Centre for Respiratory Diseases and Meningitis Sentinel Surveillance in South Africa Respiratory Pathogens Report

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

Report week: 36
Reporting period: 30 December 2024 to 07 September 2025
Date of data extraction: 2025-09-11
Data are provisional as of the date of extraction. The number of consultations/specimens is reported/analysed by date of consultation/specimen collection. Data cleaning is ongoing and this may result in some changes in subsequent reports. Refer to the end of the report for methodology and definitions.

Highlights

- In week 36 (01 September 2025 to 07 September 2025), from 147 samples tested, we detected 5 (3.4%) cases of influenza, 2 (1.4%) cases of RSV and 11 (7.5%) cases of SARS-CoV-2.
- The influenza season started in week 13 (week starting 24 March 2025), peaked in week 20 (week starting 12 May 2025) and ended in week 30 (week starting 21 July 2025). The season began earlier than in the previous years, and throughout the season, the detection rate remained within the low threshold.
- The RSV season started in week 11 (week starting 10 March 2025), peaked in week 16 (week starting 15 April 2025 at the moderate level, and ended in week 31 (week starting 28 July 2025).
- An increase in SARS-CoV-2 detections has been observed in since week 29 (week starting 14 July 2025), however the detection rate is still below the epidemic threshold. Among cases with sequencing data available since week 29, 9 (56%, 9/16)) were identified as the XFG recombinant variant.
- In August, we detected 1 (0.2%, 1/502) case of *Bordetella pertussis*.
- From 30 December 2024 to 07 September 2025, from 5500 samples tested, we detected 556 (10.1%) cases of influenza, 755 (13.7%) cases of respiratory syncytial virus (RSV), 173 (3.1%) cases of SARS-CoV-2 and 35 (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 36 (week starting 1 September 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

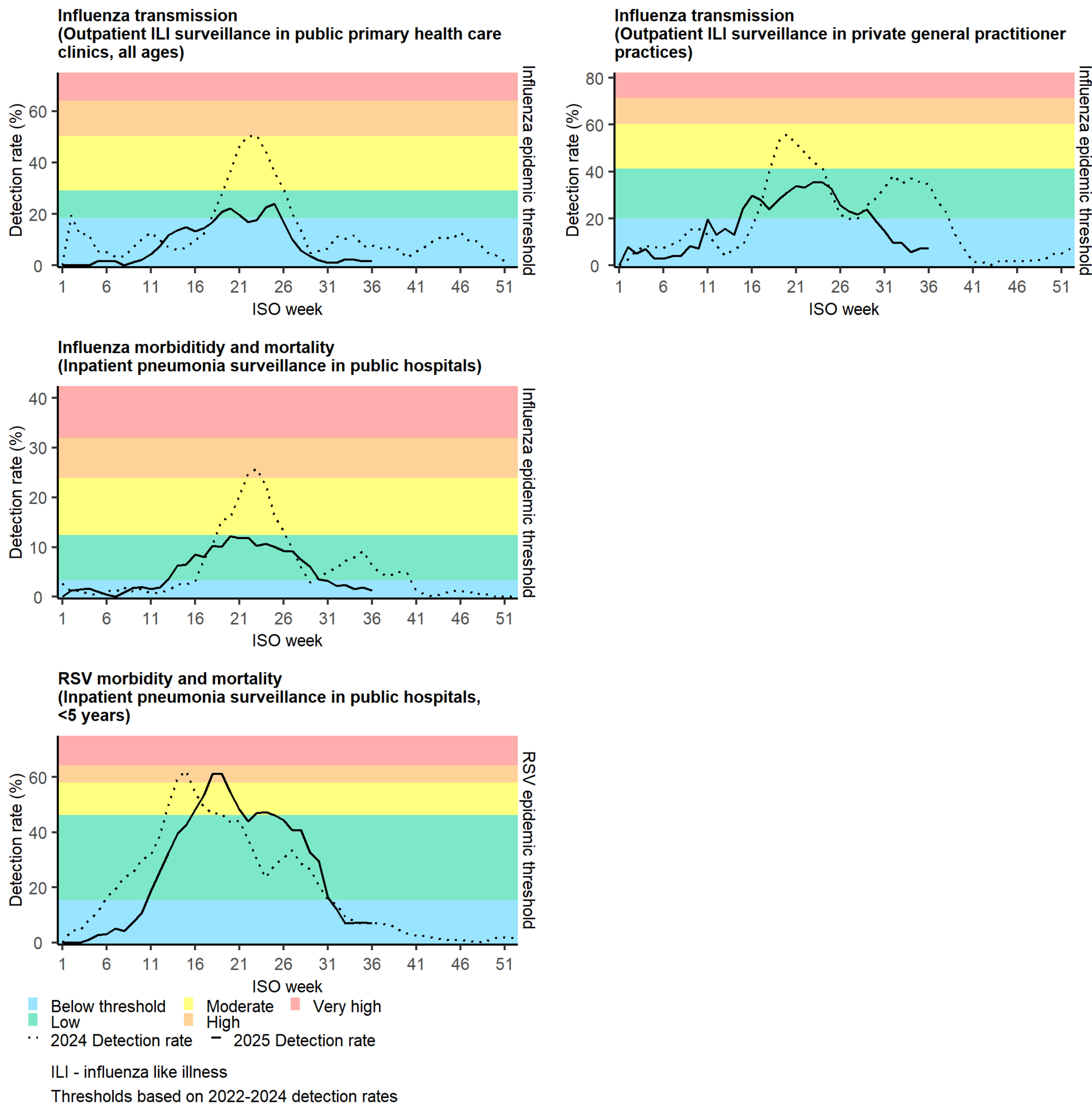

Figure 1: Influenza and Respiratory Syncytial Virus (RSV) surveillance epidemic threshold summary, Sentinel Surveillance, South Africa, 30 December 2024 to 07 September 2025.

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SARS-CoV-2 epidemic thresholds

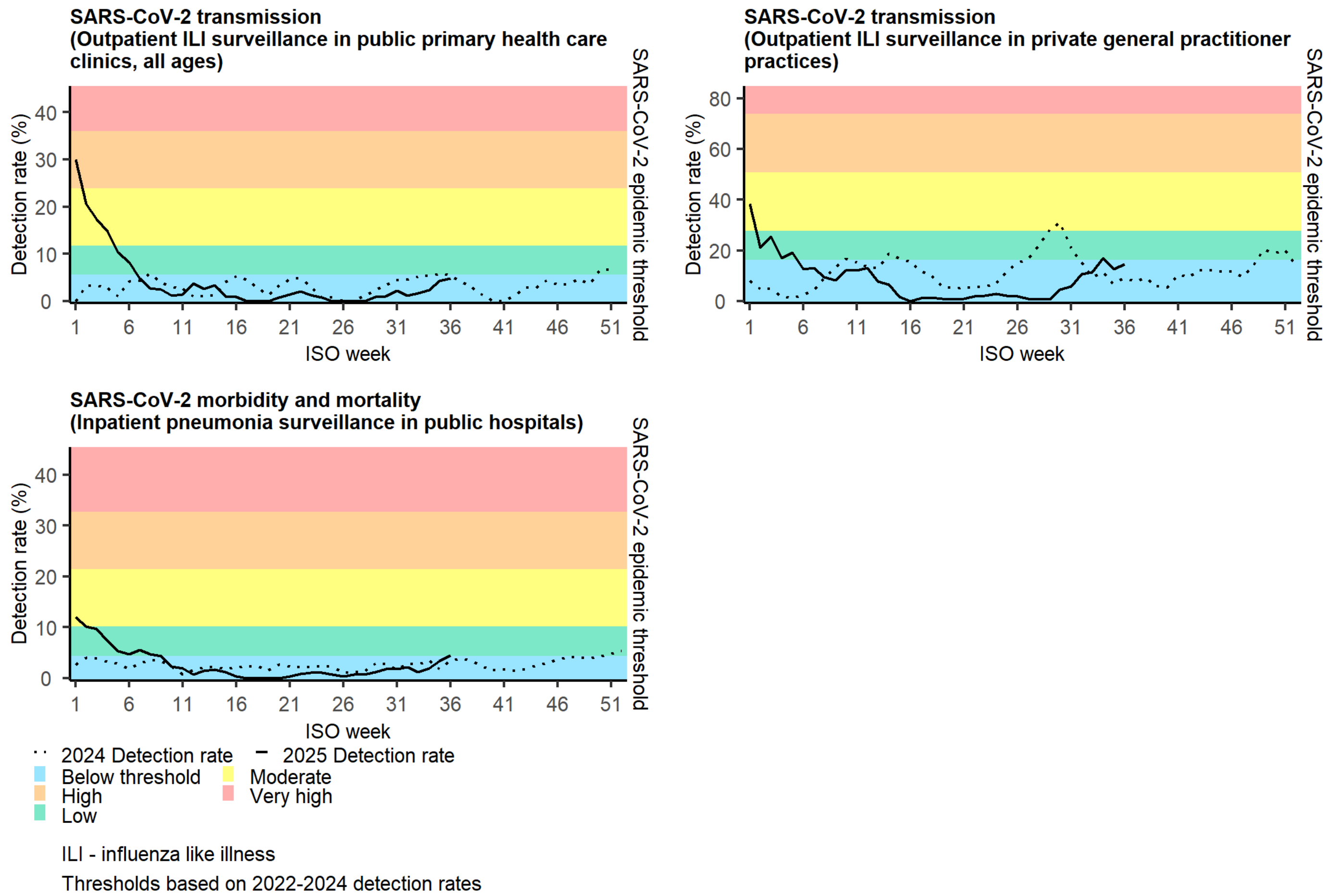

Figure 2: Severe acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) surveillance epidemic threshold summary, sentinel surveillance, South Africa, 30 December 2024 to 07 September 2025.

Influenza

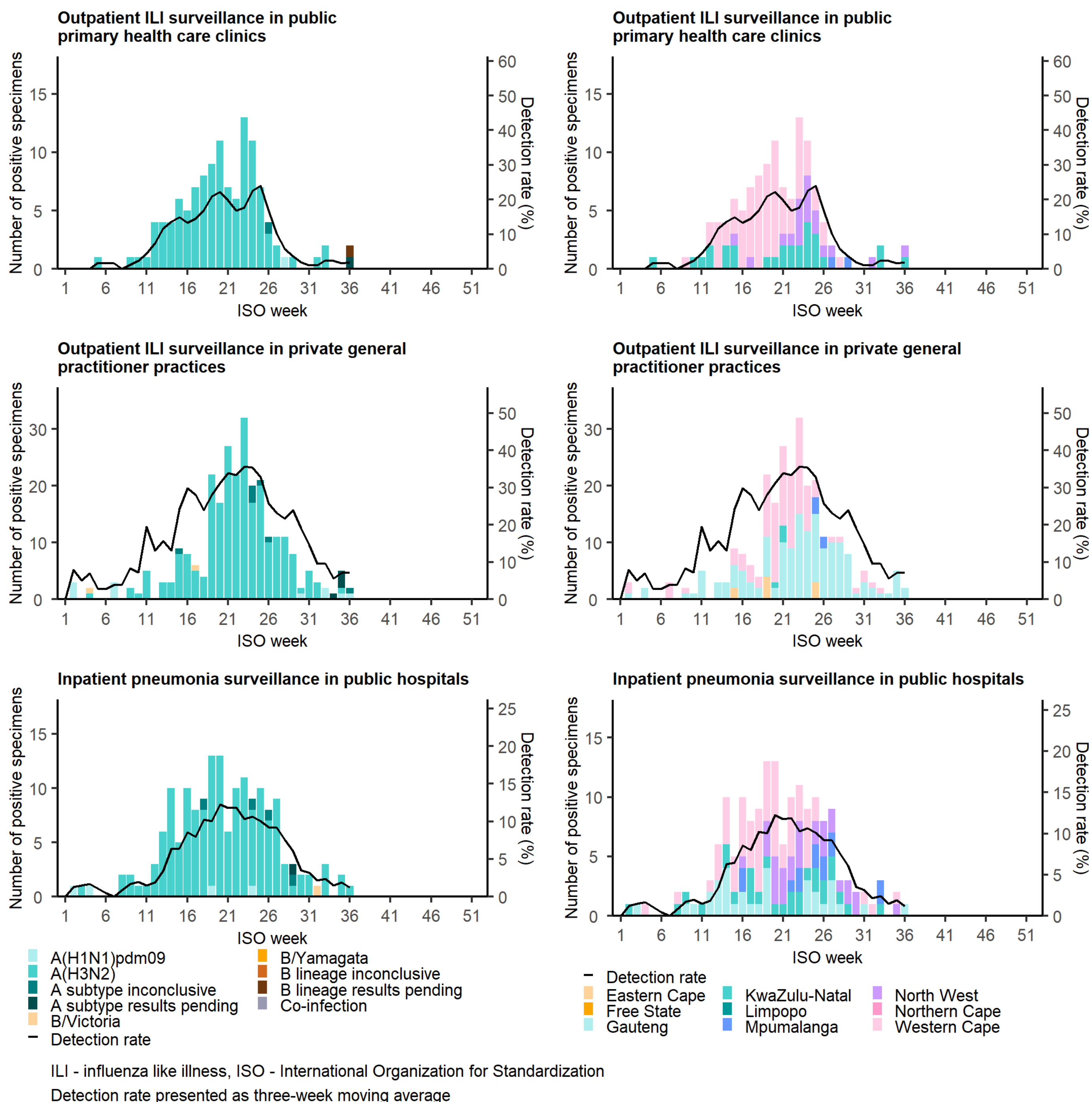

Figure 3: Number of laboratory-confirmed influenza cases and detection rate by subtype and lineage (left) and province (right) in all ages, sentinel surveillance, South Africa, 30 December 2024 to 07 September 2025.

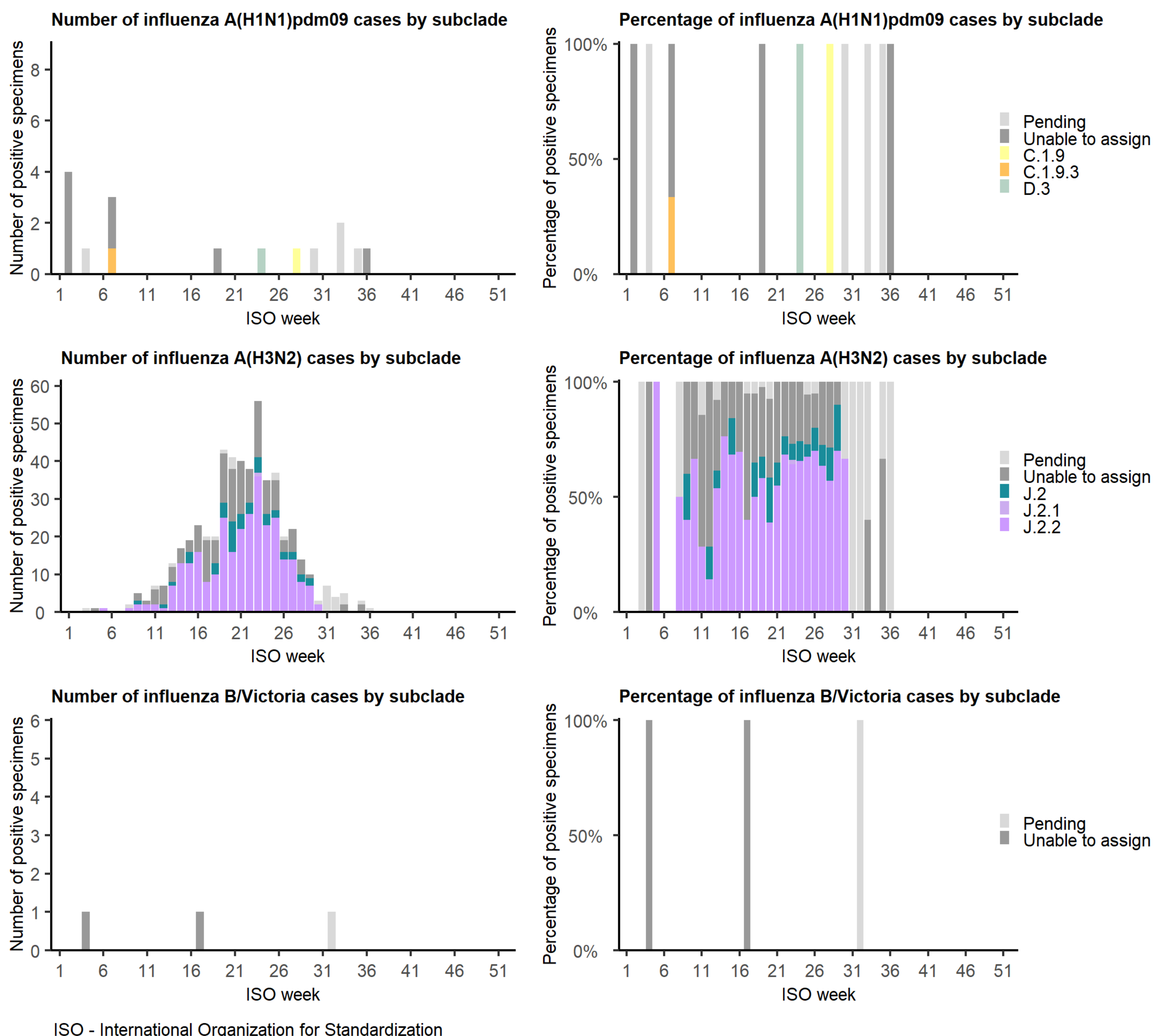

Figure 4: Combined number and percentage of influenza cases by subclade in all ages from three sentinel surveillance systems: outpatient influenza-like illness (ILI) surveillance in public primary health care clinics, outpatient ILI surveillance in private general practitioner practices, and inpatient pneumonia surveillance in public hospitals, South Africa, 30 December 2024 to 07 September 2025.

Table 1: Combined number of 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 07 September 2025.

Subtype/Lineage	Clade	Subclade	Count
A(H1N1)pdm09	6B.1A.5a.2a	C.1.9	1
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	45
A(H3N2)	3C.2a1b.2a.2a.3a.1	J.2.1	1
A(H3N2)	3C.2a1b.2a.2a.3a.1	J.2.2	294

Data are provisional as on the 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.

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 07 September 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	27	0	0	0	0	0	1	0	28	411
Agincourt (MP)	0	2	0	0	0	0	0	0	0	2	50
Jouberton (NW)	0	17	0	1	0	0	0	0	0	18	254
Eastridge (WC)	1	69	1	0	0	0	0	0	0	71	434
Mitchell's Plain (WC)	0	0	0	0	0	0	0	0	0	0	51
Total	1	115	1	1	0	0	0	1	0	119	1200

Specimens where more than one influenza subtype or lineage was detected were denoted as co-infection, and included in the counts for each separate type as well. The Agincourt clinic was not active from February to 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 07 September 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	9	0	0	0	0	0	0	0	9	17
Free State	0	0	0	0	0	0	0	0	0	0	1
Gauteng	4	136	4	4	1	0	0	0	0	149	937
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	35
North West	0	0	0	0	0	0	0	0	0	0	0
Northern Cape	0	0	0	0	0	0	0	0	0	0	0
Western Cape	7	94	2	0	1	0	0	0	0	104	283
Total	11	247	7	4	2	0	0	0	0	271	1288

Specimens where more than one influenza subtype or lineage was detected were 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 07 September 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	33	0	0	0	0	0	0	0	33	536
Harry Gwala (KZ)	1	25	2	0	0	0	0	0	0	28	447
Mapulaneng-Matikwana (MP)	0	7	0	0	0	0	0	0	0	7	172
Tintswalo (MP)	0	6	0	0	0	0	0	0	0	6	172
Klerksdorp-Tshepong (NW)	1	28	0	1	0	0	0	0	0	30	434
Mitchell's Plain (WC)	0	31	1	0	0	0	0	0	0	32	516
Red Cross (WC)	2	26	1	0	1	0	0	0	0	30	735
Total	4	156	4	1	1	0	0	0	0	166	3012

Specimens where more than one influenza subtype or lineage was detected were 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)

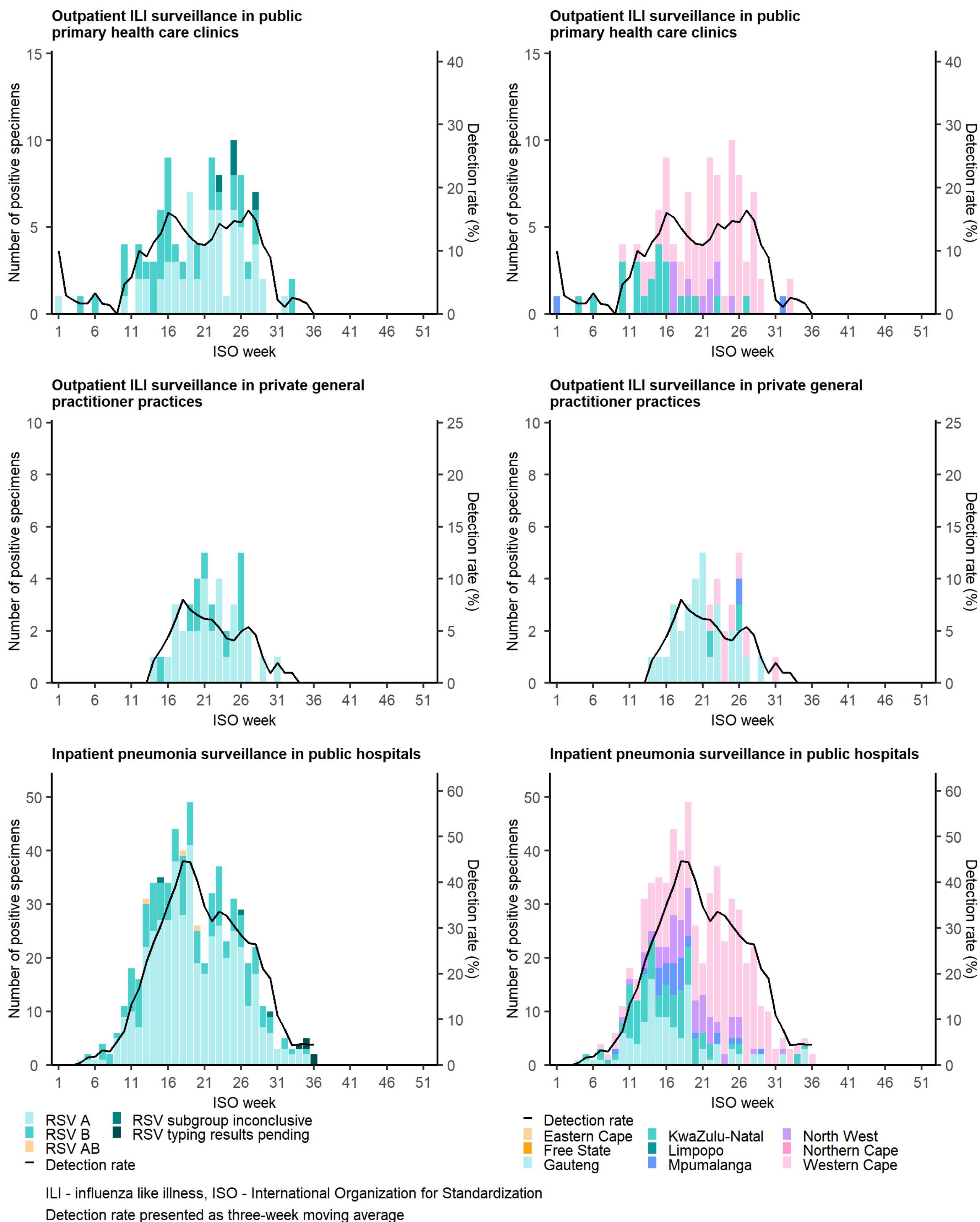

Figure 5: Number of laboratory-confirmed respiratory syncytial virus (RSV) cases and detection rate by type (left) and province (right) in all ages, sentinel surveillance, South Africa, 30 December 2024 to 07 September 2025.

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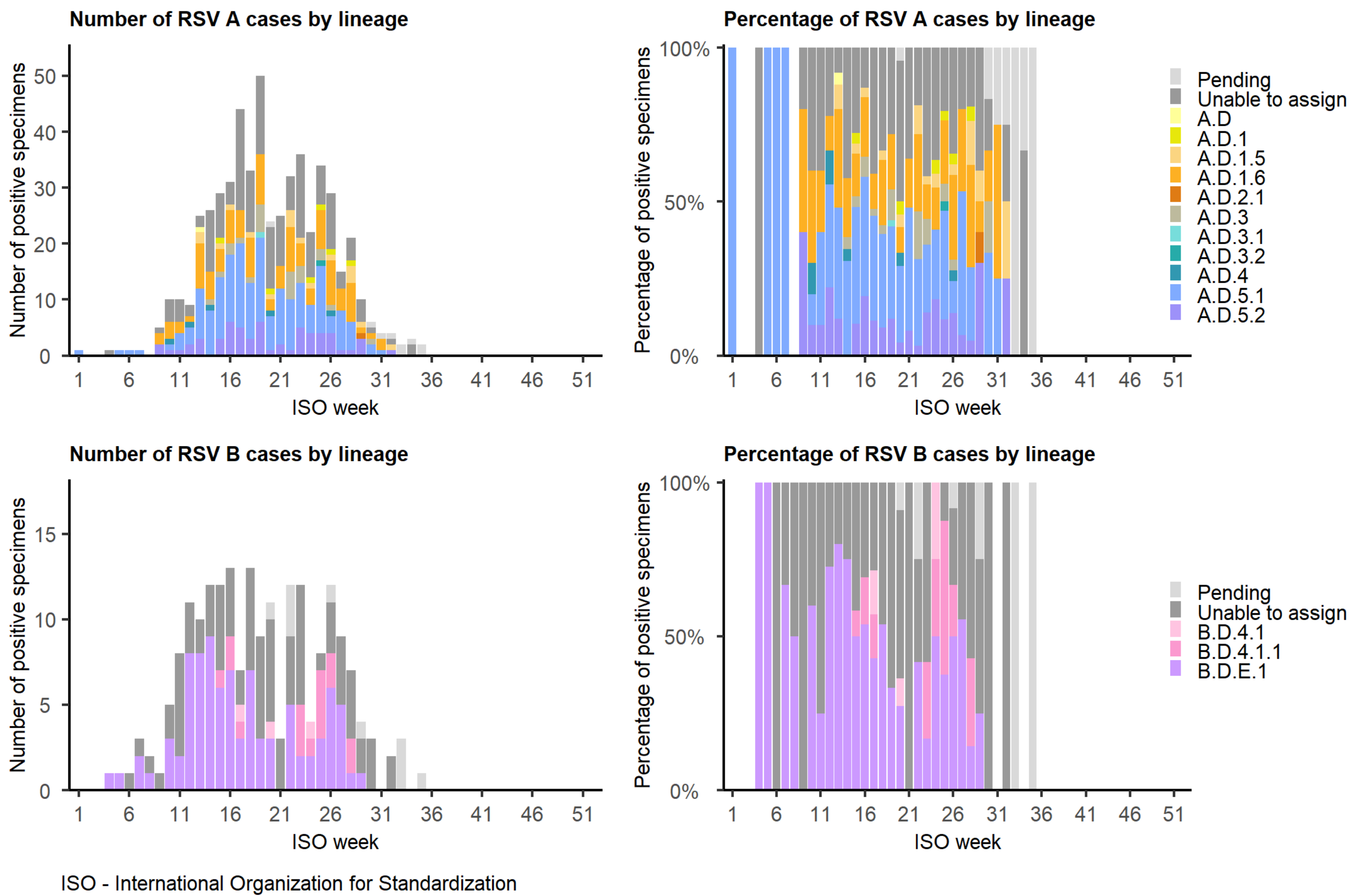

Figure 6: Combined number and percentage of RSV cases by lineage in all ages from three sentinel surveillance systems: outpatient influenza-like illness (ILI) surveillance in public primary health care clinics, outpatient ILI surveillance in private general practitioner practices, and inpatient pneumonia surveillance in public hospitals, South Africa, 30 December 2024 to 07 September 2025.

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 07 September 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	411
Agincourt (MP)	2	0	0	0	0	2	50
Jouberton (NW)	9	0	0	1	0	10	254
Eastridge (WC)	46	20	0	2	0	68	434
Mitchell's Plain (WC)	2	0	0	0	0	2	51
Total	62	39	0	4	0	105	1200

The 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 07 September 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	17
Free State	0	0	0	0	0	0	1
Gauteng	24	6	0	0	0	30	937
KwaZulu-Natal	0	2	0	0	0	2	15
Limpopo	0	0	0	0	0	0	0
Mpumalanga	0	1	0	0	0	1	35
North West	0	0	0	0	0	0	0
Northern Cape	0	0	0	0	0	0	0
Western Cape	7	1	0	0	0	8	283
Total	31	10	0	0	0	41	1288

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 07 September 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)	107	4	0	0	0	111	536
Harry Gwala (KZ)	25	64	0	0	1	90	447
Mapulaneng-Matikwana (MP)	14	1	0	0	0	15	172
Tintswalo (MP)	9	7	0	0	0	16	172
Klerksdorp-Tshepong (NW)	65	6	0	0	0	71	434
Mitchell's Plain (WC)	67	23	0	3	1	94	516
Red Cross (WC)	163	43	3	0	3	212	735
Total	450	148	3	3	5	609	3012

SARS-CoV-2

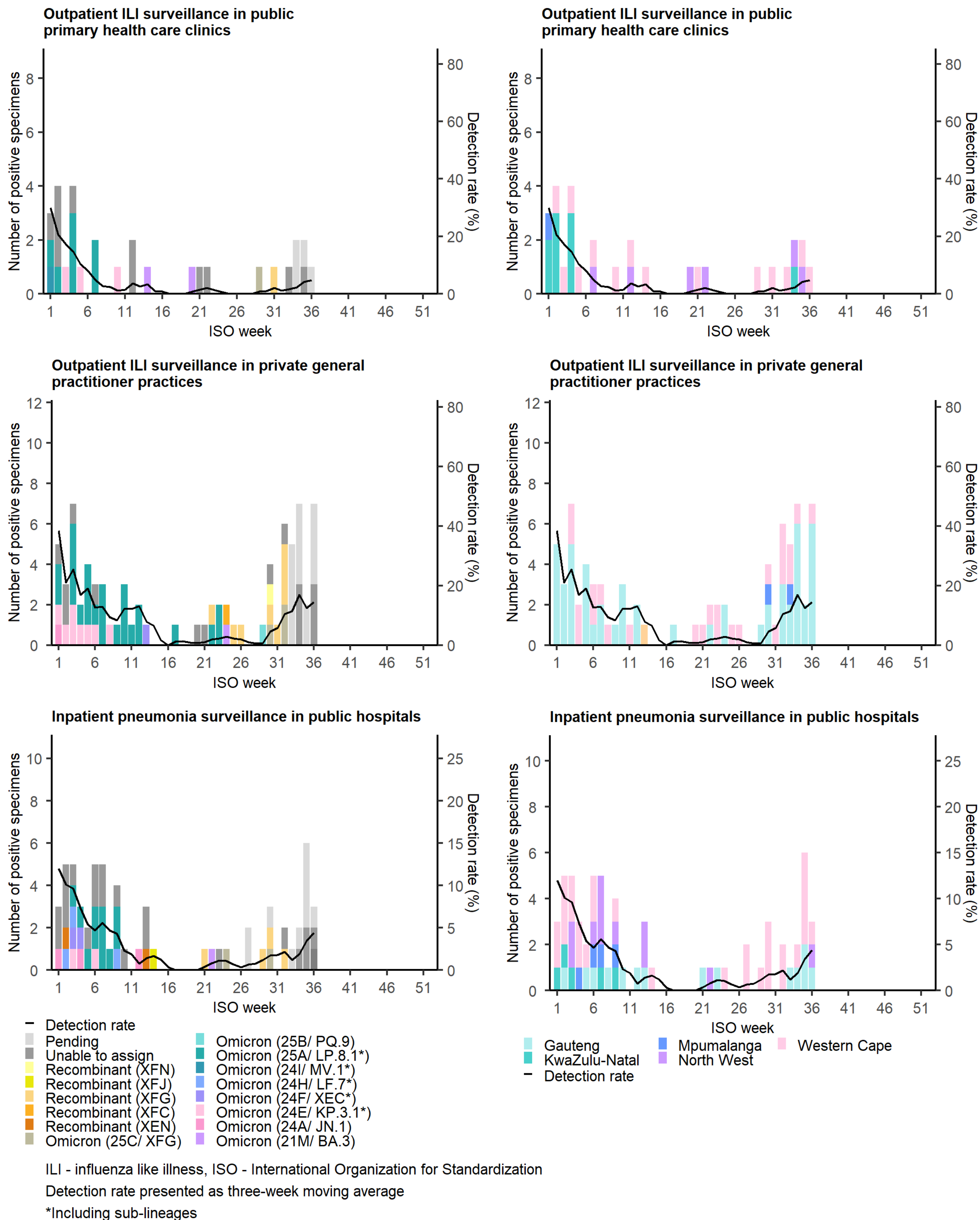

Figure 7: Number of laboratory-confirmed SARS-CoV-2 cases and detection rate by variant type (left) and province (right) in all ages, sentinel surveillance, South Africa, 30 December 2024 to 07 September 2025.

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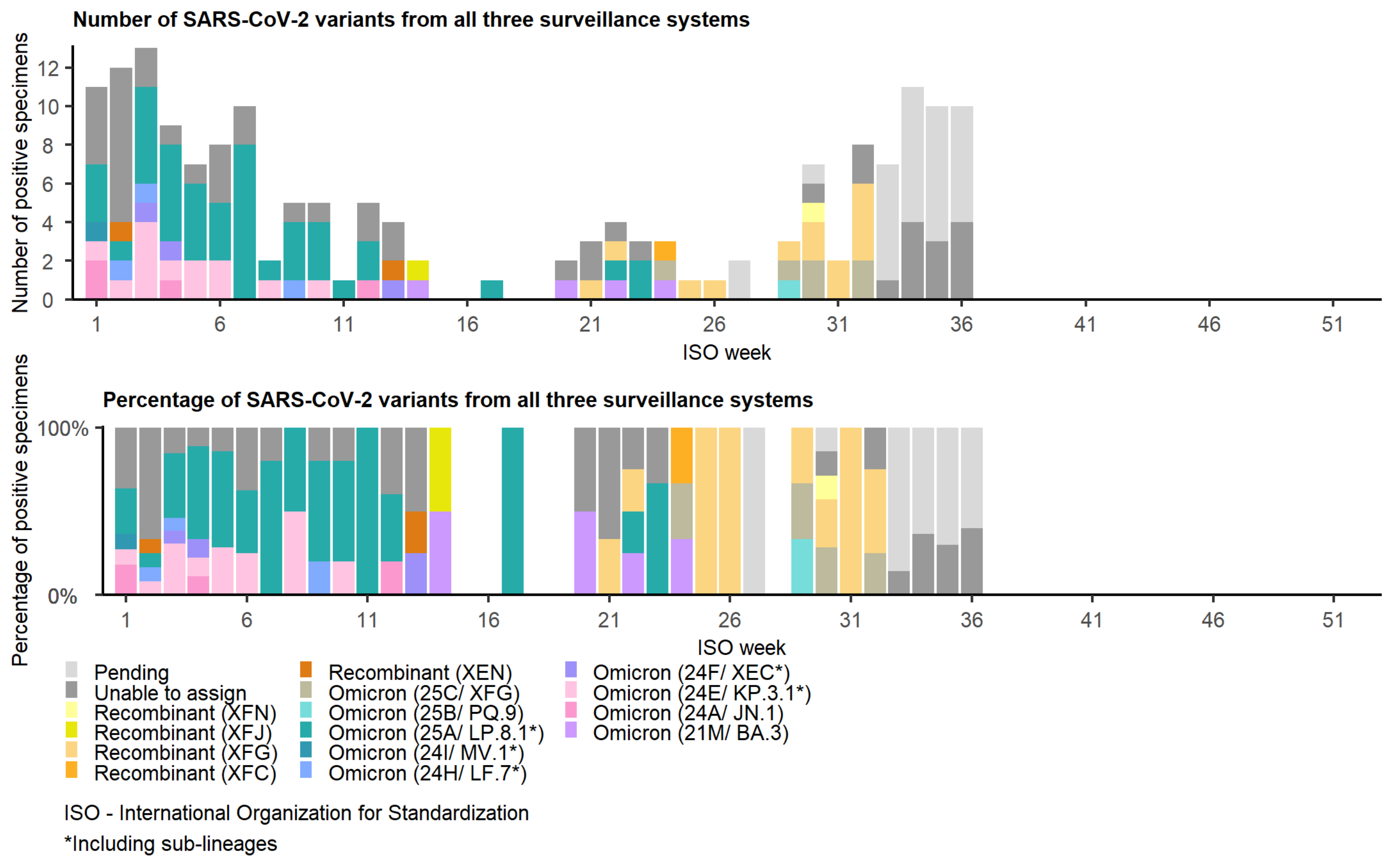

Figure 8: Combined number and percentage of SARS-CoV-2 variants in all ages from three sentinel surveillance systems: outpatient influenza-like illness (ILI) surveillance in public primary health care clinics, outpatient ILI surveillance in private general practitioner practices, and inpatient pneumonia surveillance in public hospitals, South Africa, 30 December 2024 to 07 September 2025.

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 07 September 2025.

Clinic (Province)	Om (21M/ BA.3)	Om (24A/ JN.1)	Om (24E/ KP.3.1*)	Om (24F/ XEC*)	Om (24H/ LF.7*)	Om (24I/ MV.1*)	Om (25A/ LP.8.1*)	Om (25B/ PQ.9)	Om (25C/ XFG)	Rcb (XEN/XFC/XFG/ XFJ/XFN)	Pending	Unable to assign	Total SARS-CoV-2	Total specimens
Edendale Gateway (KZ)	0	0	0	0	0	1	4	0	0	0	1	3	9	411
Agincourt (MP)	0	0	0	0	0	0	1	0	0	0	0	0	1	50
Jouberton (NW)	1	0	0	0	0	0	1	0	0	0	1	3	6	254
Eastridge (WC)	0	0	3	0	0	0	1	0	1	0	2	3	10	434
Mitchell's Plain (WC)	1	0	0	0	0	0	0	0	0	1	0	2	4	51
Total	2	0	3	0	0	1	7	0	1	1	4	11	30	1200

The Agincourt clinic was not active from February – June 2025. Om = Omicron; Rcb = Recombinant.
*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 07 September 2025.

Province	Om (21M/ BA.3)	Om (24A/ JN.1)	Om (24E/ KP.3.1*)	Om (24F/ XEC*)	Om (24H/ LF.7*)	Om (24I/ MV.1*)	Om (25A/ LP.8.1*)	Om (25B/ PQ.9)	Om (25C/ XFG)	Rcb (XEN/XFC/XFG/ XFJ/XFN)	Pending	Unable to assign	Total SARS-CoV-2	Total specimens
Eastern Cape	0	0	0	1	0	0	0	0	0	0	0	0	1	17
Free State	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Gauteng	1	1	4	0	0	0	17	1	3	3	11	11	52	937
KwaZulu-Natal	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
Mpumalanga	0	0	0	0	0	0	0	0	0	1	1	0	2	35
North West	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
Western Cape	0	0	4	0	0	0	8	0	0	6	3	4	25	283
Total	1	1	8	1	0	0	25	1	3	10	15	15	80	1288

Om = Omicron; Rcb = Recombinant. *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 07 September 2025.

Hospital (Province)	Om (21M/ BA.3)	Om (24A/ JN.1)	Om (24E/ KP.3.1*)	Om (24F/ XEC*)	Om (24H/ LF.7*)	Om (24I/ MV.1*)	Om (25A/ LP.8.1*)	Om (25B/ PQ.9)	Om (25C/ XFG)	Rcb (XEN/XFC/XFG/ XFJ/XFN)	Pending	Unable to assign	Total SARS-CoV-2	Total specimens
Helen Joseph-Rahima Moosa (GP)	0	1	0	0	0	0	2	0	0	2	2	7	14	536
Harry Gwala (KZ)	0	0	0	1	0	0	2	0	0	0	0	2	5	447
Mapulaneng-Matikwana (MP)	0	0	0	0	0	0	1	0	0	0	0	1	2	172
Tintswalo (MP)	0	0	0	0	0	0	0	0	0	0	0	2	2	172
Klerksdorp-Tshepong (NW)	1	0	1	0	0	0	3	0	0	0	1	5	11	434
Mitchell's Plain (WC)	0	1	1	0	0	0	0	0	1	3	5	3	13	516
Red Cross (WC)	0	1	0	1	3	0	3	0	1	2	3	1	15	735
Total	1	3	2	2	3	0	11	0	2	7	11	21	63	3013

Om = Omicron; Rcb = Recombinant. *Including sub-lineages

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Bordetella pertussis

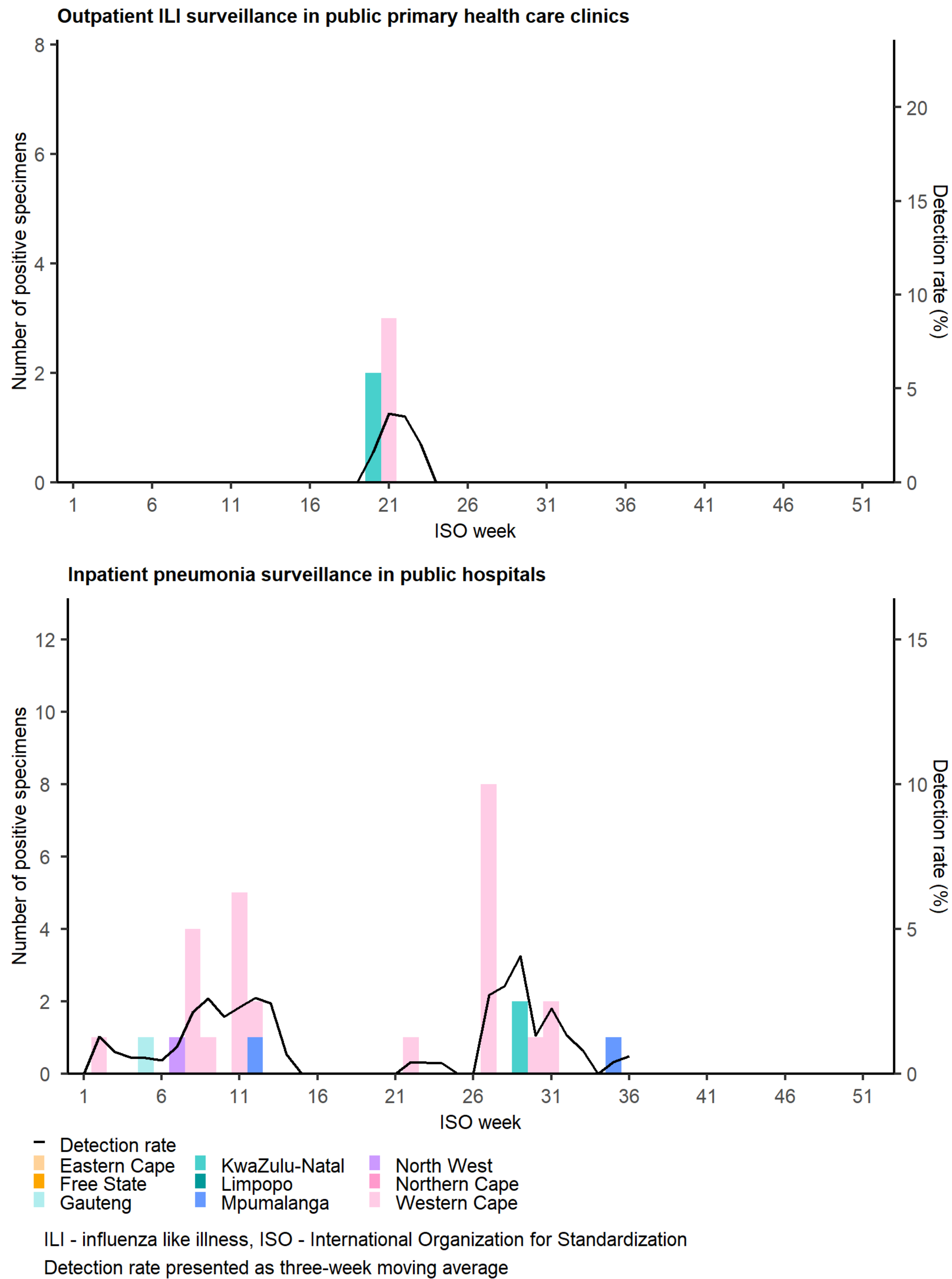

Figure 9: Number of laboratory-confirmed *Bordetella pertussis* cases and detection rate by province in all ages, sentinel surveillance, South Africa, 30 December 2024 to 07 September 2025.

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 07 September 2025.

Province	Positive	Pending testing	Total specimens
KwaZulu-Natal	2	12	411
Mpumalanga	0	6	50
North West	0	18	254
Western Cape	3	9	485
Total	5	45	1200

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 07 September 2025.

Province	Positive	Pending testing	Total specimens
Gauteng	1	12	536
KwaZulu-Natal	2	13	447
Mpumalanga	2	7	344
North West	1	12	434
Western Cape	24	41	1251
Total	30	85	3012

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 (≥38°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 (≥38°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 <5years: 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, and B. pertussis.	Influenza virus, RSV, SARS-CoV-2.	Influenza virus, RSV, SARS-CoV-2, and B. pertussis.
Testing Methods	Influenza virus, RSV, SARS-CoV-2: Allplex™ SARS-CoV-2/FluA/FluB/RSV PCR kit. B. pertussis: Multiplex real-time PCR (Tatti et al., 2011)	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. B. pertussis: Multiplex real-time PCR (Tatti et al., 2011)

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 characterise further
- Subtype/lineage/subgroup pending: Further characterisation in progress
- Unable to assign (lineage/subclade): No lineage/subclade assigned due to poor sequence quality OR low viral load (Ct≥35 for SARS-CoV-2 and Ct≥30 for influenza/RSV)
- 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, and 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 are 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 deviations for moderate, high and very high thresholds, respectively. The MSD method has been detailed by Sinnathamby et al.2024.

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

Influenza A and B viruses, RSV and SARS-CoV-2 were identified 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 et al., 2011). A specimen was considered positive when the IS481 and/or ptxS1 gene targets were detected with a Ct <45.

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

Influenza A, B and RSV positive specimens were subtyped using the US Centers for Disease Control and Prevention (CDC) RT-PCR protocol and reagents (International Reagent Resource (IRR) [Available from: https://www.internationalreagentresource.org/). All influenza-positive and RSV-positive specimens with Ct<30, and all SARS-CoV-2 positive specimens with Ct≤35 were characterised by whole genome sequencing.

RNA extraction for influenza, RSV and SARS-CoV-2 whole genome sequencing: RNA was extracted from 300µl of specimen using the Chemagic360 automated extractor and the CMG-1049 kit (Revitiy, Massachusetts, USA) and eluted in 60µl elution buffer.

SARS-CoV-2 whole-genome sequencing and analysis:

PCR and library preparation: SARS-CoV-2 was sequenced using the Illumina COVIDSeq Kit (Illumina Inc., CA, USA) with nCoV-2019 ARTIC network tiling primers v5.4.2 (https://artic.network/ncov-2019). Complementary DNA (cDNA) was synthesised using random hexamers from the kit. Using tiling PCR, two amplicon pools of SARS-CoV-2 (400bp) were multiplexed and processed for libraries. The pooled amplicons underwent bead-based tagmentation and the adapter-tagged amplicons were purified and amplified using one round of PCR. Amplicons were indexed using the Illumina UDI indexes (Illumina) according to the manufacturer's instructions. Further enrichment and clean-up were performed as per the manufacturer's instructions. Purified libraries were quantified using the Qubit 4.0 fluorimeter (Invitrogen Inc., MA, USA) using the Qubit dsDNA High Sensitivity assay according to the manufacturer's instructions. The fragment sizes were analysed using TapeStation 4200 (Agilent Technologies, California, USA). Pooled libraries were normalised to 4nM concentration, and spiked with 1% PhiX (PhiX Control v3 adaptor-ligated library used as a control). Libraries were sequenced at 0.65pM using the NextSeq1000/2000 instruments with the P1 reagent cartridge (300 cycles) and the P1 Flow Cell (Illumina).

Assembly, processing and quality control of genomic sequences: Raw reads from Illumina sequencing were assembled using the CZID SARS-CoV-2 pipeline v1.6.1 (https://github.com/chanzuckerberg/czid-workflows/tree/main/workflows/consensus-genome/). The resulting consensus sequence was further manually polished by considering and correcting indels in homopolymer regions that break the open reading frame (probably sequencing errors) using Aliview v1.27 (http://ormbunkar.se/aliview/). All assemblies determined to have acceptable quality (defined as having at least 1,000,000 reads and at least 50% 10x coverage) were deposited on GISAID (https://www.gisaid.org/).

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. SARS-CoV-2 genomes 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 analysis:

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 for influenza A (Zhou et al., 2009), and eight universal primers sets for influenza B viruses (Zhou et al.,2014). Before library preparation, amplicons underwent quality verification and quantification using the Qubit 4.0 fluorometer (Thermofischer Scientific) 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 the 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). 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 at 0.65pM 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 GISAID EpiFlu database if they met the quality criteria of >1000 reads and 90% coverage at ≥50x depth for the HA and NA segments. Sequences were assigned to clades and subclades using Nextclade (https://clades.nextstrain.org/).

RSV whole-genome sequencing and analysis:

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 (Talts et al., 2024). 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 Illumina CovidSeqLibrary Prep 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 using NextSeq 1000/2000 instruments with the P1 reagent cartridge (300 cycles) and the P1 Flow Cell (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 mapping 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. 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 lineage determination. Nextclade (https://clades.nextstrain.org/) was used for lineage assignment based on the Goya et. al. 2023 classifications.

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