

Report week: 31

Reporting period: 30 December 2024 to 03 August 2025

Date of data extraction: 2025-08-07

Data are provisional as on date data extracted. Number of consultations/specimens are reported/analysed by date of consultation/specimen collection. Data cleaning is ongoing and this may result in some changes in subsequent reports. Refer to end of report for methodology and definitions.

Highlights

- In week 31 (28 July 2025 to 03 August 2025), from 117 samples tested, we detected 7 (6.0%) cases of influenza, 3 (2.6%) cases of RSV and 2 (1.7%) cases of SARS-CoV-2.
- The influenza season started in week 13 (week starting 24 March 2025) when the detection rate of influenza in hospitalised patients crossed the seasonal threshold. This is early compared to the start of the season historically. The detection rate reduced below the seasonal threshold in week 30, but is still being monitored closely for a potential second increase for the season, as commonly observed in past seasons.
- The RSV season started in week 11 (week starting 10 March 2025) when the detection rate of RSV in hospitalised children aged <5 years enrolled into surveillance crossed the seasonal threshold. RSV activity peaked in week 19 (week starting 05 May 2025) and is currently in low threshold since week 22 (week starting 26 May 2025).
- In the month of July, we detected 10 (2.0%, 10/502) cases of *Bordetella pertussis*.
- From 30 December 2024 to 03 August 2025, from 4605 samples tested, we detected 502 (10.9%) cases of influenza, 730 (15.9%) cases of respiratory syncytial virus (RSV), 125 (2.7%) cases of SARS-CoV-2 and 32 (0.9%) cases of *B. pertussis*.

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Monitoring potentially imported cases of respiratory viruses

No specimens were received from the OR Tambo International Airport clinic in week 31 (week starting 28 July 2025). Since 30 December 2024, one specimen has been received and tested, which was positive for influenza. This case was excluded from subsequent tables and figures, as it was likely not acquired in South Africa.

Influenza & respiratory syncytial virus (RSV) epidemic thresholds

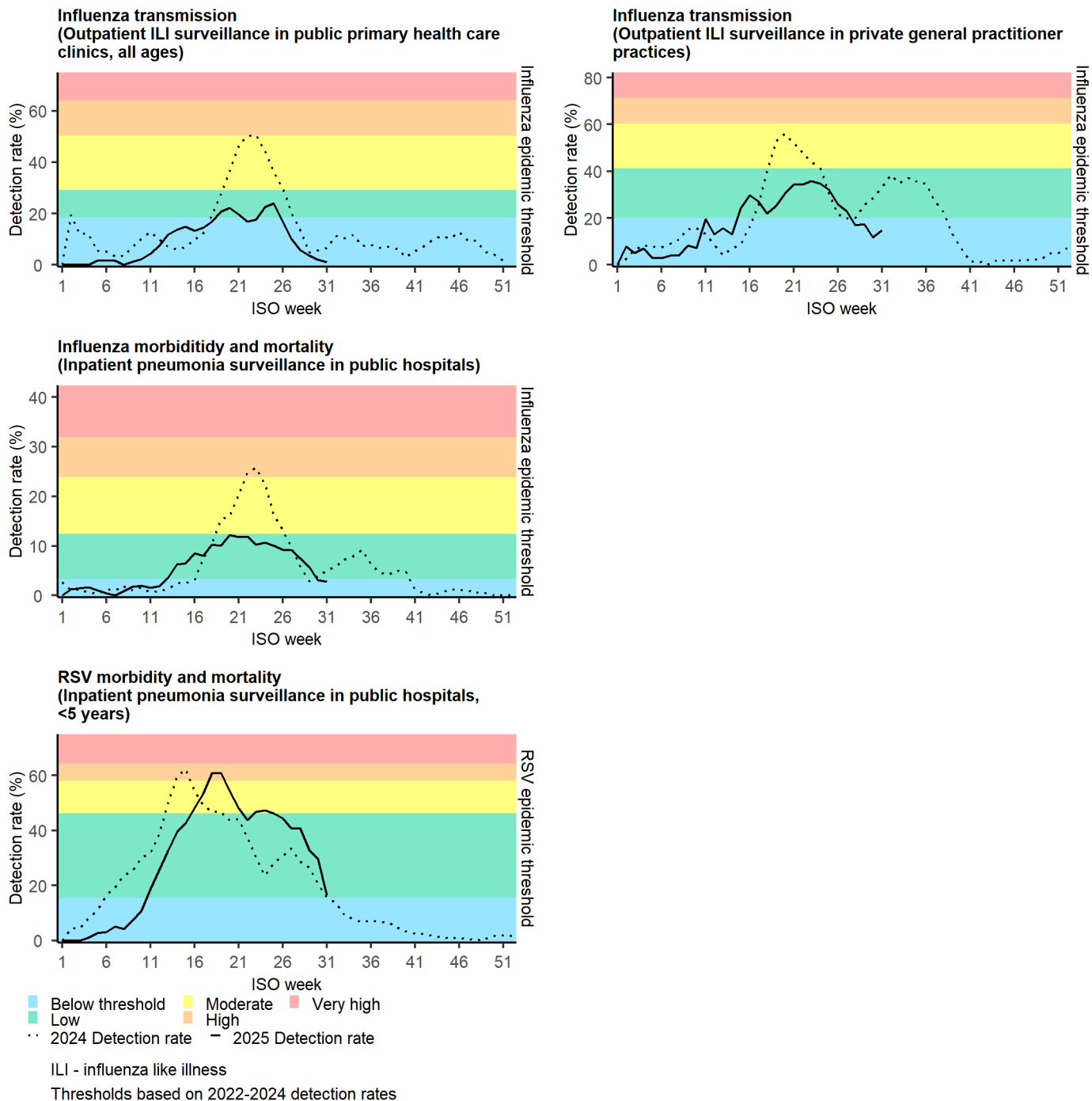


Figure 1: Influenza and respiratory syncytial virus (RSV) surveillance epidemic threshold summary, sentinel surveillance, South Africa, 30 December 2024 to 03 August 2025.

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SARS-CoV-2 epidemic thresholds

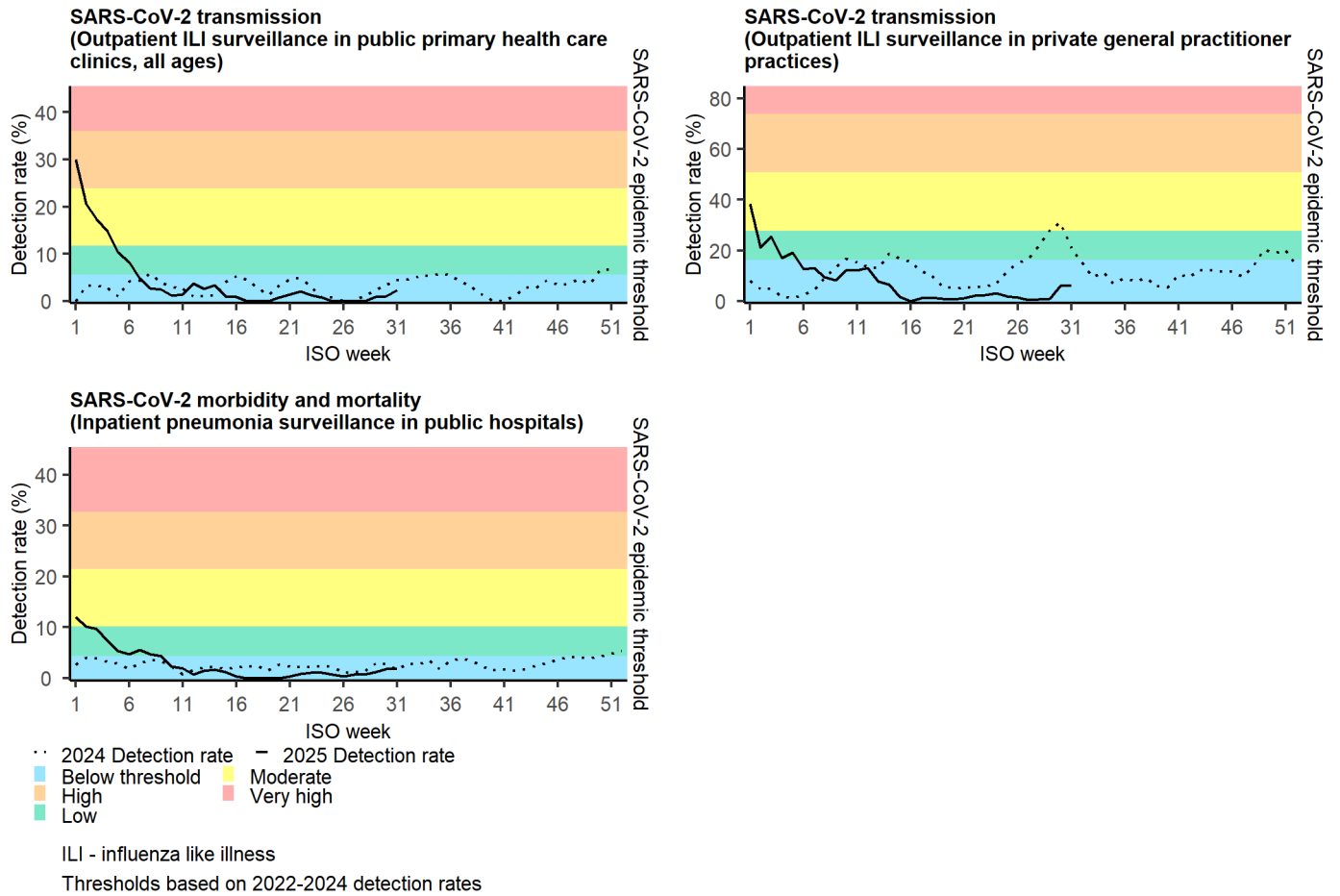


Figure 2: Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) surveillance epidemic threshold summary, sentinel surveillance, South Africa, 30 December 2024 to 03 August 2025.

Influenza

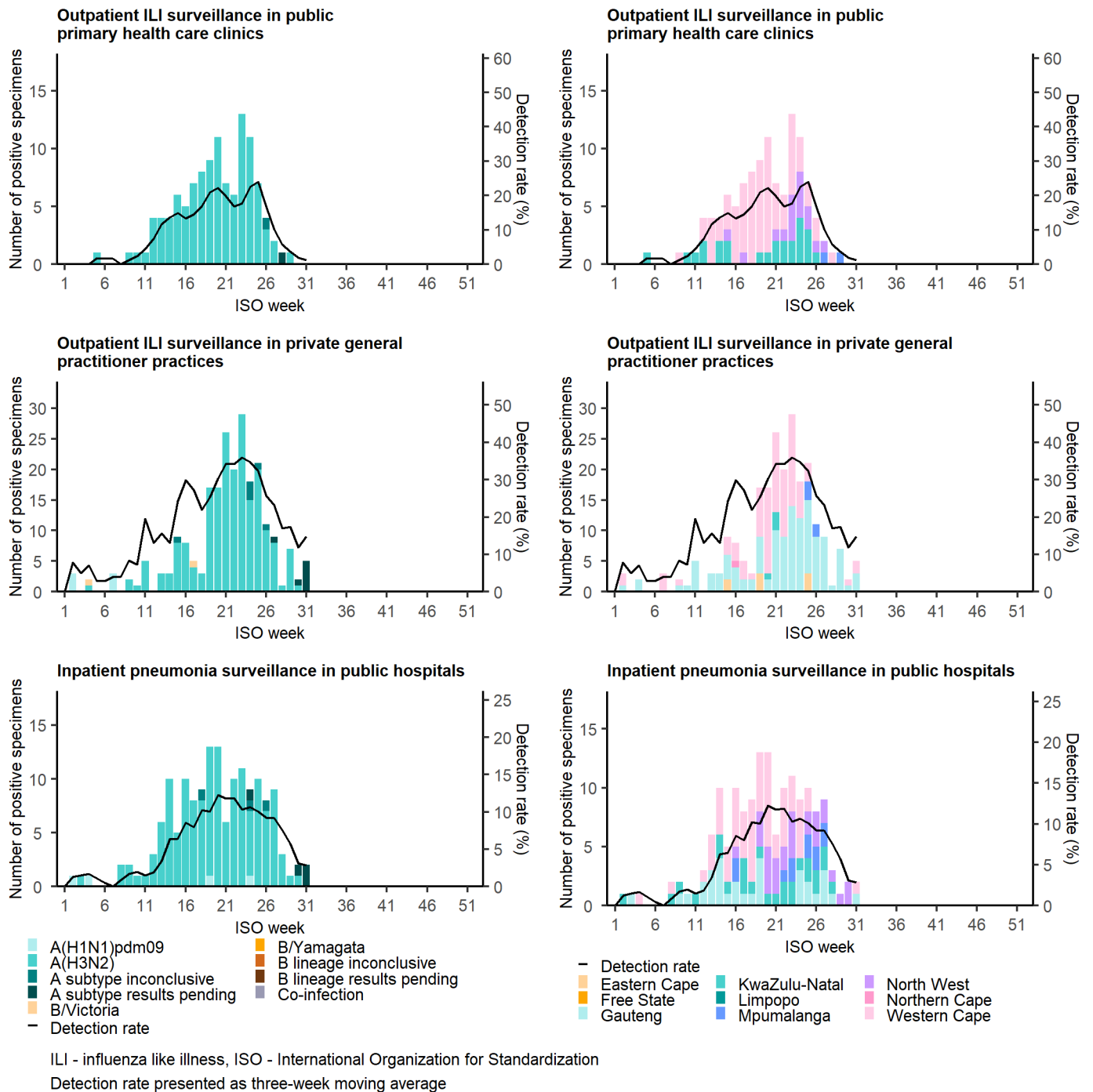


Figure 3: Number of laboratory-confirmed influenza cases and detection rate by subtype and lineage (left) and province (right) in all ages, sentinel surveillance, South Africa, 30 December 2024 to 03 August 2025.

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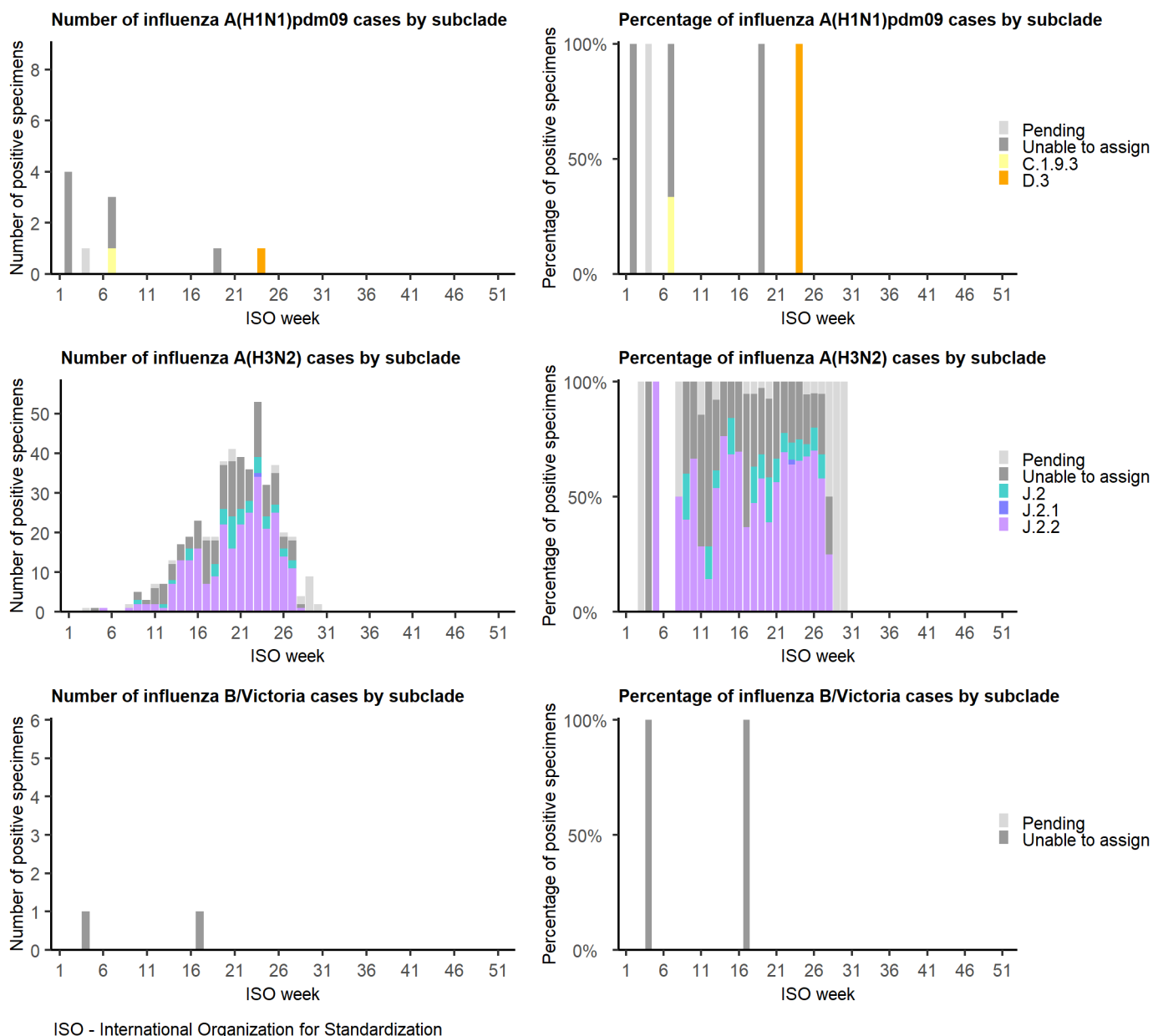


Figure 4: Combined number and percentage of influenza cases by subclade in all ages from three sentinel surveillance systems: outpatient influenza like illness (ILI) surveillance in public primary health care clinics, outpatient ILI surveillance in private general practitioner practices, and inpatient pneumonia surveillance in public hospitals, South Africa, 30 December 2024 to 03 August 2025.

Table 1: Combined number influenza cases by subclade and clade in all ages from three sentinel surveillance systems: outpatient influenza like illness (ILI) surveillance in public primary health care clinics, outpatient ILI surveillance in private general practitioner practices, and inpatient pneumonia surveillance in public hospitals, South Africa, 30 December 2024 to 03 August 2025.

Subtype/Lineage	Clade	Subclade	Count
A(H1N1)pdm09	6B.1A.5a.2a	C.1.9.3	1
A(H1N1)pdm09	6B.1A.5a.2a.1	D.3	1
A(H3N2)	3C.2a1b.2a.2a.3a.1	J.2	41
A(H3N2)	3C.2a1b.2a.2a.3a.1	J.2.1	1
A(H3N2)	3C.2a1b.2a.2a.3a.1	J.2.2	265

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Table 2: Number of laboratory-confirmed influenza cases by subtype and lineage and total number of samples tested by clinic and province in all ages, outpatient ILI surveillance in public primary health care clinics, South Africa, 30 December 2024 to 03 August 2025.

Clinic (Province)	A(H1N1) pdm09	A(H3N2)	A subtype inconclusive	A subtype pending	B/ Victoria	B/ Yamagata	B lineage inconclusive	B lineage pending	Co-infection	Total influenza	Total specimens
Edendale Gateway (KZ)	0	25	0	0	0	0	0	0	0	25	351
Agincourt (MP)	0	2	0	0	0	0	0	0	0	2	21
Jouberton (NW)	0	16	0	0	0	0	0	0	0	16	204
Eastridge (WC)	0	69	1	1	0	0	0	0	0	71	382
Mitchell's Plain (WC)	0	0	0	0	0	0	0	0	0	0	45
Total	0	112	1	1	0	0	0	0	0	114	1003

Specimens where more than one influenza subtype or lineage was detected denoted as co-infection, and included in the counts for each separate type as well. Agincourt clinic was not active from February – June 2025.

Table 3: Number of laboratory-confirmed influenza cases by subtype and lineage and total number of samples tested by province in all ages, outpatient ILI surveillance in private general practitioner practices, South Africa, 30 December 2024 to 03 August 2025.

Province	A(H1N1) pdm09	A(H3N2)	A subtype inconclusive	A subtype pending	B/ Victoria	B/ Yamagata	B lineage inconclusive	B lineage pending	Co-infection	Total influenza	Total specimens
Eastern Cape	0	8	0	0	0	0	0	0	0	8	15
Free State	0	0	0	0	0	0	0	0	0	0	0
Gauteng	1	114	3	4	1	0	0	0	0	123	750
KwaZulu-Natal	0	4	0	0	0	0	0	0	0	4	15
Limpopo	0	0	0	0	0	0	0	0	0	0	0
Mpumalanga	0	4	1	0	0	0	0	0	0	5	30
North West	0	0	0	0	0	0	0	0	0	0	0
Northern Cape	0	1	0	0	0	0	0	0	0	1	1
Western Cape	5	78	2	3	1	0	0	0	0	89	221
Total	6	209	6	7	2	0	0	0	0	230	1032

Specimens where more than one influenza subtype or lineage was detected denoted as co-infection, and included in the counts for each separate type as well.

Table 4: Number of laboratory-confirmed influenza cases by subtype and lineage and total number of samples tested by hospital and province in all ages, inpatient pneumonia surveillance in public hospitals, South Africa, 30 December 2024 to 03 August 2025.

Hospital (Province)	A(H1N1) pdm09	A(H3N2)	A subtype inconclusive	A subtype pending	B/ Victoria	B/ Yamagata	B lineage inconclusive	B lineage pending	Co-infection	Total influenza	Total specimens
Helen Joseph-Rahima Moosa (GP)	0	30	0	2	0	0	0	0	0	32	453
Harry Gwala (KZ)	1	24	1	0	0	0	0	0	0	27	394
Mapulaneng-Matikwana (MP)	0	5	0	0	0	0	0	0	0	5	145
Tintswalo (MP)	0	6	0	0	0	0	0	0	0	6	142
Klerksdorp-Tshepong (NW)	1	26	0	1	0	0	0	0	0	28	370
Mitchell's Plain (WC)	0	30	1	0	0	0	0	0	0	31	439
Red Cross (WC)	2	25	1	1	0	0	0	0	0	29	627
Total	4	146	3	4	0	0	0	0	0	158	2570

Specimens where more than one influenza subtype or lineage was detected denoted as co-infection, and included in the counts for each separate type as well. Enrolment ended on the 31st of January 2025 at Matikwana Hospital.

Respiratory syncytial virus (RSV)

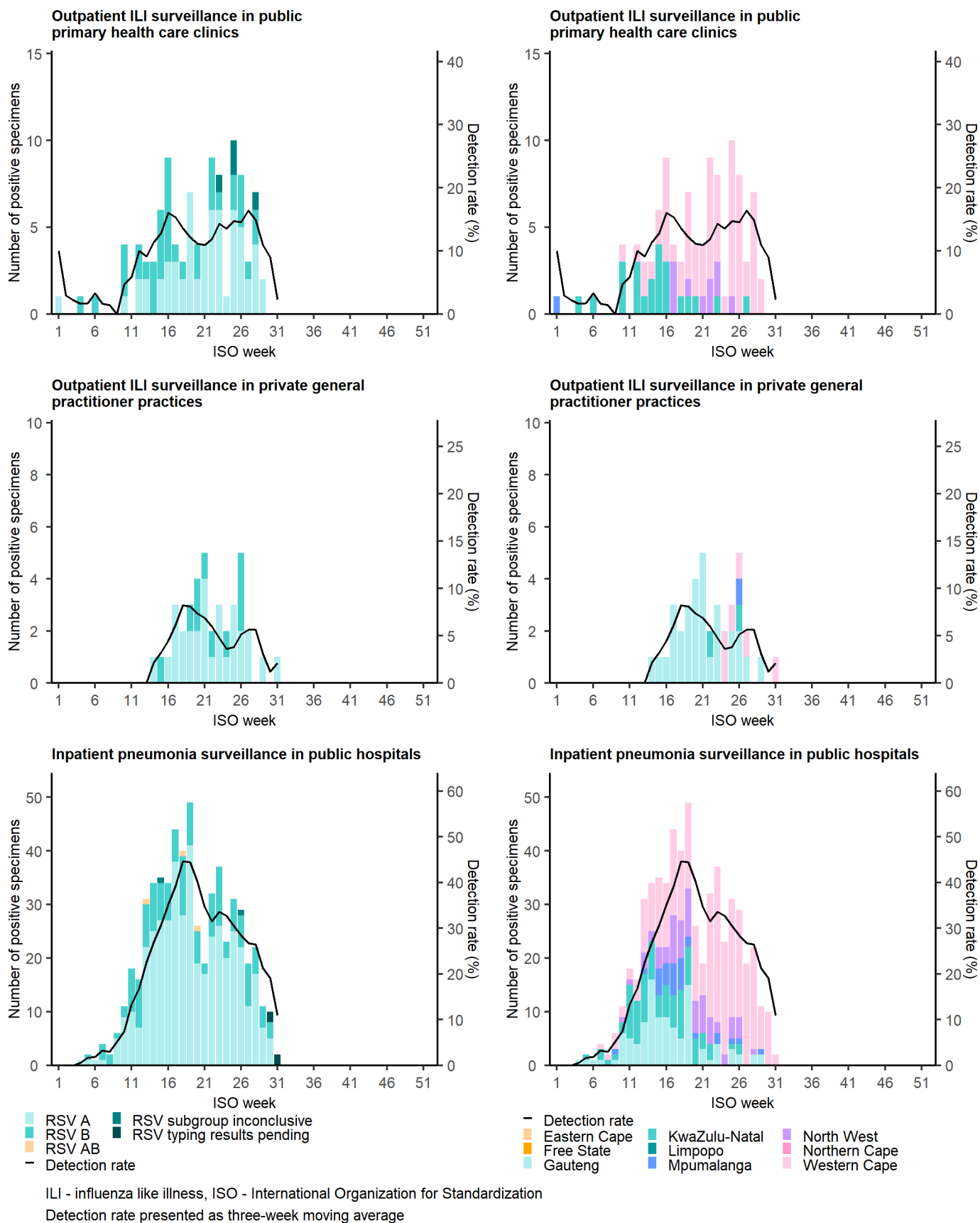


Figure 5: Number of laboratory-confirmed respiratory syncytial virus (RSV) cases and detection rate by type (left) and province (right) in all ages, sentinel surveillance, South Africa, 30 December 2024 to 03 August 2025.

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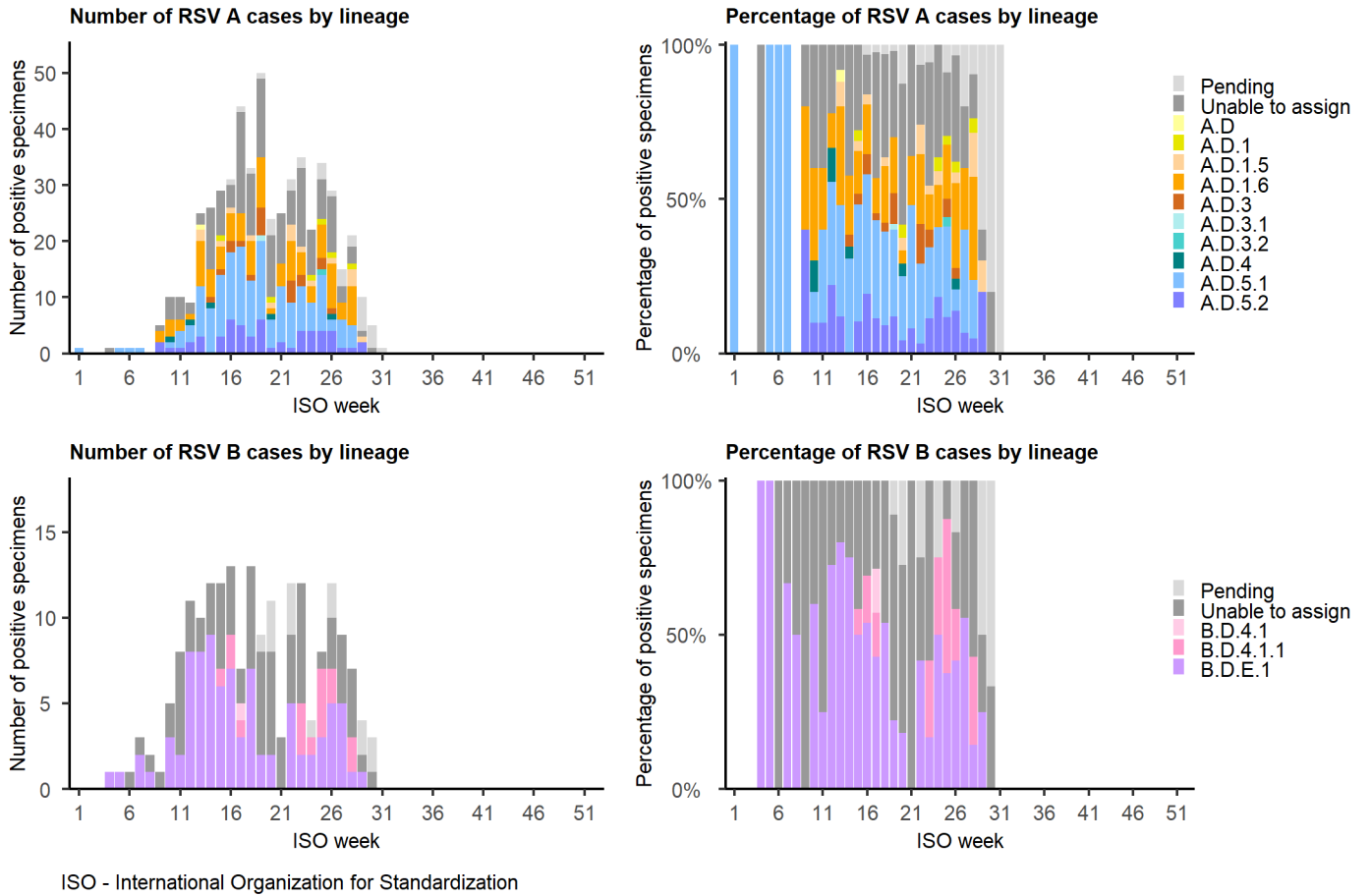


Figure 6: Combined number and percentage of RSV cases by lineage in all ages from three sentinel surveillance systems: outpatient influenza like illness (ILI) surveillance in public primary health care clinics, outpatient ILI surveillance in private general practitioner practices, and inpatient pneumonia surveillance in public hospitals, South Africa, 30 December 2024 to 03 August 2025.

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Table 5: Number of laboratory-confirmed respiratory syncytial virus (RSV) cases by type and total number of samples tested by clinic and province in all ages, outpatient ILI surveillance in public primary health care clinics, South Africa, 30 December 2024 to 03 August 2025.

Clinic (Province)	RSV A	RSV B	RSV AB	RSV subgroup inconclusive	RSV typing results pending	Total RSV	Total specimens
Edendale Gateway (KZ)	3	19	0	1	0	23	351
Agincourt (MP)	1	0	0	0	0	1	21
Jouberton (NW)	9	0	0	1	0	10	204
Eastridge (WC)	46	18	0	2	0	66	382
Mitchell's Plain (WC)	2	0	0	0	0	2	45
Total	61	37	0	4	0	102	1003

Agincourt clinic was not active from February – June 2025.

Table 6: Number of laboratory-confirmed respiratory syncytial virus (RSV) cases by type and total number of samples tested by province in all ages, outpatient ILI surveillance in private general practitioner practices, South Africa, 30 December 2024 to 03 August 2025.

Province	RSV A	RSV B	RSV AB	RSV subgroup inconclusive	RSV typing results pending	Total RSV	Total specimens
Eastern Cape	0	0	0	0	0	0	15
Free State	0	0	0	0	0	0	0
Gauteng	24	6	0	0	0	30	750
KwaZulu-Natal	0	2	0	0	0	2	15
Limpopo	0	0	0	0	0	0	0
Mpumalanga	0	1	0	0	0	1	30
North West	0	0	0	0	0	0	0
Northern Cape	0	0	0	0	0	0	1
Western Cape	5	1	0	0	0	6	221
Total	29	10	0	0	0	39	1032

Table 7: Number of laboratory-confirmed respiratory syncytial virus (RSV) cases by type and total number of samples tested by hospital and province in all ages, inpatient pneumonia surveillance in public hospitals, South Africa, 30 December 2024 to 03 August 2025.

Hospital (Province)	RSV A	RSV B	RSV AB	RSV subgroup inconclusive	RSV typing results pending	Total RSV	Total specimens
Helen Joseph-Rahima Moosa (GP)	103	3	0	0	0	106	453
Harry Gwala (KZ)	23	64	0	0	0	87	394
Mapulaneng-Matikwana (MP)	14	1	0	0	0	15	145
Tintswalo (MP)	9	7	0	0	0	16	142
Klerksdorp-Tshepong (NW)	65	5	0	0	0	70	370
Mitchell's Plain (WC)	64	23	0	2	3	92	439
Red Cross (WC)	158	41	3	0	1	203	627
Total	436	144	3	2	4	589	2570

SARS-CoV-2

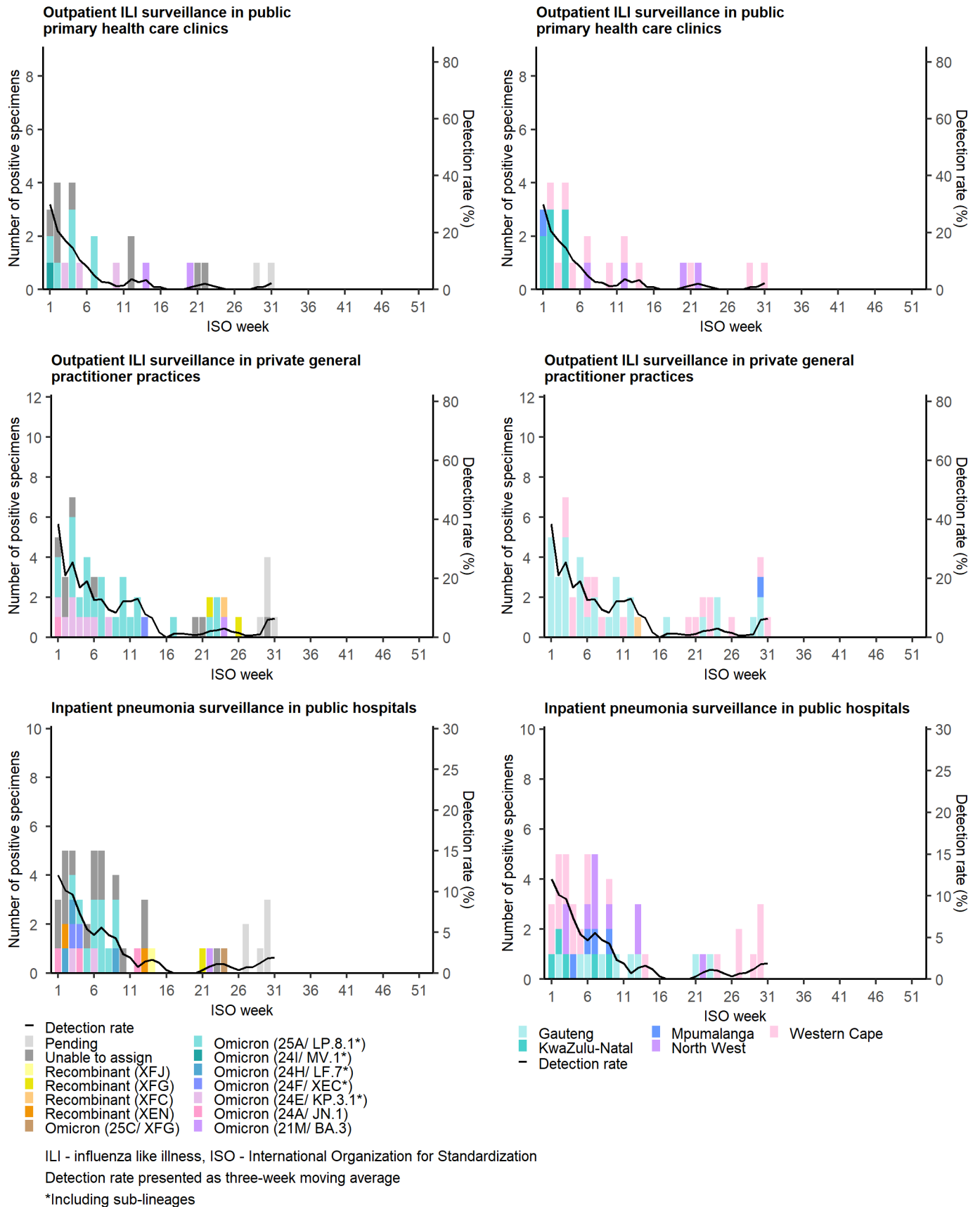


Figure 7: Number of laboratory-confirmed SARS-CoV-2 cases and detection rate by variant type (left) and province (right) in all ages, sentinel surveillance, South Africa, 30 December 2024 to 03 August 2025.

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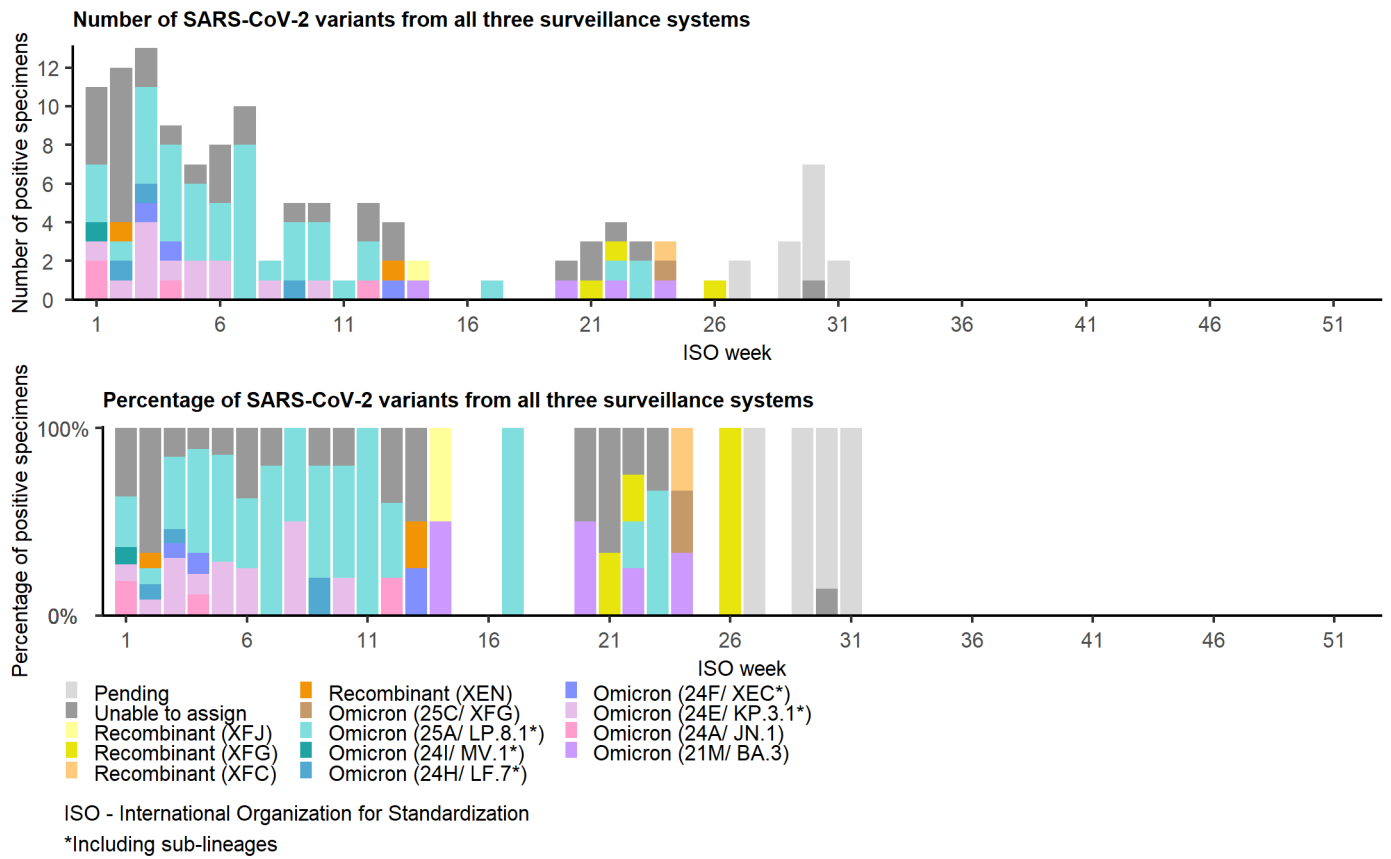


Figure 8: Combined number and percentage of SARS-CoV-2 variants in all ages from three sentinel surveillance systems: outpatient influenza like illness (ILI) surveillance in public primary health care clinics, outpatient ILI surveillance in private general practitioner practices, and inpatient pneumonia surveillance in public hospitals, South Africa, 30 December 2024 to 03 August 2025.

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Table 8: Number of laboratory-confirmed SARS-CoV-2 cases by variant type and total number of samples tested by clinic and province in all ages, outpatient ILI surveillance in public primary health care clinics, South Africa, 30 December 2024 to 03 August 2025.

Clinic (Province)	Omicron (21M/BA.3)	Omicron (24A/JN.1)	Omicron (24E/KP.3.1*)	Omicron (24F/XEC*)	Omicron (24H/LF.7*)	Omicron (24I/MV.1*)	Omicron (25A/LP.8.1*)	Omicron (25C/XFG)	Recombinant (XEN)	Recombinant (XFC)	Recombinant (XFG)	Recombinant (XFJ)	Pending	Unable to assign	Total SARS-CoV-2	Total specimens
Edendale Gateway (KZ)	0	0	0	0	0	1	4	0	0	0	0	0	0	3	8	351
Agincourt (MP)	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	21
Jouberton (NW)	1	0	0	0	0	0	1	0	0	0	0	0	0	2	4	204
Eastridge (WC)	0	0	3	0	0	0	1	0	0	0	0	0	1	2	7	382
Mitchell's Plain (WC)	1	0	0	0	0	0	0	0	0	0	0	0	1	2	4	45
Total	2	0	3	0	0	1	7	0	0	0	0	0	2	9	24	1003

Agincourt clinic was not active from February – June 2025. *Including sub-lineages

Table 9: Number of laboratory-confirmed SARS-CoV-2 cases by variant type and total number of samples tested by province in all ages, outpatient ILI surveillance in private general practitioner practices, South Africa, 30 December 2024 to 03 August 2025.

Province	Omicron n(21M/BA.3)	Omicron n(24A/JN.1)	Omicron n(24E/KP.3.1*)	Omicron n(24F/XEC*)	Omicron n(24H/LF.7*)	Omicron n(24I/MV.1*)	Omicron n(25A/LP.8.1*)	Omicron n(25C/XFG)	Recombinant t(XEN)	Recombinant t(XFC)	Recombinant t(XFG)	Recombinant t(XFJ)	Pending	Unable to assign	Total SARS-CoV-2	Total specimens
Eastern Cape	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	15
Free State	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Gauteng	1	1	4	0	0	0	17	0	0	1	1	0	3	5	33	750
KwaZulu-Natal	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	15
Limpopo	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Mpumalanga	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	30
North West	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Northern Cape	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Western Cape	0	0	4	0	0	0	8	0	0	0	1	0	1	3	17	221
Total	1	1	8	1	0	0	25	0	0	1	2	0	5	8	52	1032

*Including sub-lineages

Table 10: Number of laboratory-confirmed SARS-CoV-2 cases by variant type and total number of samples tested by hospital and province in all ages, inpatient pneumonia surveillance in public hospitals, South Africa, 30 December 2024 to 03 August 2025.

Hospital (Province)	Omicron n(21M/BA.3)	Omicron n(24A/JN.1)	Omicron n(24E/KP.3.1*)	Omicron n(24F/XEC*)	Omicron n(24H/LF.7*)	Omicron n(24I/MV.1*)	Omicron n(25A/LP.8.1*)	Omicron n(25C/XFG)	Recombinant t(XEN)	Recombinant t(XFC)	Recombinant t(XFG)	Recombinant t(XFJ)	Pending	Unable to assign	Total SARS-CoV-2	Total specimens
Helen Joseph-Rahima	0	1	0	0	0	0	2	0	1	0	1	0	0	4	9	453
Moosa (GP)																
Harry Gwala (KZ)	0	0	0	1	0	0	2	0	0	0	0	0	0	2	5	394
Mapulaneng-Matikwana (MP)	0	0	0	0	0	0	1	0	0	0	0	0	0	1	2	145
Tintswalo (MP)	0	0	0	0	0	0	0	0	0	0	0	0	0	2	2	142
Klerksdorp-Tshepong (NW)	1	0	1	0	0	0	3	0	0	0	0	0	0	5	10	370
Mitchell's Plain (WC)	0	1	1	0	0	0	0	0	0	0	0	1	3	2	8	439
Red Cross (WC)	0	1	0	1	3	0	3	1	1	0	0	0	3	0	13	627
Total	1	3	2	2	3	0	11	1	2	0	1	1	6	16	49	2570

*Including sub-lineages

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Bordetella pertussis

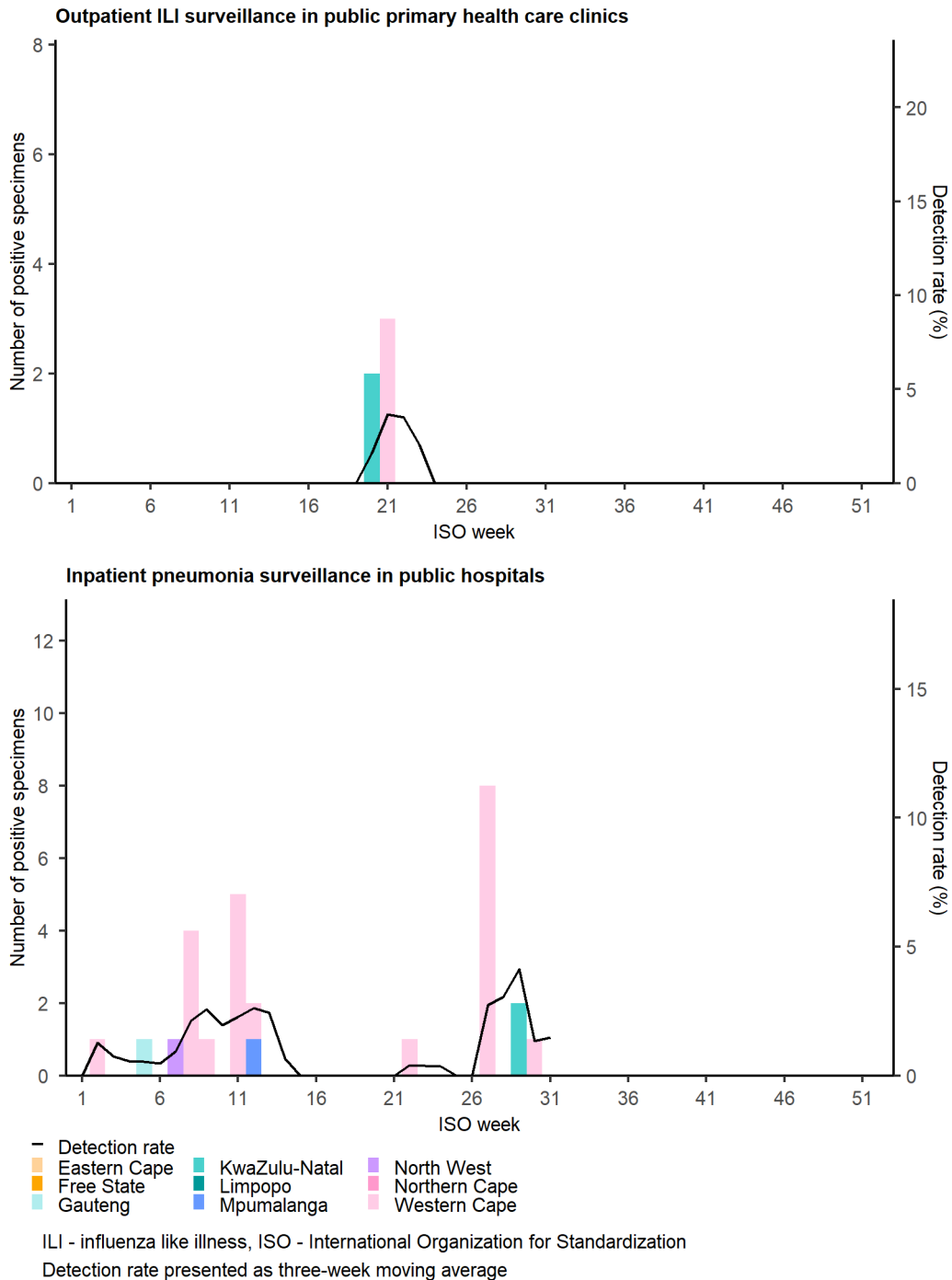


Figure 9: Number of laboratory-confirmed *Bordetella pertussis* cases and detection rate by province in all ages, sentinel surveillance, South Africa, 30 December 2024 to 03 August 2025.

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Table 11: Number of laboratory-confirmed *Bordetella pertussis* cases and total number of samples tested by province in all ages, outpatient ILI surveillance in public primary health care clinics, South Africa, 30 December 2024 to 03 August 2025.

Province	Positive	Pending testing	Total specimens
KwaZulu-Natal	2	1	351
Mpumalanga	0	0	21
North West	0	0	204
Western Cape	3	0	427
Total	5	1	1003

Table 12: Number of laboratory-confirmed *Bordetella pertussis* cases and total number of samples tested by province in all ages, inpatient pneumonia surveillance in public hospitals, South Africa, 30 December 2024 to 03 August 2025.

Province	Positive	Pending testing	Total specimens
Gauteng	1	0	453
KwaZulu-Natal	2	1	394
Mpumalanga	1	0	287
North West	1	0	370
Western Cape	22	8	1066
Total	27	9	2570

Methods

Table 13: Programme descriptions for sentinel surveillance in South Africa

Programme	Influenza-like illness (ILI)	Viral Watch	National Syndromic Surveillance for Pneumonia
Description	Outpatient ILI surveillance in public primary health care clinics	Outpatient ILI surveillance in private general practitioner practices	Inpatient pneumonia surveillance in public hospitals
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	ILI: An acute respiratory illness with a temperature ($\geq 38^{\circ}\text{C}$) or history of fever and cough, & onset ≤ 10 days. 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: paroxysms of coughing, or inspiratory "whoop", or post-tussive vomiting or apnoea in children < 1 year; OR Any person in whom a clinician suspects pertussis.	ILI: An acute respiratory illness with a temperature ($\geq 38^{\circ}\text{C}$) or history of fever and cough, & onset ≤ 10 days.	SRI: Patients aged 2 days to < 3 months: Diagnosis of sepsis or suspected sepsis, or physician diagnosed LRTI AND symptoms of any duration. Patients aged 3 months to < 5 years: Physician diagnosed LRTI, symptoms of any duration. Patients aged ≥ 5 years with fever (≥ 38) or history of fever AND cough AND symptoms of any duration. 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: paroxysms of coughing, or inspiratory "whoop", or post-tussive vomiting or apnoea in children < 1 year; OR Any person in whom a clinician suspects pertussis.
Specimens collected	Mid-turbinate nasal swabs.	Throat and/or nasal swabs or Nasopharyngeal swabs.	Mid-turbinate nasal swabs.
Main pathogens tested	Influenza virus, RSV, SARS-CoV-2, <i>B. pertussis</i> .	Influenza virus, RSV, SARS-CoV-2.	Influenza virus, RSV, SARS-CoV-2, <i>B. pertussis</i> .
Testing Methods	Influenza virus, RSV, SARS-CoV-2: Allplex™ SARS-CoV-2/FluA/FluB/RSV PCR kit. <i>B. pertussis</i> : Multiplex real-time PCR (Tatti et al., J Clin Microbiol 2011) and culture.	Influenza virus, RSV, SARS-CoV-2: Allplex™ SARS-CoV-2/FluA/FluB/RSV PCR kit.	Influenza virus, RSV, SARS-CoV-2: Allplex™ SARS-CoV-2/FluA/FluB/RSV PCR kit. <i>B. pertussis</i> : Multiplex real-time PCR (Tatti et al., J Clin Microbiol 2011) and culture.

Abbreviations and definitions:

- ILI: Influenza-like illness
- SRI: Severe respiratory infection
- EC: Eastern Cape
- FS: Free State
- GP: Gauteng
- KZ: KwaZulu-Natal
- LP: Limpopo Province
- MP: Mpumalanga
- NW: North West
- NC: Northern Cape
- WC: Western Cape
- Subtype/lineage/subgroup inconclusive: Insufficient viral load in sample and unable to characterize further
- Subtype/lineage/subgroup pending: Further characterization in progress
- Unable to assign SARS-CoV-2 lineage: No lineage assigned due to poor sequence quality OR low viral load ($\text{Ct} \geq 35$)
- Epidemic threshold: Flu and RSV 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. We used the "original method" included in the package to determine the start of the season. 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 influenza-associated morbidity and mortality. For influenza the start and end of the season is defined as once the three week moving average of the detection rate remains above or below the seasonal threshold for two consecutive weeks, respectively. For RSV, thresholds from outpatient influenza like illness (ILI in primary health care clinics) from children aged < 5 years are used as an indicator of disease transmission in the community and thresholds from pneumonia surveillance from children aged < 5 years are used as an indicator of RSV-associated morbidity and mortality. For RSV the start and end of the season is defined as once the three week moving average of the detection rate in children < 5 years from inpatient pneumonia surveillance in public hospitals remains above or below 15% for two consecutive weeks, respectively. SARS-CoV-2 thresholds were calculated using the mean standard deviation (MSD) method, where the seasonal threshold level is determined using the mean three week moving average of the detection rate of the selected historical years and severity levels are based on the mean plus one, three, or five standard deviation for moderate, high and very high thresholds respectively. The MSD method has been detailed by Sinnathamby et al. 2024. Euro Surveill. doi: 10.2807/1560-7917.ES.2024.29.45.2400696

Laboratory testing for influenza, RSV, SARS-CoV-2 and *B. pertussis*:

Influenza A and B viruses, RSV and SARS-CoV-2 were tested using a commercial multiplex RT-PCR assay (Allpex SARS-CoV-2/FluA/FluB/RSV PCR kit, Seegene Inc., Seoul, South Korea). A specimen was considered positive for influenza A, B or RSV if the PCR cycle threshold (Ct) was < 40 for the respective target, and considered positive for SARS-CoV-2 when the Ct was < 40 for ≥ 1 of the S, N or RdRp gene targets. *B. pertussis* was tested using a previously described RT-PCR method (Tatti KM, et al. Journal of Clinical Microbiology. 2011;49(12):4059-4066). A specimen was considered positive when the IS481 and/or ptxS1 gene targets

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are detected with a Ct <45.

Further characterization of influenza, RSV, and SARS-CoV-2:

Influenza A and B positive specimens were subtyped using the US Centres for Disease Control and Prevention (CDC) RT-PCR protocol and reagents (International Reagent Resource (IRR) [Available from: <https://www.internationalreagentresource.org/>]). RSV positive specimens were subgrouped using an in-house assay (Pretorius M, et al. *Journal of Infectious Diseases*. 2012(1537-6613)). SARS-CoV-2 positive specimens were sequenced using the Illumina COVIDSeq protocol (Illumina, CA, USA).

SARS-CoV-2 whole-genome sequencing and genome assembly for SARS-CoV-2 genomic surveillance:

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://orombunkar.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).

Influenza whole-genome sequencing and genome assembly:

RNA extraction: RNA was extracted using the Chemagic360 automated extractor (PerkinElmer, Massachusetts, USA), and eluted in 60µl elution buffer. **PCR and library preparation:** cDNA synthesis was performed using Invitrogen™ SuperScript™ III One-Step RT-PCR System with Platinum™ Taq High Fidelity DNA Polymerase (ThermoFischer Scientific, Massachusetts, USA) and three universal primer sets namely; Uni13/Inf-1, Uni12/Inf-1 and MBTuni-12.4 (Zhou et al., 2009). Prior to library preparation, amplicons underwent quality verification and quantification using the Qubit 4.0 fluorometer (ThermoFischer Scientific (Invitrogen), Massachusetts, USA) and the Qubit dsDNA High Sensitivity assay kit. Fragment sizes were analysed using the TapeStation 4200 (Agilent Technologies, California, USA). Libraries were prepared using the Illumina DNA Library Preparation kit as per manufacturer's protocol (Illumina, San Diego, CA, USA). Amplicons were fragmented and tagmented, then indexed using different sets of UDI indexes by IDT for Illumina DNA library preparation kit (Illumina, San Diego, CA, USA). The indexed libraries were cleaned and normalised at 4nM for pooling. The pooled library was spiked with 1% PhiX (PhiX Control v3 adaptor-ligated library, used as a control). Libraries were sequenced using the NextSeq1000/2000 instrument with the P1 reagent cartridge (300 cycles) and the P1 Flow Cell (Illumina).

Assembly, processing and quality control of genomic sequences: Sequencing reads were analysed using the IRMA/MIRA pipeline (<https://wonder.cdc.gov/amd/flu/irma/irma.html>) with default parameters (Shepard et al., 2016). The quality of the mapping was assessed using QualiMap (García-Alcalde et al., 2012). Consensus sequences were uploaded to the Global Initiative to Share All Influenza Data (GISAID) EpiFlu database if they met the quality criteria of >1000 reads and 90% coverage at 50x depths for the HA and NA segment. Sequences were assigned to clades and subclades using Nextclade (Aksamentov et al., 2021).

RSV whole-genome sequencing and genome assembly:

RNA extraction: Automated RNA extraction was performed using the CMG-1049 kit and the Chemagic 360 (PerkinElmer, USA) using the purification protocol for Viral DNA/RNA from 300 µL from respiratory specimens. **PCR and library preparation:** cDNA synthesis was performed using LunaScript® RT SuperMix (New England Biolabs, Massachusetts, USA) according to the manufacturer's instructions, followed by amplification with Q5® Hot Start High-Fidelity 2X Master Mix (New England Biolabs, Massachusetts, USA) and eight pooled primer sets targeting RSV-A and RSV-B genome regions (UKHSA tiling PCR). Amplicons were quantified using the Qubit 4.0 fluorometer (ThermoFisher Scientific, Massachusetts, USA) and the Qubit 1X dsDNA High Sensitivity assay kit. Primer pools were normalised to equimolar concentrations prior to library preparation. Libraries were prepared using the Nextera DNA Flex or DNA Prep Library Preparation kits (Illumina, San Diego, CA, USA) following the manufacturer's protocol, indexed with unique dual indexes (IDT for Illumina), normalised, and pooled with 1% PhiX Control v3. Sequencing was performed on the Illumina NextSeq 550 or NextSeq 1000/2000 platforms using appropriate reagent cartridges and flow cells (Illumina). **Assembly, processing and quality control of genomic sequences:** RSV-GenoScan (<https://github.com/AlexandreD-bio/RSV-GenoScan>) was used for the assembly of RSV reads which included trimming, and quality control of raw reads, and then mapped reads to the RSV-A and RSV-B references (GenBank accession: NC_001803.1 and AY353550.1 for RSV-A and RSV-B respectively). The minimum depth for consensus sequence generation was set at 50x. Nextclade was used for lineage assignment based on the Goya et. al. 2023 classification. A sequence was considered high quality if the whole genome coverage was 90%, and the coverage of the G and F genes was 100%. A whole genome coverage of at least 70% was accepted for subtype and lineage calling.

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