

# Weekly respiratory pathogens report Week 37 of 2023

### Highlights

- Influenza transmission is below the seasonal threshold. The 2023 influenza season started in week 17 (week starting 24 April 2023) when the influenza detection rate (3-week moving average) breached the seasonal threshold, peaked in week 22 (week starting on 4 June 2023) and ended in week 27 (week starting 10 July 2023). The detection of influenza B has been increasing in the Viral Watch surveillance programme in recent weeks. Of the 25 influenza positive samples in weeks 31-37 the majority, 88% (22/25) were influenza B. However, the detection rate remains below seasonal threshold.
- In 2023 to date, 970 influenza cases have been detected from all surveillance programmes, of which 97% (908/938) of those with typing information available were influenza A(H3N2), <1% (8/938) were A (H1N1)pdm09 and 2% (23/938) were influenza B Victoria.
- In 2023 to date, 777 respiratory syncytial virus (RSV) cases have been detected from all surveillance programmes. The RSV season ended in week 21 (week starting 22 May 2023), although circulation of RSV continues.
- In 2023 to date, 179 cases of *Bordetella pertussis* were detected, of which 30% (53/179) were from Gauteng Province, 22% (39/179) North West Province, 17% (30/179) from Mpumalanga Province, 15% (27/179) Western Cape Province, 13% (24/179) from KwaZulu-Natal Province and 3% (6/179) from Eastern Cape Province.
- In 2023 to date, 362 COVID-19 cases were detected from all surveillance programmes. Weekly numbers of SARS-CoV-2 cases have increased since week 31 in all surveillance programmes. Ongoing monitoring will determine whether these increases are sustained. Of the 267 specimens sequenced, a variant could be assigned in 65% (174/267). Of these, 98% (171/174) were assigned the Omicron variant, majority of which 56% (96/171) were Omicron (23A/XBB.1.5) followed by Omicron (22B/BA.5) and Omicron (22E/BQ.1.1) 13% (22/171) each. The rest of Omicron lineages contributed <10% each, among which was the 23D/XBB.1.9 lineage which has been detected in 15 samples since June 2023. One Omicron (23F/EG.5.1.1) sequences belonged to the newly added variant of interest. Variant could not be assigned for 35% (93/267) due to a low viral load or insufficient sample.

#### **Programme Descriptions**

| Programme           | Influenza-like illness (ILI)  | Viral Watch   | National Syndromic Surveillance for<br>Pneumonia                             |  |  |
|---------------------|---|---|--|--|--|
| Start year          | 2012  | 1984  | 2009   |  |  |
| Provinces*          | KZ  | EC  | EC   |  |  |
|                     | NW  | FS  | GP   |  |  |
|                     | wc  | GP  | KZ   |  |  |
|                     | MP  | LP  | MP   |  |  |
|                     |   | MP  | NW   |  |  |
|                     |   | NC  | WC   |  |  |
|                     |   | NW  |  |  |  |
|                     |   | WC  |  |  |  |
| Type of site        | Primary health care clinics   | General practitioners   | Public hospitals   |  |  |
| Case definition     | ILI: An acute respiratory illness with a                                      | ILI: An acute respiratory illness with a                            | SRI: Acute (symptom onset≤10 days) or  |  |  |
|                     | temperature (≥38°C) and cough, & onset  | temperature (≥38°C) and cough, & onset                              | chronic (symptom onset >10) lower  |  |  |
|                     | ≤10 days  | ≤10 days  | respiratory tract illness  |  |  |
|                     | ===   | ===   | respiratory tract initiation   |  |  |
|                     | Suspected pertussis   |   | Suspected pertussis  |  |  |
|                     | Any person with an acute cough illness  |   | Any person with an acute cough illness                                       |  |  |
|                     | lasting ≥14 days (or cough illness of any                                     |   | lasting ≥14 days (or cough illness of any                                    |  |  |
|                     | duration for children <1 year), without a                                     |   | duration for children <1 year), without a                                    |  |  |
|                     | more likely diagnosis AND one or more of                                      |   | more likely diagnosis AND one or more of                                     |  |  |
|                     | the following signs or symptoms:  |   | the following signs or symptoms:   |  |  |
|                     | <ul> <li>paroxysms of coughing,</li> </ul>                                    |   | <ul> <li>paroxysms of coughing,</li> </ul>                                   |  |  |
|                     |   |   |  |  |  |
|                     | • or inspiratory "whoop",   |   | or inspiratory "whoop",  |  |  |
|                     | or post-tussive vomiting  |   | or post-tussive vomiting   |  |  |
|                     | <ul> <li>or apnoea in children &lt;1 year;</li> <li>OR</li> </ul>             |   | <ul> <li>or apnoea in children &lt;1 year;</li> <li>OR</li> </ul>            |  |  |
|                     | Any person in whom a clinician suspects pertussis                             |   | Any person in whom a clinician suspects pertussis.                           |  |  |
|                     | Suspected SARS-CoV-2  |   | Suspected SARS-CoV-2   |  |  |
|                     |   | Suspected SARS-CoV-2  | -  |  |  |
|                     | Any person presenting with an acute (≤14 days) respiratory tract infection or | Any person presenting with an acute                                 | Any person admitted with a physician-<br>diagnosis of suspected COVID-19 and |  |  |
|                     |   | (≤14 days) respiratory tract infection or                           | =  |  |  |
|                     | other clinical illness compatible with COVID-19**                             | other clinical illness compatible with                              | not meeting SRI case definition.   |  |  |
|                     |   | COVID-19**  |  |  |  |
| Specimens collected | Oropharyngeal & nasopharyngeal swabs  | Throat and/or nasal swabs or<br>Nasopharyngeal swabs                | Oropharyngeal & nasopharyngeal swabs   |  |  |
| Main pathogens      | INF   | INF   | INF  |  |  |
| tested***           | RSV   | RSV   | RSV  |  |  |
|                     | ВР  | SARS-CoV-2  | BP   |  |  |
|                     | SARS-CoV-2  |   | SARS-CoV-2   |  |  |
| Testing Methods     | INF and RSV   | INF and RSV   | INF and RSV  |  |  |
| <del>-</del>        | - Fast-Track Diagnostics multiplex real-                                      | - Fast-Track Diagnostics multiplex real-                            | - Fast Track Diagnostics multiplex real-                                     |  |  |
|                     | time reverse transcription polymerase   | time reverse transcription polymerase                               | time reverse transcription polymerase  |  |  |
|                     | chain reaction (until 31 March 2021)  | chain reaction (until 31 March 2021)                                | chain reaction (until 31 March 2021)   |  |  |
|                     | B. pertussis  | B. pertussis  | B. pertussis   |  |  |
|                     | Multiplex real-time PCR (Tatti et al., J Clin                                 | Multiplex real-time PCR (Tatti et al., J Clin                       | Multiplex real-time PCR (Tatti et al., J Clin                                |  |  |
|                     | Microbiol 2011) and culture (if PCR cycle                                     | Microbiol 2011) and culture (if PCR cycle                           | Microbiol 2011) and culture (if PCR cycle                                    |  |  |
|                     | threshold ≤25)  | threshold ≤25)  | threshold ≤25)   |  |  |
|                     | SARS-CoV-2  | SARS-CoV-2  | SARS-CoV-2   |  |  |
|                     |   |   |  |  |  |
|                     | 1 April 2020 – 31 March 2021: Roche E   | 1 April 2020 – 31 March 2021: Roche E                               | 1 April 2020 – 31 March 2021: Roche E  |  |  |
|                     | gene real-time PCR essay (Corman et al.,                                      | gene real-time PCR essay Corman et al.,                             | gene real-time PCR essay (Corman et al.,                                     |  |  |
|                     | Euro Surv 2020)   | Euro Surv 2020)   | Euro Surv 2020)  |  |  |
|                     | 1 April 2021 to date: Allplex™ SARS-CoV-<br>2/FluA/FluB/RSV PCR kit           | 1 April 2021 to date: Allplex™ SARS-CoV-<br>2/FluA/FluB/RSV PCR kit | 1 April 2021 to date: Allplex™ SARS-CoV-<br>2/FluA/FluB/RSV PCR kit          |  |  |
|                     | <ul> <li>positivity assigned if PCR cycle</li> </ul>                          | <ul> <li>positivity assigned if PCR cycle</li> </ul>                | <ul> <li>positivity assigned if PCR cycle</li> </ul>                         |  |  |
|                     | threshold is <40 for ≥1 gene targets  | threshold is <40 for ≥1 gene targets                                | threshold is <40 for ≥1 gene targets   |  |  |
|                     | (N, S, OR RdRp)   | (N, S, OR RdRp)   | (N, S, OR RdRp)  |  |  |

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.

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

<sup>\*\*</sup>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  $virus; RSV: respiratory \ syncytial \ virus; BP: \textit{Bordetella pertussis}; SARS-CoV-2: severe \ acute \ respiratory \ syndrome \ coronavirus \ 2$ 

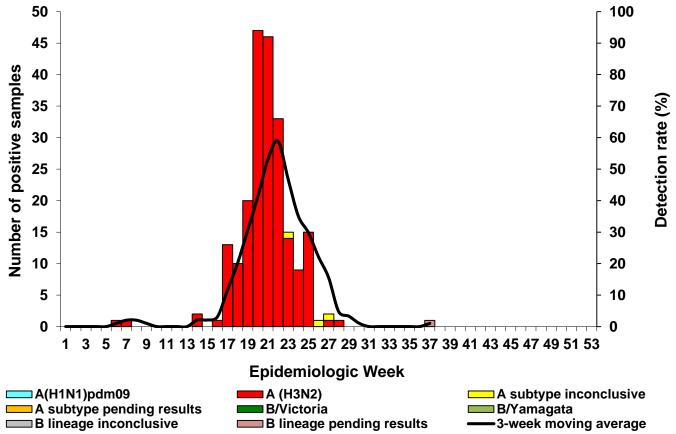


Figure 1. Number of influenza positive cases\* by influenza subtype and lineage\*\* and 3-week moving average by week, influenza-like illness (ILI) surveillance in primary health care clinics, 01/01/2023 – 17/09/2023

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

Table 1. Number of laboratory-confirmed influenza\* cases by subtype and lineage and total number of samples tested by clinic and province, influenza-like illness (ILI) surveillance in primary health care clinics, 01/01/2023 – 17/09/2023

| Clinic (Province)     | A(H1N1)<br>pdm09 | A(H3N2) | A<br>subtype<br>in-<br>conclusiv<br>e** | A subtype pending results*** | B/<br>Victoria | B/<br>Yamagata | B<br>lineag<br>e in-<br>conclu<br>sive* | B<br>lineage<br>pending<br>results*<br>** | Total<br>sample<br>s |
|-----------------------|------------------|---------|---|------------------------------|----------------|----------------|---|---|----------------------|
| Agincourt (MP)        | 0                | 41      | 0                                       | 0                            | 0              | 0              | 0                                       | 0   | 230                  |
| Eastridge (WC)        | 0                | 34      | 1                                       | 0                            | 0              | 0              | 0                                       | 0   | 250                  |
| Edendale Gateway (KZ) | 0                | 53      | 2                                       | 0                            | 0              | 0              | 0                                       | 0   | 425                  |
| Jouberton (NW)        | 0                | 76      | 0                                       | 0                            | 0              | 0              | 0                                       | 0   | 308                  |
| Mitchell's Plain (WC) | 0                | 10      | 0                                       | 0                            | 0              | 0              | 0                                       | 1   | 135                  |
| Total:                | 0                | 214     | 3                                       | 0                            | 0              | 0              | 0                                       | 1   | 1348                 |

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

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

<sup>\*\*</sup> Influenza A(H3N2) was detected in 10/58, 17% of specimens from patients who met suspected SARS-CoV-2 or *B. pertussis* case definition but did not meet influenza-like illness (ILI) case definition.

<sup>\*</sup> Influenza A(H3N2) was detected in 10/58, 17% of specimens from patients who met suspected SARS-CoV-2 or *B. pertussis* case definition but did not meet influenza-like illness (ILI) case definition.

<sup>\*\*</sup>Inconclusive: insufficient viral load in sample and unable to characterise further

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

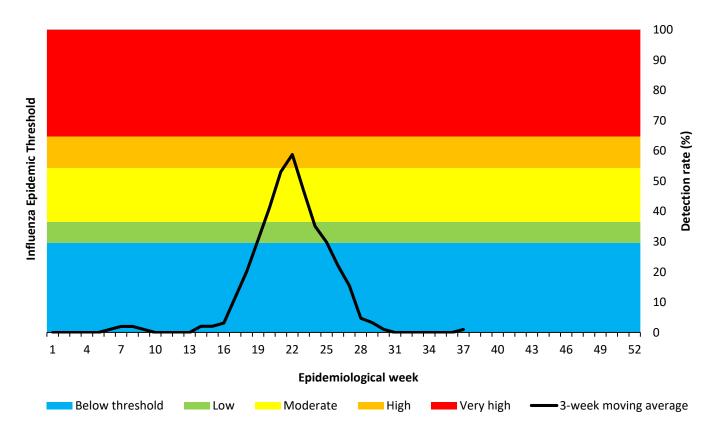


Figure 2. Influenza percentage detections and epidemic thresholds\* among cases of all ages, influenza-like illness (ILI) surveillance in primary health care clinics, 01/01/2023 –17/09/2023

\*Thresholds based on 2012-2019 data

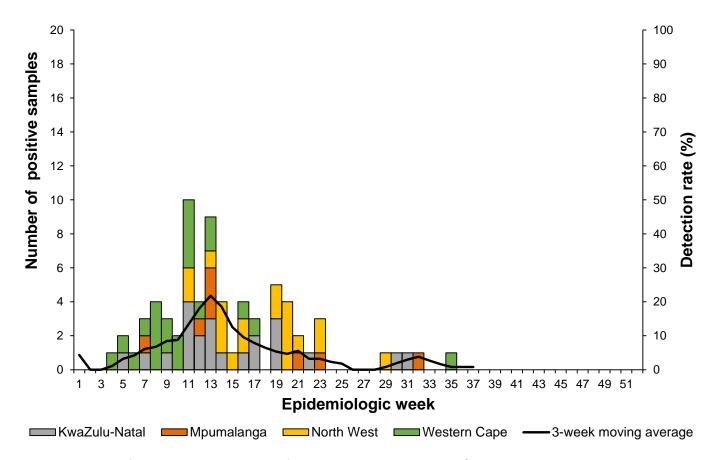


Figure 3. Number of patients testing positive for respiratory syncytial virus\* by province and 3-week moving average by week, influenza-like illness (ILI) surveillance in primary health care clinics, 01/01/2023 –17/09/2023

\*RSV was detected in 1/56, 1.8% of specimens of patients who met suspected SARS-CoV-2 or *B. pertussis* case definition but did not meet influenza-like illness (ILI) case definition.

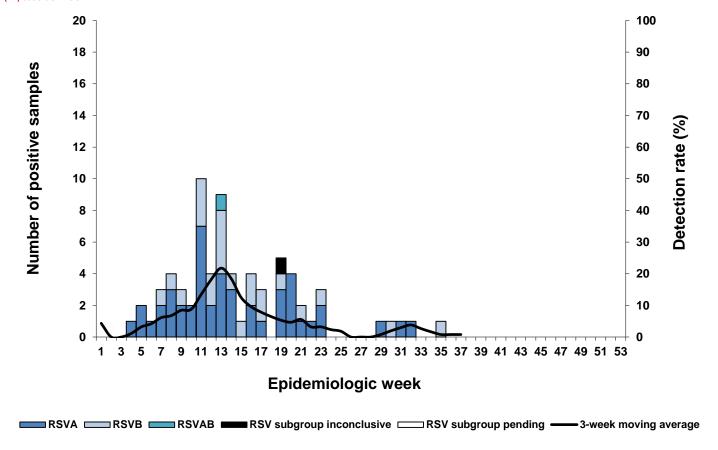


Figure 4. Number of patients testing positive for respiratory syncytial virus\* by subgroup and 3-week moving average by week, influenza-like illness (ILI) surveillance in primary health care clinics, 01/01/2023 – 17/09/2023

RSV AB: Both RSV A and B subgroups identified.

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

Table 2. Number of patients testing positive for respiratory syncytial virus (RSV)\* by subgroups identified and total number of samples tested by clinic and province, influenza-like illness (ILI) surveillance in primary health care clinics, 01/01/2023 - 17/09/2023

| Clinic (Province)     | RSVA | RSVB | RSVAB** | RSV subgroup inconclusive* | RSV<br>subgroup<br>pending**<br>** | Total<br>samples |
|-----------------------|------|------|---------|----------------------------|------------------------------------|------------------|
| Agincourt (MP)        | 3    | 4    | 1       | 0                          | 0                                  | 230              |
| Eastridge (WC)        | 18   | 3    | 0       | 0                          | 0                                  | 250              |
| Edendale Gateway (KZ) | 9    | 12   | 0       | 1                          | 0                                  | 425              |
| Jouberton (NW)        | 15   | 4    | 0       | 0                          | 0                                  | 308              |
| Mitchell's Plain (WC) | 1    | 0    | 0       | 0                          | 0                                  | 135              |
| Total                 | 46   | 23   | 1       | 1                          | 0                                  | 1348             |

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

<sup>\*</sup>RSV was detected in 1/58, 1.7% of specimens of patients who met suspected SARS-CoV-2 or *B. pertussis* case definition but did not meet influenza-like illness (ILI) case definition.

<sup>\*</sup>RSV was detected in 1/58, 1.7% of specimens of patients who met suspected SARS-CoV-2 or *B. pertussis* case definition but did not meet influenza-like illness (ILI) case definition.

<sup>\*\*</sup>RSV AB: Both RSV A and B subgroups identified

<sup>\*\*\*</sup>Inconclusive: insufficient viral load in sample and unable to characterise further

<sup>\*\*\*\*</sup>RSV results for subgroups are pending

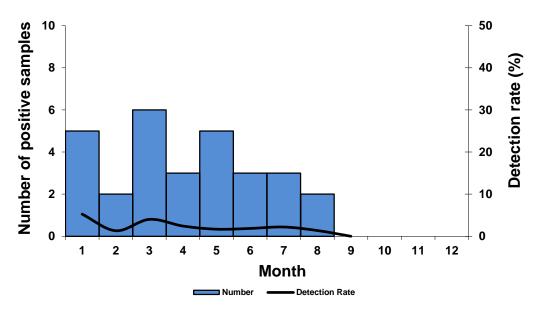


Figure 5. Number of patients testing positive for *B. pertussis\** and detection rate by month, influenza-like illness (ILI) surveillance primary health care clinics\*\*, 01/01/2023 –17/09/2023

Table 3. Number of patients testing positive for *B. pertussis\** identified and total number of samples tested by province, influenza-like illness (ILI) surveillance primary health care clinics, 01/01/2023 –17/09/2023

| Clinic (Province)     | <i>B. pertussis</i><br>Positive | Total samples |
|-----------------------|---------------------------------|---------------|
| Agincourt (MP)        | 5                               | 212           |
| Eastridge (WC)        | 3                               | 250           |
| Edendale Gateway (KZ) | 7                               | 417           |
| Jouberton (NW)        | 14                              | 299           |
| Mitchell's Plain (WC) | 0                               | 135           |
| Total:                | 29                              | 1313          |

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

<sup>\*</sup>B. pertussis was detected in 1/56, 1.8% of specimens of patients who met the suspected SARS-CoV-2 or B. pertussis case definition but did not meet influenza-like illness (ILI) case definition. These are not included in the epidemiological curve.

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

<sup>\*</sup>B. pertussis was detected in 1/56, 1.8% of specimens of patients who met the suspected SARS-CoV-2 or B. pertussis case definition but did not meet influenzalike illness (ILI) case definition. These are not included in the epidemiological curve.

NB: Results pending for 49 samples.

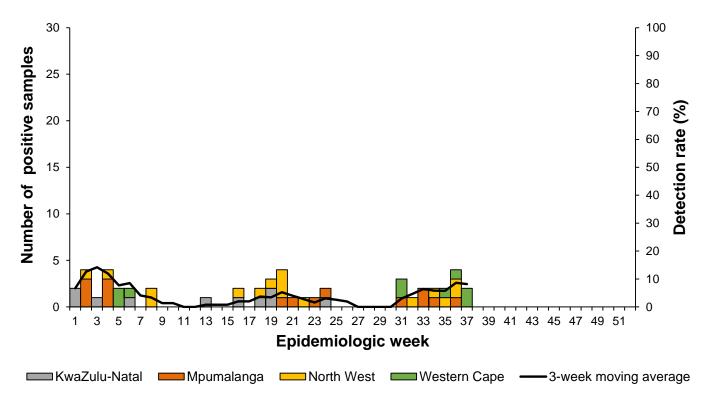


Figure 6. Number of patients\* testing positive for SARS-CoV-2\*\* by province and 3-week moving average by week, influenza-like illness (ILI) surveillance in primary health care clinics, 01/01/2023 – 17/09/2023

Table 4. Number of patients positive for SARS-CoV-2\* identified and total number of samples tested by clinic and province, influenza-like illness (ILI) surveillance primary health care clinics, 01/01/2023 – 17/09/2023

| Clinic (Province)     | SARS-CoV-2 positive | Total samples tested |
|-----------------------|---------------------|----------------------|
| Agincourt (MP)        | 15                  | 230                  |
| Eastridge (WC)        | 5                   | 250                  |
| Edendale Gateway (KZ) | 10                  | 425                  |
| Jouberton (NW)        | 16                  | 308                  |
| Mitchell's Plain (WC) | 4                   | 135                  |
| Total:                | 50                  | 1348                 |

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

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

<sup>\*\*</sup>SARS-CoV-2 was detected in 1/58, 1.7% 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.

<sup>\*</sup>SARS-CoV-2 was detected in 1/58, 1.7% specimens patients who met suspected SARS-CoV-2 or *B. pertussis* case definition but did not meet influenza-like illness (ILI) case definition. These are not included in the epidemiological curve.

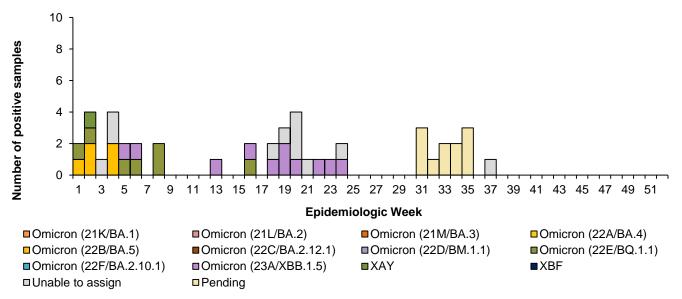


Figure 7. Number of laboratory-confirmed SARS-CoV-2\* cases by variant type (variant PCR/sequencing) and week, influenza-like illness (ILI) surveillance in primary health care clinics, 01/01/2023 – 17/09/2023

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

Table 5. Number of cases positive for SARS-CoV-2\* by variant\*\* (variant PCR and/or sequencing) identified and total number of samples tested by clinic and province, influenza-like illness (ILI) surveillance primary health care clinics, 01/01/2023 – 17/09/2023

| Province                           | Omicron<br>(21L/BA.2) | Omicron<br>(21M/ BA.3) | Omicron<br>(22A/BA.4) | Omicron<br>(22B/ BA.5) | Omicron<br>(22C/BA.2.12.1) | Omicron<br>(22D/BM.1.1) | Omicron<br>(22E/BQ.1.1) | Omicron<br>(22F/XXB.1) | Omicron<br>(23A/XBB.1.5) | ХАУ | Unable to<br>assign** | Pending*** | SARS-CoV-2<br>positive | Total samples<br>tested |
|------------------------------------|-----------------------|------------------------|-----------------------|------------------------|----------------------------|-------------------------|-------------------------|------------------------|--------------------------|-----|-----------------------|------------|------------------------|-------------------------|
| Agincourt<br>Clinic (MP)           | 0                     | 0                      | 0                     | 4                      | 0                          | 0                       | 0                       | 0                      | 3                        | 1   | 2                     | 5          | 15                     | 230                     |
| Eastridge<br>Clinic (WC)           | 0                     | 0                      | 0                     | 0                      | 0                          | 0                       | 0                       | 0                      | 1                        | 0   | 0                     | 4          | 5                      | 250                     |
| Edendale<br>Clinic (KZ)            | 0                     | 0                      | 0                     | 1                      | 0                          | 0                       | 3                       | 0                      | 4                        | 0   | 2                     | 1          | 11                     | 473                     |
| Jouberton<br>Clinic (NW)           | 0                     | 0                      | 0                     | 0                      | 0                          | 0                       | 3                       | 0                      | 2                        | 0   | 6                     | 5          | 16                     | 318                     |
| Mitchell's<br>Plain Clinic<br>(WC) | 0                     | 0                      | 0                     | 0                      | 0                          | 0                       | 1                       | 0                      | 1                        | 0   | 1                     | 1          | 4                      | 135                     |
| Total:                             | 0                     | 0                      | 0                     | 5                      | 0                          | 0                       | 7                       | 0                      | 11                       | 1   | 11                    | 16         | 51                     | 1406                    |

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

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

<sup>\*</sup>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

<sup>\*</sup>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

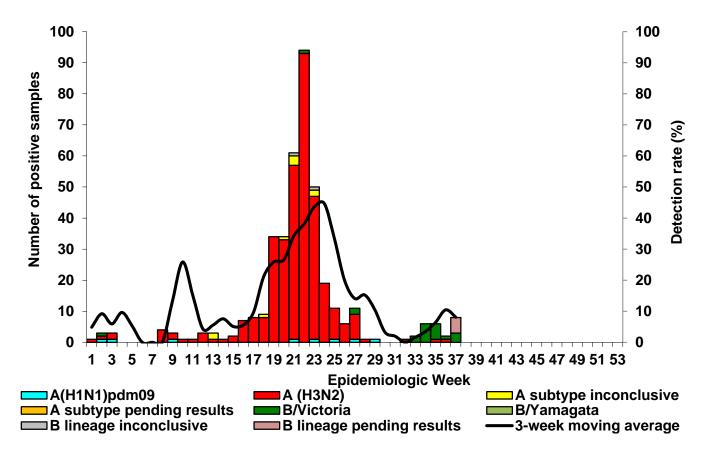


Figure 8a. Number of positive patients\* by influenza subtype and lineage and 3-week moving average by week, influenza-like illness (ILI) surveillance - Viral Watch, 01/01/2023 – 17/09/2023

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

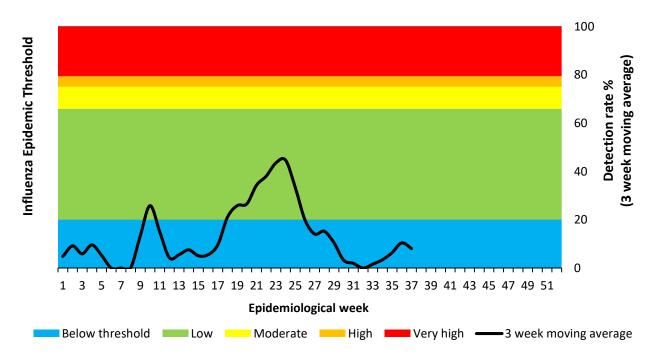


Figure 8b. Influenza percentage detections and epidemic thresholds\* among cases of all ages, Influenza-like Illness (ILI) surveillance - Viral Watch, 01/01/2023 – 17/09/2023

<sup>\*</sup>Thresholds based on 2015-2019 data

Table 6. Number of laboratory-confirmed influenza cases by influenza subtype and lineage and total number of samples tested by province, ILI surveillance - Viral Watch, 01/01/2023 – 17/09/2023

| Province      | A(H1N1)<br>pdm09 | A(H3N2) | A subtype inconclusive | A subtype pending results* | B/Victori<br>a | B/Yamaga<br>ta | B lineage<br>inconclusi<br>ve | B lineage<br>pending<br>results* | Total<br>samples |
|---------------|------------------|---------|------------------------|----------------------------|----------------|----------------|-------------------------------|----------------------------------|------------------|
| Eastern Cape  | 0                | 17      | 0                      | 0                          | 3              | 0              | 0                             | 3                                | 50               |
| Free State    | 0                | 2       | 0                      | 0                          | 0              | 0              | 0                             | 0                                | 2                |
| Gauteng       | 3                | 127     | 4                      | 0                          | 1              | 0              | 1                             | 1                                | 730              |
| Limpopo       | 0                | 2       | 0                      | 0                          | 0              | 0              | 0                             | 0                                | 6                |
| Mpumalanga    | 1                | 19      | 2                      | 0                          | 0              | 0              | 0                             | 0                                | 52               |
| North West    | 0                | 3       | 0                      | 0                          | 0              | 0              | 0                             | 0                                | 3                |
| Northern Cape | 0                | 0       | 0                      | 0                          | 0              | 0              | 0                             | 0                                | 0                |
| Western Cape  | 4                | 181     | 3                      | 0                          | 17             | 0              | 1                             | 1                                | 438              |
| Total:        | 8                | 351     | 9                      | 0                          | 21             | 0              | 2                             | 5                                | 1281             |

<sup>\*</sup>Inconclusive: insufficient viral load in sample and unable to characterise further

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

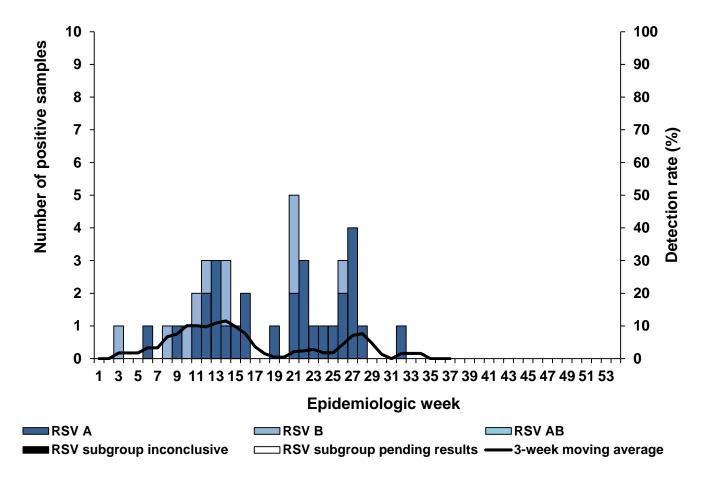


Figure 9. Number of RSV positive cases testing positive for respiratory syncytial virus (RSV)\* by subgroup and 3-week moving average by week, Influenza-like Illness (ILI) surveillance - Viral Watch, 01/01/2023 – 17/09/2023

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

Table 7. Number of RSV positive cases identified and total number of samples tested by province, influenza-like illness surveillance - Viral Watch, 01/01/2023 – 17/09/2023

| Province      | RSV A | RSV B | RSV AB* | RSV<br>subgroup<br>inconclusive<br>** | RSV subgroup pending results*** | Total samples<br>tested |
|---------------|-------|-------|---------|---------------------------------------|---------------------------------|-------------------------|
| Eastern Cape  | 1     | 1     | 0       | 0                                     | 0                               | 50                      |
| Free State    | 0     | 0     | 0       | 0                                     | 0                               | 2                       |
| Gauteng       | 17    | 7     | 0       | 0                                     | 0                               | 730                     |
| Limpopo       | 0     | 0     | 0       | 0                                     | 0                               | 6                       |
| Mpumalanga    | 2     | 0     | 0       | 0                                     | 0                               | 52                      |
| North West    | 0     | 0     | 0       | 0                                     | 0                               | 3                       |
| Northern Cape | 0     | 0     | 0       | 0                                     | 0                               |                         |
| Western Cape  | 9     | 3     | 0       | 0                                     | 0                               | 438                     |
| Total:        | 29    | 11    | 0       | 0                                     | 0                               | 1281                    |

<sup>\*</sup>RSV AB: Both RSV A and B subgroup identified

<sup>\*\*\*</sup>RSV results for subgroups are pending

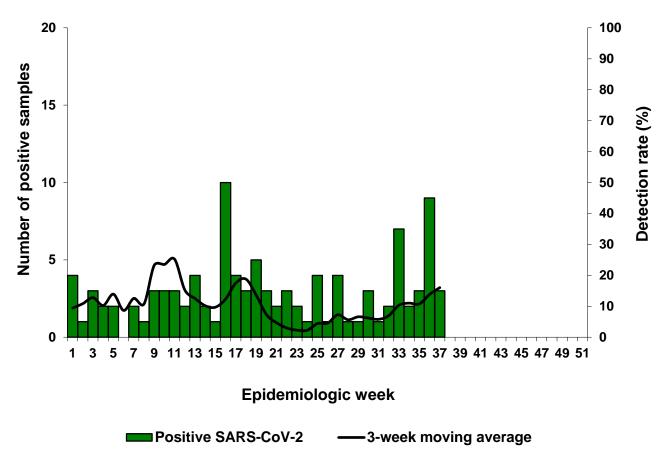


Figure 10. Number of patients testing positive for SARS-CoV-2\*, by site and 3-week moving average\*\* by week, influenza-like illness(ILI) surveillance - Viral Watch, 01/01/2023 – 17/09/2023

<sup>\*\*</sup>Inconclusive: insufficient viral load in sample and unable to characterise further

<sup>\*</sup>Specimens from patients with influenza-like illnesses at 92 sentinel sites in 8 provinces

Table 8. Number of SARS-CoV-2 positive cases identified and total number tested by province, influenza-like illness (ILI) surveillance - Viral Watch, 01/01/2023 – 17/09/2023

| Province      | SARS-CoV-2 positive | Total samples tested |
|---------------|---------------------|----------------------|
| Eastern Cape  | 5                   | 50                   |
| Free State    | 0                   | 2                    |
| Gauteng       | 70                  | 730                  |
| Limpopo       | 0                   | 6                    |
| Mpumalanga    | 4                   | 52                   |
| North West    | 0                   | 3                    |
| Northern Cape | 0                   | 0                    |
| Western Cape  | 28                  | 438                  |
| Total:        | 107                 | 1281                 |

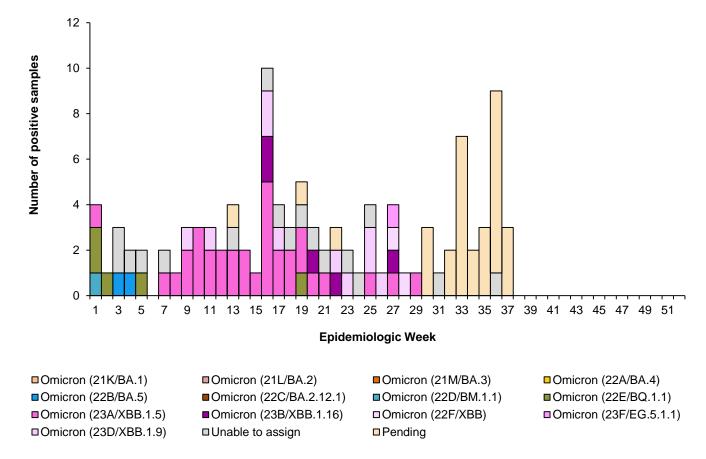


Figure 11. Number of laboratory confirmed SARS-CoV-2\* cases by variant type (variant PCR/sequencing) and week, influenza-like illness (ILI) surveillance - Viral Watch, 01/01/2023 – 17/09/2023

<sup>\*</sup>Specimens from patients with influenza-like illnesses at 92 sentinel sites in 8 provinces

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

Table 9. Number of SARS-CoV-2\* positive cases by variant (variant PCR and/or sequencing) identified and total number of samples tested by province, ILI surveillance - Viral Watch, 01/01/2023 – 17/09/2023

| Clinic<br>(Province) | Omicron (21L/BA.2) | Omicron (21M/BA.3) | Omicron (22A/BA.4) | Omicron (22B/BA.5) | Omicron (22C/<br>BA.2.12.1) | Omicron (22D/BM.1.1) | Omicron (22E/BQ.1.1) | Omicron (23A/XBB.1.5) | Omicron (23B/XBB.1.16) | Omicron (22F/XBB) | Omicron<br>23F/23F/EG.5.1 | Omicron (23D/XBB.1.9) | Unable to assign** | Pending*** | Total SARS-CoV-2 positive | Total samples tested |
|----------------------|--------------------|--------------------|--------------------|--------------------|-----------------------------|----------------------|----------------------|-----------------------|------------------------|-------------------|---------------------------|-----------------------|--------------------|------------|---------------------------|----------------------|
| Eastern<br>Cape      | 0                  | 0                  | 0                  | 0                  | 0                           | 0                    | 0                    | 2                     | 0                      | 0                 | 0                         | 1                     | 1                  | 1          | 5                         | 50                   |
| Free State           | 0                  | 0                  | 0                  | 0                  | 0                           | 0                    | 0                    | 0                     | 0                      | 0                 | 0                         | 0                     | 0                  | 0          | 0                         | 2                    |
| Gauteng              | 0                  | 0                  | 0                  | 2                  | 0                           | 1                    | 3                    | 25                    | 2                      | 1                 | 1                         | 7                     | 7                  | 21         | 70                        | 730                  |
| Limpopo              | 0                  | 0                  | 0                  | 0                  | 0                           | 0                    | 0                    | 0                     | 0                      | 0                 | 0                         | 0                     | 0                  | 0          | 0                         | 6                    |
| Mpumala<br>nga       | 0                  | 0                  | 0                  | 0                  | 0                           | 0                    | 0                    | 1                     | 0                      | 1                 | 0                         | 0                     | 1                  | 1          | 4                         | 52                   |
| North<br>West        | 0                  | 0                  | 0                  | 0                  | 0                           | 0                    | 0                    | 0                     | 0                      | 0                 | 0                         | 0                     | 0                  | 0          | 0                         | 3                    |
| Northern<br>Cape     | 0                  | 0                  | 0                  | 0                  | 0                           | 0                    | 0                    | 0                     | 0                      | 0                 | 0                         | 0                     | 0                  | 0          | 0                         | 0                    |
| Western<br>Cape      | 0                  | 0                  | 0                  | 0                  | 0                           | 0                    | 2                    | 5                     | 3                      | 0                 | 0                         | 2                     | 8                  | 8          | 28                        | 438                  |
| Total:               | 0                  | 0                  | 0                  | 2                  | 0                           | 1                    | 5                    | 33                    | 5                      | 2                 | 1                         | 10                    | 17                 | 31         | 107                       | 1281                 |

<sup>\*</sup>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

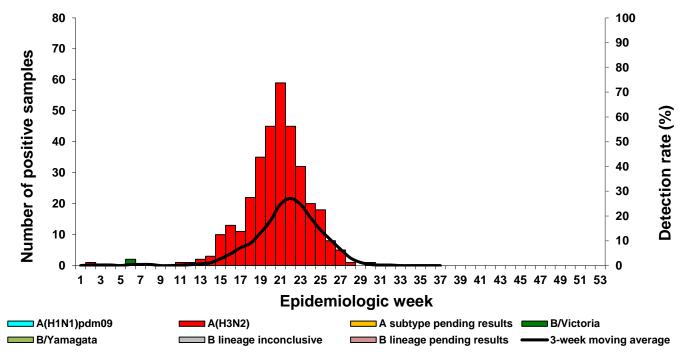


Figure 12. Number of positive influenza positive cases\* by influenza subtype and lineage\*\* and 3-week moving average by week, pneumonia surveillance public hospitals, 01/01/2023 – 17/09/2023

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

<sup>\*\*</sup>No cases of Alpha, Beta or 20D (C.1.2) variants detected.

<sup>\*</sup>Specimens from patients hospitalised with pneumonia at 15 sentinel sites in 6 provinces

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

Table 10. Number of laboratory confirmed influenza cases by subtype and lineage\* and total number of samples tested by hospital, pneumonia surveillance public hospitals, 01/01/2023-17/09/2023

| Hospital (Province)               | A(H1N1)pd<br>m09 | A(H3N2) | A subtype inconclusive | A subtype<br>pending<br>results*** | B/Victoria | B/Yamagat<br>a | B lineage inconclusive | B lineage<br>pending<br>results*** | Total<br>samples |
|-----------------------------------|------------------|---------|------------------------|------------------------------------|------------|----------------|------------------------|------------------------------------|------------------|
| Edendale (KZ)                     | 0                | 29      | 1                      | 0                                  | 1          | 0              | 0                      | 0                                  | 563              |
| Helen Joseph-Rahima<br>Moosa (GP) | 0                | 64      | 1                      | 0                                  | 0          | 0              | 0                      | 0                                  | 1075             |
| Khayelitsha (WC)                  | 0                | 29      | 1                      | 0                                  | 1          | 0              | 0                      | 0                                  | 495              |
| Klerksdorp-Tshepong<br>(NW)       | 0                | 47      | 1                      | 0                                  | 0          | 0              | 0                      | 0                                  | 445              |
| Livingstone (EC)                  | 0                | 21      | 1                      | 0                                  | 0          | 0              | 0                      | 0                                  | 541              |
| Mapulaneng-<br>Matikwana (MP)     | 0                | 22      | 2                      | 0                                  | 0          | 0              | 0                      | 0                                  | 398              |
| Mitchell's Plain (WC)             | 0                | 16      | 0                      | 0                                  | 0          | 0              | 0                      | 0                                  | 383              |
| Red Cross (WC)                    | 0                | 26      | 1                      | 0                                  | 0          | 0              | 0                      | 0                                  | 754              |
| Tambo Memorial (GP)               | 0                | 33      | 1                      | 0                                  | 0          | 0              | 0                      | 0                                  | 456              |
| Tembisa (GP)                      | 0                | 26      | 3                      | 0                                  | 0          | 0              | 0                      | 0                                  | 464              |
| Tintswalo (MP)                    | 0                | 11      | 0                      | 0                                  | 0          | 0              | 0                      | 0                                  | 234              |
| Tygerberg (WC)                    | 0                | 9       | 0                      | 0                                  | 0          | 0              | 0                      | 0                                  | 113              |
| Total:                            | 0                | 333     | 12                     | 0                                  | 2          | 0              | 0                      | 0                                  | 5921             |

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

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

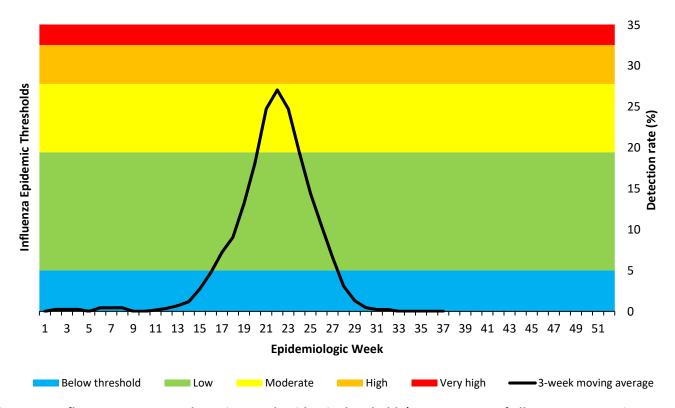


Figure 13. Influenza percentage detections and epidemic thresholds\* among cases of all ages, pneumonia surveillance public hospitals, 01/01/2023 - 17/09/2023

<sup>\*\*</sup>Inconclusive: insufficient viral load in sample and unable to characterise further

<sup>\*</sup>Thresholds based on 2010-2019 data

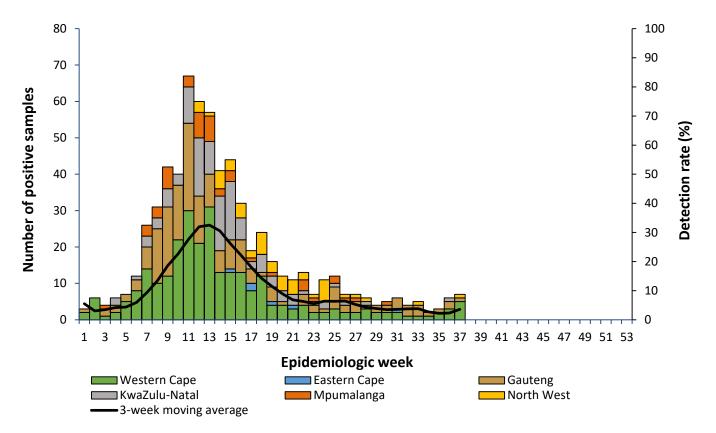


Figure 14. Number of patients (all ages) testing positive for respiratory syncytial virus\* by province and 3-week moving average by week, pneumonia surveillance public hospitals, 01/01/2023 – 17/09/2023

Specimens from patients hospitalised with pneumonia at 15 sentinel sites in 6 provinces.

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

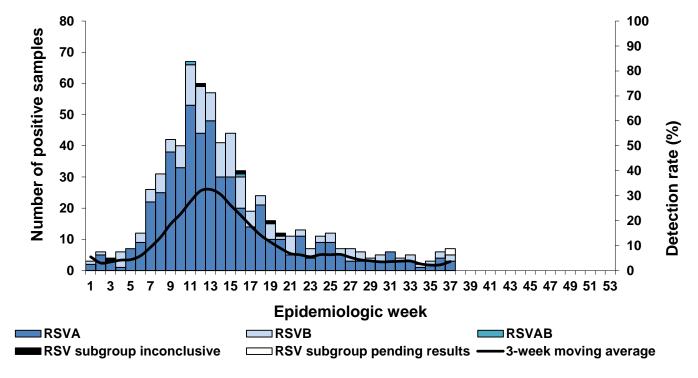


Figure 15. Number of patients (all ages) testing positive for respiratory syncytial virus\* by subgroup and 3-week moving average by week, pneumonia surveillance public hospitals, 01/01/2023 – 17/09/2023

Specimens from patients hospitalised with pneumonia at 15 sentinel sites in 6 provinces.

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

RSV AB: Both RSV A and B subgroup identified

RSV subgroup pending: RSV results for subgroups are pending

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

Table 11. Number of patients (all ages) positive for respiratory syncytial virus subgroups\* by subgroups identified and total number of samples tested by hospital, pneumonia surveillance public hospitals, 01/01/2023 – 17/09/2023

| Hospital (Province)            | RSVA | RSVB | RSVAB** | RSV subgroup inconclusive** | RSV<br>subgroup<br>pending**<br>** | Total<br>samples |
|--------------------------------|------|------|---------|-----------------------------|------------------------------------|------------------|
| Edendale (KZ)                  | 38   | 73   | 1       | 1                           | 0                                  | 563              |
| Helen Joseph-Rahima Moosa (GP) | 153  | 17   | 0       | 1                           | 0                                  | 1075             |
| Khayelitsha (WC)               | 5    | 4    | 0       | 0                           | 0                                  | 495              |
| Klerksdorp-Tshepong (NW)       | 45   | 3    | 0       | 1                           | 0                                  | 445              |
| Livingstone (EC)               | 4    | 2    | 0       | 0                           | 0                                  | 541              |
| Mapulaneng-Matikwana (MP)      | 13   | 6    | 1       | 0                           | 0                                  | 398              |
| Mitchell's Plain (WC)          | 68   | 11   | 0       | 0                           | 1                                  | 383              |
| Red Cross (WC)                 | 145  | 32   | 0       | 2                           | 1                                  | 754              |
| Tambo Memorial (GP)            | 3    | 2    | 0       | 0                           | 0                                  | 456              |
| Tembisa (GP)                   | 2    | 2    | 0       | 0                           | 0                                  | 464              |
| Tintswalo (MP)                 | 25   | 1    | 0       | 0                           | 0                                  | 234              |
| Tygerberg (WC)                 | 1    | 1    | 0       | 0                           | 0                                  | 113              |
| Total:                         | 502  | 154  | 2       | 5                           | 2                                  | 5921             |

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

<sup>\*\*\*\*</sup>RSV results for subgroups are pending

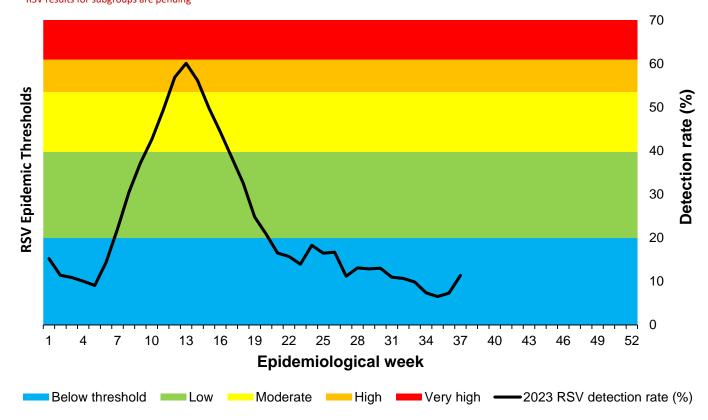


Figure 16. RSV percentage 3-week moving average and epidemic thresholds\* among children aged < 5 years, pneumonia surveillance public hospitals, 01/01/2023 - 17/09/2023

<sup>\*\*</sup>RSV AB: Both RSV A and B subgroup identified

<sup>\*\*\*</sup>Inconclusive: insufficient viral load in sample and unable to characterise further

<sup>\*</sup>Thresholds based on 2010-2019 data

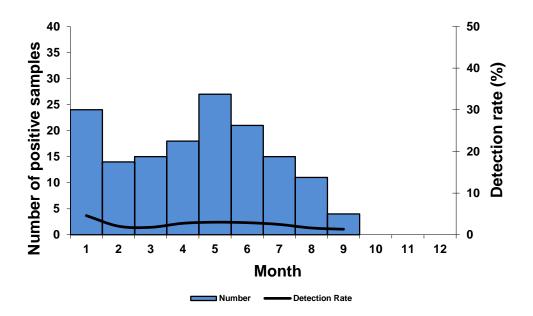


Figure 17. Number of patients testing positive for *B. pertussis\** and 3-week moving average by month, pneumonia surveillance public hospitals\*\*, 01/01/2023 – 17/09/2023

Table 12. Number of patients testing positive for *B. pertussis\** identified and total number of samples tested by hospital and province, pneumonia surveillance public hospitals, 01/01/2023 – 17/09/2023

| Hospital (Province)            | <i>B. pertussis</i><br>Positive | Total samples |  |  |  |
|--------------------------------|---------------------------------|---------------|--|--|--|
| Edendale (KZ)                  | 17                              | 552           |  |  |  |
| Helen Joseph-Rahima Moosa (GP) | 40                              | 1054          |  |  |  |
| Khayelitsha (WC)               | 3                               | 484           |  |  |  |
| Klerksdorp-Tshepong(NW)        | 24                              | 438           |  |  |  |
| Livingstone (EC)               | 6                               | 538           |  |  |  |
| Mapulaneng-Matikwana (MP)      | 21                              | 387           |  |  |  |
| Mitchell's Plain (WC)          | 3                               | 383           |  |  |  |
| Red Cross (WC)                 | 16                              | 751           |  |  |  |
| Tambo Memorial (GP)            | 6                               | 445           |  |  |  |
| Tembisa (GP)                   | 7                               | 459           |  |  |  |
| Tintswalo (MP)                 | 4                               | 233           |  |  |  |
| Tygerberg (WC)                 | 2                               | 113           |  |  |  |
| Total:                         | 149                             | 5837          |  |  |  |

<sup>\*</sup>No cases who met the suspected SARS-CoV-2 or *B. pertussis* case definition but did not meet the pneumonia (SRI) case definition. These are not included in the table.

<sup>\*</sup>No cases who met the suspected SARS-CoV-2 or *B. pertussis* case definition but did not meet Pneumonia Surveillance case definition.

<sup>\*</sup>Specimens from patients hospitalised with pneumonia at 15 sentinel sites in 6 provinces.

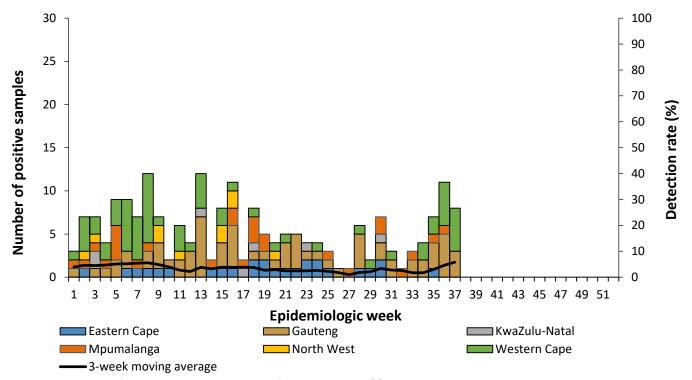


Figure 18. Number of patients testing positive for SARS-CoV-2\*\* by province and 3-week moving average by week, pneumonia surveillance public hospitals, 01/01/2023 – 17/09/2023

Table 13. Number of patients positive for SARS-CoV-2\* and total number of samples tested by hospital, pneumonia surveillance public hospitals, 01/01/2023 – 17/09/2023

| Hospital (Province)            | SARS-CoV-2 positive | Total samples<br>tested |  |  |  |  |
|--------------------------------|---------------------|-------------------------|--|--|--|--|
| Edendale (KZ)                  | 7                   | 563                     |  |  |  |  |
| Helen Joseph-Rahima Moosa (GP) | 31                  | 1075                    |  |  |  |  |
| Khayelitsha (WC)               | 19                  | 495                     |  |  |  |  |
| Klerksdorp-Tshepong (NW)       | 10                  | 445                     |  |  |  |  |
| Livingstone (EC)               | 26                  | 541                     |  |  |  |  |
| Mapulaneng-Matikwana (MP)      | 18                  | 398                     |  |  |  |  |
| Mitchell's Plain (WC)          | 18                  | 383                     |  |  |  |  |
| Red Cross (WC)                 | 23                  | 754                     |  |  |  |  |
| Tambo Memorial (GP)            | 26                  | 456                     |  |  |  |  |
| Tembisa (GP)                   | 13                  | 464                     |  |  |  |  |
| Tintswalo (MP)                 | 9                   | 234                     |  |  |  |  |
| Tygerberg (WC)                 | 4                   | 113                     |  |  |  |  |
| Total:                         | 204                 | 5921                    |  |  |  |  |

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

<sup>\*</sup>Specimens from patients hospitalized with pneumonia at 15 sentinel sites in 6 provinces.

<sup>\*\*</sup>No cases met suspected SARS-CoV-2 or B. pertussis case definition but did not meet pneumonia (SRI) case definition.

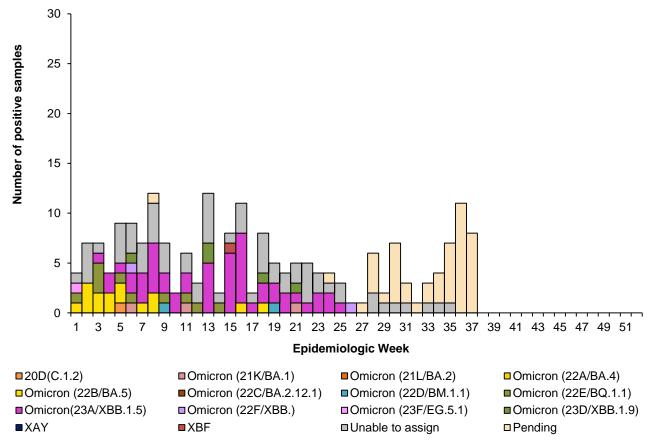


Figure 19. Number and 3-week moving average of laboratory-confirmed SARS-CoV-2 cases\* by variant type (variant PCR/sequencing), pneumonia surveillance public hospitals, 01/01/2023 – 17/09/2023

Table 14. Number of SARS-CoV-2 positive cases\* by variant (variant PCR and/or sequencing) identified and total number of samples tested by hospital, pneumonia surveillance public hospitals, 01/01/2023 – 17/09/2023

| Hospital<br>(Province)         | 20D (C.1.2) | Omicron<br>(21K/BA.1) | Omicron<br>(22B/BA.5) | Omicron<br>(22C/BA.2.12.1) | Omicron<br>(22D/BM.1.1) | Omicron<br>(22E/BQ.1.1) | Omicron(23A/XB<br>B.1.5) | Omicron<br>(22F/XBB.) | Omicron<br>(23F/EG.5.1) | Omicron<br>(23D/XBB.1.9) | ХАУ | XBF | XBL | Unable to assign | Pending | SARS-CoV-2<br>positive | Total samples<br>tested |
|--------------------------------|-------------|-----------------------|-----------------------|----------------------------|-------------------------|-------------------------|--------------------------|-----------------------|-------------------------|--------------------------|-----|-----|-----|------------------|---------|------------------------|-------------------------|
| Edendale (KZ)<br>Helen Joseph- | 0           | 0                     | 0                     | 0                          | 0                       | 1                       | 1                        | 0                     | 0                       | 1                        | 0   | 0   | 0   | 3                | 1       | 7                      | 563                     |
| Rahima Moosa<br>(GP)           | 0           | 1                     | 1                     | 0                          | 0                       | 1                       | 9                        | 1                     | 0                       | 2                        | 0   | 0   | 0   | 6                | 10      | 31                     | 1075                    |
| Khayelitsha (WC)               | 0           | 1                     | 1                     | 0                          | 0                       | 1                       | 5                        | 0                     | 0                       | 0                        | 0   | 0   | 0   | 8                | 3       | 19                     | 495                     |
| Klerksdorp-<br>Tshepong (NW)   | 0           | 0                     | 1                     | 0                          | 0                       | 2                       | 5                        | 0                     | 0                       | 0                        | 0   | 0   | 0   | 2                | 0       | 10                     | 445                     |
| Livingstone (EC)               | 0           | 1                     | 1                     | 0                          | 2                       | 0                       | 6                        | 0                     | 0                       | 0                        | 0   | 1   | 0   | 9                | 6       | 26                     | 541                     |
| Mapulaneng-<br>Matikwana (MP)  | 0           | 0                     | 3                     | 0                          | 0                       | 1                       | 2                        | 0                     | 0                       | 0                        | 0   | 0   | 0   | 9                | 3       | 18                     | 398                     |
| Mitchell's Plain<br>(WC)       | 0           | 0                     | 1                     | 0                          | 0                       | 0                       | 7                        | 1                     | 0                       | 1                        | 0   | 0   | 0   | 5                | 3       | 18                     | 383                     |
| Red Cross (WC)                 | 1           | 0                     | 4                     | 0                          | 0                       | 0                       | 4                        | 0                     | 0                       | 0                        | 0   | 0   | 1   | 3                | 10      | 23                     | 754                     |
| Tambo Memorial<br>(GP)         | 0           | 0                     | 0                     | 0                          | 0                       | 3                       | 10                       | 0                     | 0                       | 0                        | 0   | 0   | 0   | 6                | 7       | 26                     | 456                     |
| Tembisa (GP)                   | 0           | 0                     | 1                     | 0                          | 0                       | 1                       | 1                        | 0                     | 0                       | 1                        | 0   | 0   | 0   | 9                | 0       | 13                     | 464                     |
| Tintswalo (MP)                 | 0           | 0                     | 1                     | 0                          | 0                       | 0                       | 0                        | 0                     | 0                       | 0                        | 0   | 0   | 0   | 4                | 4       | 9                      | 234                     |
| Tygerberg (WC)                 | 0           | 0                     | 1                     | 0                          | 0                       | 0                       | 2                        | 0                     | 0                       | 0                        | 0   | 0   | 0   | 1                | 0       | 4                      | 113                     |
| Total:                         | 1           | 3                     | 15                    | 0                          | 2                       | 10                      | 52                       | 2                     | 0                       | 5                        | 0   | 1   | 1   | 65               | 47      | 204                    | 5921                    |

<sup>\*</sup>Specimens are from hospitalized patients at 15 sentinel sites in 6 provinces who met the pneumonia (SRI), suspected SARS-CoV-2 or *B. pertussis* case definition **Unable to assign**: no lineage assigned due to poor- sequence quality **OR** low viral load (C₁≥35) **OR** variant PCR could not assign variant and no sequencing result **Pending**: outstanding variant results

<sup>\*</sup>Specimens are from hospitalized patients at 15 sentinel sites in 6 provinces who met the pneumonia (SRI), suspected SARS-CoV-2 or *B. pertussis* case definition **Unable to assign**: no lineage assigned due to poor- sequence quality **OR** low viral load (Ct≥35) **OR** variant PCR could not assign variant and no sequencing result **Pending**: outstanding variant results

#### **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<sup>™</sup> 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.

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  $\mu$ l per sample, in order to increase yields. 300  $\mu$ l of each sample was used for automated magnetic bead-based extraction using the Chemagic 360. RNA was eluted in 60  $\mu$ l 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, (<a href="https://sars-cov-2.exatype.com/">https://sars-cov-2.exatype.com/</a>). 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, (<a href="https://ormbunkar.se/aliview/">https://ormbunkar.se/aliview/</a>) (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 (<a href="https://www.gisaid.org/">https://www.gisaid.org/</a>) (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).