|-----------------------|-----------------------|-----------------------|-----------------------|-----------------------|-----------------------------|-------------------------|------------------|-----------|---------------------------|----------------------|
| Edendale (KZ) | 1 | 24 | 13 | 1 | 3 | 23 | 0 | 0 | 28 | 2 | 96 | 931 |
| Helen Joseph-Rahima Moosa (GP) | 0 | 8 | 9 | 0 | 13 | 16 | 0 | 0 | 17 | 6 | 70 | 1365 |
| Klerksdorp-Tshepong (NW) | 0 | 11 | 2 | 1 | 5 | 10 | 0 | 0 | 19 | 3 | 51 | 580 |
| Livingstone (EC) | 0 | 2 | 3 | 0 | 8 | 8 | 0 | 0 | 7 | 3 | 31 | 563 |
| Mapulaneng-Matikwana (MP) | 0 | 6 | 8 | 0 | 4 | 2 | 0 | 1 | 18 | 2 | 41 | 599 |
| Mitchell's Plain (WC) | 0 | 13 | 1 | 0 | 15 | 7 | 0 | 0 | 18 | 5 | 59 | 747 |
| Red Cross (WC) | 0 | 4 | 6 | 0 | 13 | 13 | 0 | 0 | 13 | 5 | 54 | 1320 |
| Tambo Memorial (GP) | 0 | 0 | 0 | 0 | 1 | 4 | 0 | 0 | 0 | 0 | 5 | 76 |
| Tembisa (GP) | 2 | 2 | 0 | 0 | 4 | 6 | 0 | 0 | 7 | 0 | 21 | 358 |
| Tintswalo (MP) | 0 | 4 | 5 | 0 | 1 | 1 | 0 | 0 | 10 | 0 | 21 | 367 |
| Tygerberg (WC) | 0 | 1 | 1 | 0 | 1 | 2 | 0 | 0 | 1 | 0 | 6 | 178 |
| Total: | 3 | 75 | 48 | 2 | 68 | 92 | 0 | 1 | 138 | 26 | 455 | 7084 |

EC: Eastern Cape (Livingstone started enrolling on the 3rd of May 2022); GP: Gauteng (Tembisa started enrolling on the 10th of March 2022 and Tambo Memorial on the 21st of September 2022); KZ: KwaZulu-Natal; NW: North West; MP: Mpumalanga; WC: Western Cape (Tygerberg started enrolling on the 20th April 2022)

*Specimens are from hospitalized patients at 11 sentinel sites in 6 provinces who met the pneumonia (SRI), suspected SARS-CoV-2 or *B. pertussis* case definition

**One case of Alpha variant from Helen Joseph-Rahima Moosa (GP), no cases of Beta variant and one case of 20D (C.1.2) variant detected from Edendale (KZ).

Unable to assign: no lineage assigned due to poor- sequence quality **OR** low viral load ($C_t \geq 35$) **OR** variant PCR could not assign variant and no sequencing result

Pending: outstanding variant results

Summary of individuals with laboratory-confirmed SARS-CoV-2

Table 15: Characteristics of individuals with laboratory-confirmed SARS-CoV-2, enrolled in influenza-like illness (ILI) and pneumonia surveillance programmes, South Africa, 03/01/2022 – 10/12/2022

| Characteristic | Influenza-like illness (ILI), public-sector, n=178 (%) | Pneumonia, public-sector, n=455(%) |
|------------------------------------------|--------------------------------------------------------|------------------------------------|
| Age group (years) | | |
| 0-9 | 38/178 (21) | 117/455 (26) |
| 10-19 | 17/178 (10) | 9/455 (2) |
| 20-39 | 48/178 (27) | 124/455 (27) |
| 40-59 | 57/178 (32) | 104/455 (23) |
| 60-79 | 16/178 (9) | 84/455 (18) |
| ≥80 | 2/178 (1) | 17/455 (4) |
| Sex-female | 110/178 (62) | 232/455 (51) |
| Province* | | |
| Eastern Cape | 0/178 (0) | 31/455(7) |
| Gauteng | 0/178 (0) | 96/455 (21) |
| KwaZulu-Natal | 29/178 (16) | 96/455 (21) |
| Mpumalanga | 37/178 (21) | 62/455 (14) |
| North West | 53/178 (30) | 51/455 (11) |
| Western Cape | 59/178 (33) | 119/455 (26) |
| Race | | |
| Black | 106/178 (60) | 335/455 (74) |
| Coloured | 45/178 (25) | 127/455 (18) |
| Asian/Indian | 0/178 (0) | 3/455 (0) |
| White | 15/178 (8) | 21/455 (5) |
| Other | 13/178 (7) | 14/455 (3) |
| Variant | | |
| Non-Alpha/Beta/Delta | 0/178 (0) | 0/454 (0) |
| Alpha(20I) | 0/178 (0) | 1/454 (0) |
| Beta(20H) | 0/178 (0) | 0/454 (0) |
| Delta(21A, 21I, 21J) | 0/178 (0) | 3/454 (1) |
| C.1.2(20D) | 0/178 (0) | 1/454 (0) |
| Omicron (21K/BA.1) | 11/178 (6) | 75/454 (17) |
| Omicron (21L/BA.2) | 13/178 (7) | 48/454 (11) |
| Omicron (21M/BA.3) | 0/178 (0) | 2/454 |
| Omicron (22A/BA.4) | 33/178 (19) | 68/454 (15) |
| Omicron (22B/BA.5) | 39/178 (22) | 92/454 (20) |
| Omicron (22C/ BA.2.12.1) | 0/178 (0) | 0/454 (0) |
| XAY | 0/178 (0) | 0/454 (0) |
| Unable to assign** | 77/178 (43) | 138/454 (30) |
| Pending results*** | 5/178 (3) | 26/454 (6) |
| Presentation | | |
| Fever | 119/166 (72) | 179/435 (41) |
| Cough | 165/167 (99) | 416/443 (94) |
| Shortness of breath | 61/163 (37) | 363/438 (69) |
| Chest pain | 67/163 (41) | 170/438 (39) |
| Diarrhoea | 20/163 (12) | 41/438(9) |
| Underlying conditions | | |
| Hypertension | 31/165(19) | 77/438 (18) |
| Cardiac | 3/178 (2) | 15/455 (3) |
| Lung disease | 0/165 (0) | 1/438 (0) |
| Diabetes | 9/165 (5) | 48/438 (11) |
| Cancer | 0/178 (0) | 4/455 (1) |
| Tuberculosis - Previous | 1/178 (1) | 4/455 (1) |
| Tuberculosis - Current | 2/178 (1) | 56/455 (12) |
| HIV-infection | 21/178 (12) | 167/455 (37) |
| Other **** | 6/161 | 47/436 (11) |
| SARS-CoV-2 Vaccine | | |
| Pfizer-BioNTech (1 st dose) | 25/178 (14) | 42/455 (9) |
| Pfizer-BioNTech (2 nd dose) | 23/178 (13) | 35/455 (8) |
| Johnson & Johnson (1 st dose) | 19/178 (11) | 29/455 (6) |
| Johnson & Johnson (2 nd dose) | 3/178 (2) | 2/455 (0) |
| Unknown | 16/178 (9) | 24/455 (5) |
| No vaccine | 97/178 (54) | 342/455 (75) |
| Management | | |
| Oxygen therapy | 0/166 (0) | 246/427 (58) |
| ICU admission | 0/166 (0) | 3/427(1) |
| Ventilation | 0/166 (0) | 41/427 (10) |
| Outcome***** | | |
| Died | 0/166 (0) | 31/416 (7) |

*ILI surveillance not conducted in Gauteng or Eastern Cape province

**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 results: outstanding variant results

****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

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

Of the 31 patients who died, nine were in the 20-39-year age group, ten were in 40-59 age group and twelve were ≥60 years; 19/31 (64.5%) 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 (C_t) <40 interpreted as positive for SARS-CoV-2. From April 2021 to date the laboratory changed to the Allplex™ SARS-CoV-2/FluA/FluB/RSV kit (Seegene Inc., Seoul, South Korea), with positivity assigned if the PCR cycle threshold (C_t) was <40 for ≥1 gene targets (N, S or RdRp).

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

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

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

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

SARS-CoV-2 genomic surveillance

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

RNA Extraction

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

PCR and Library Preparation

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

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

Assembly, Processing and Quality Control of Genomic Sequences

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

Classification of Lineage, Clade and Associated Mutations

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