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: 28
Reporting period: 29 December 2025 to 12 July 2026
Date of data extraction: 16 July 2026
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 28 (6 July 2026 to 12 July 2026), from 103 samples tested, we detected 8 (7.8%) cases of influenza, 17 (16.5%) cases of respiratory syncytial virus (RSV) and no cases of SARS-CoV-2.
- The influenza season started in week 11 (week beginning 9 March 2026). The start of the influenza season was earlier than in previous seasons, like that seen in 2025. In week 28, influenza transmission remains below threshold in outpatient surveillance from public clinics and decreased to low level in inpatient pneumonia surveillance from public hospitals and outpatient surveillance from private general practitioner practices.
- The RSV season started in week 11 (week starting 9 March 2026). The start of the season was late compared to the start of the previous seasons, but like what was observed in 2025. RSV activity is currently at a low level.
- From 29 December 2025 to 12 July 2026, from 4502 samples tested, we detected 873 (19.4%) cases of influenza, 726 (16.1%) cases of RSV, 57 (1.3%) cases of SARS-CoV-2 and 36 (1.0%) cases of *B. pertussis*.

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Monitoring potentially imported cases of respiratory viruses

No specimens were tested from the OR Tambo International Airport clinic during the reporting period.

Influenza & respiratory syncytial virus (RSV) epidemic thresholds

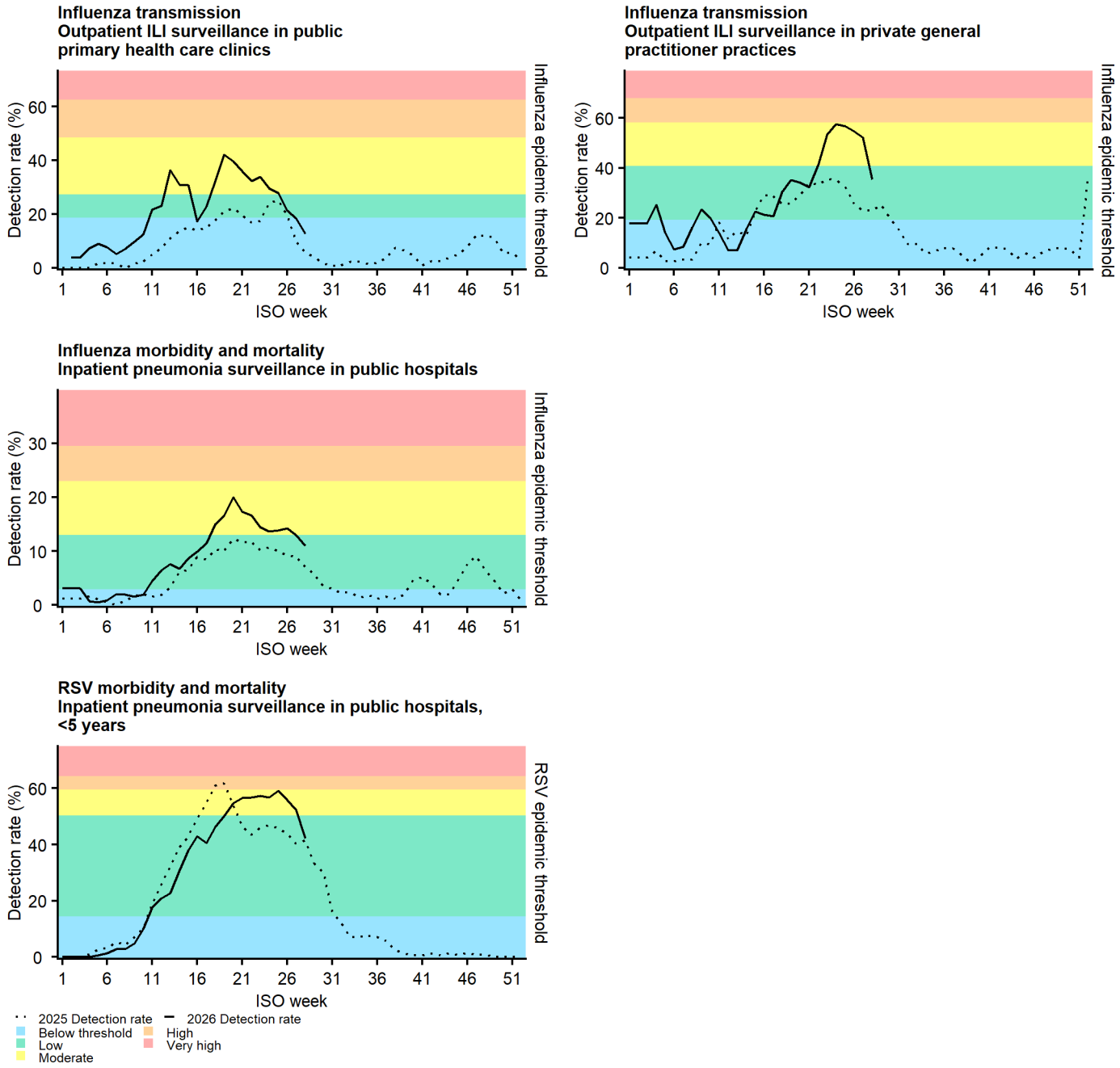

Figure 1: Influenza and respiratory syncytial virus (RSV) surveillance epidemic threshold summary, sentinel surveillance, South Africa, 29 December 2025 to 12 July 2026.

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SARS-CoV-2 epidemic thresholds

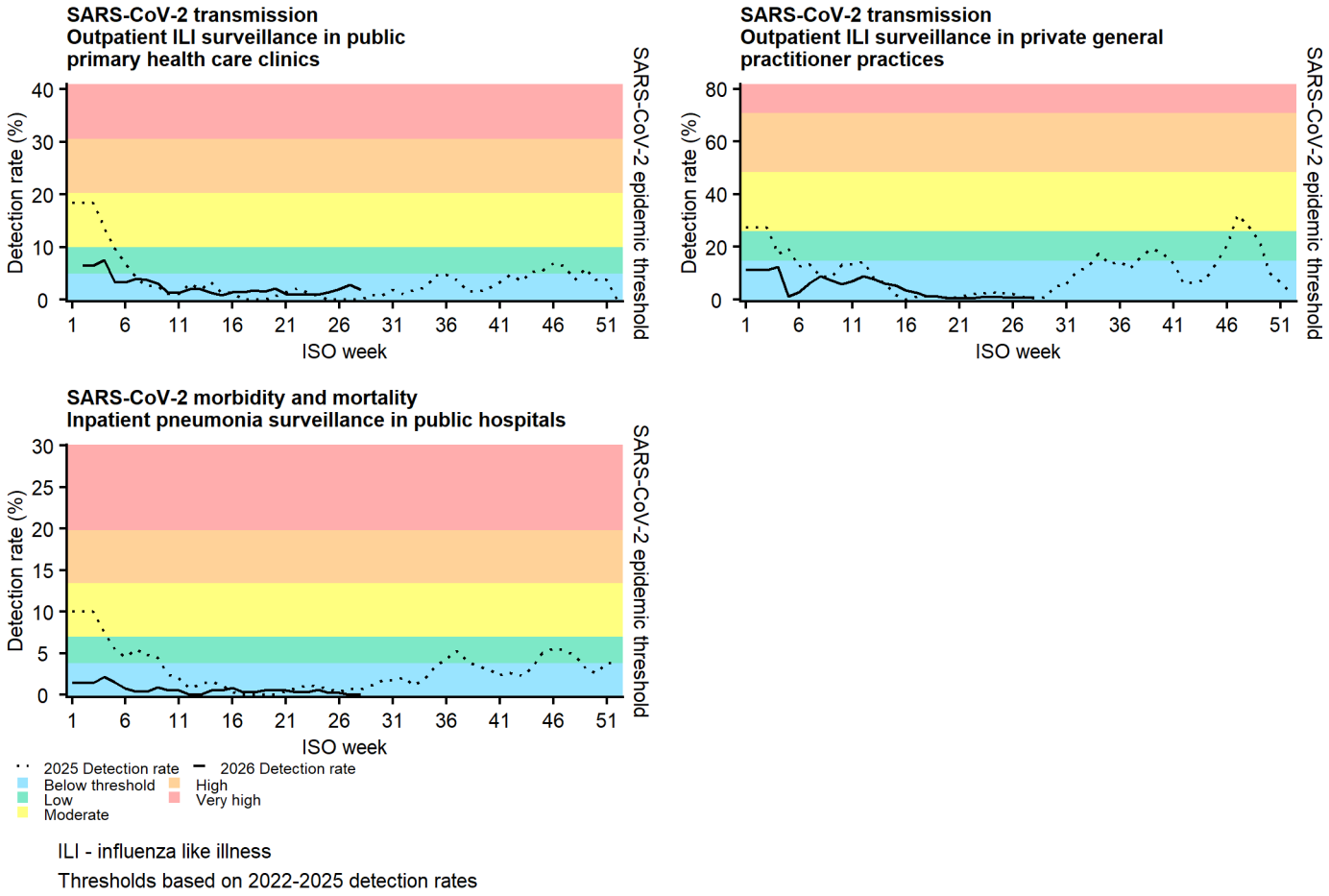

Figure 2: Severe acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) surveillance epidemic threshold summary, sentinel surveillance, South Africa, 29 December 2025 to 12 July 2026.

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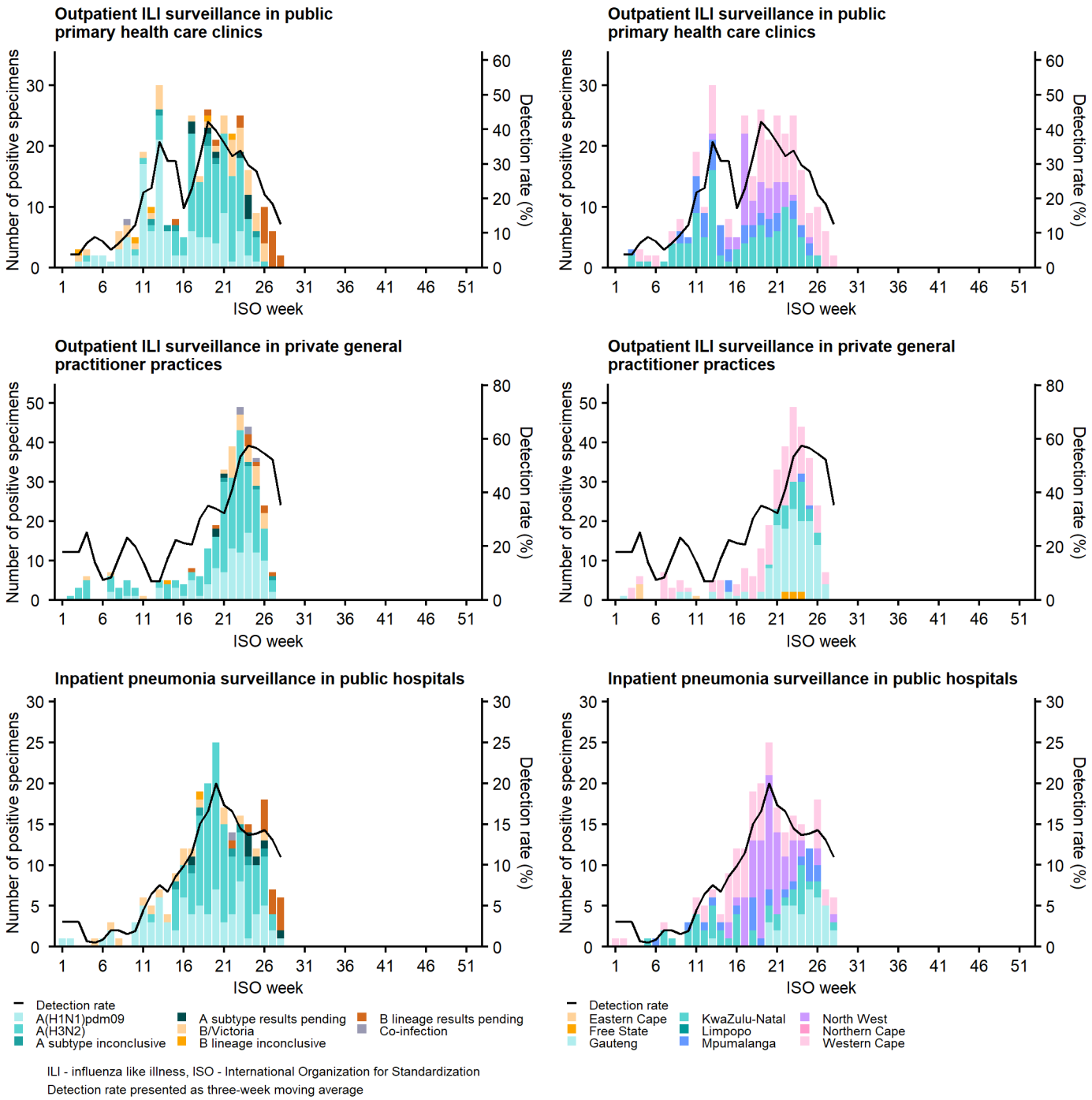

Figure 3: Number of laboratory-confirmed influenza cases and detection rate by subtype and lineage (left) and province (right) for all ages, sentinel surveillance, South Africa, 29 December 2025 to 12 July 2026.

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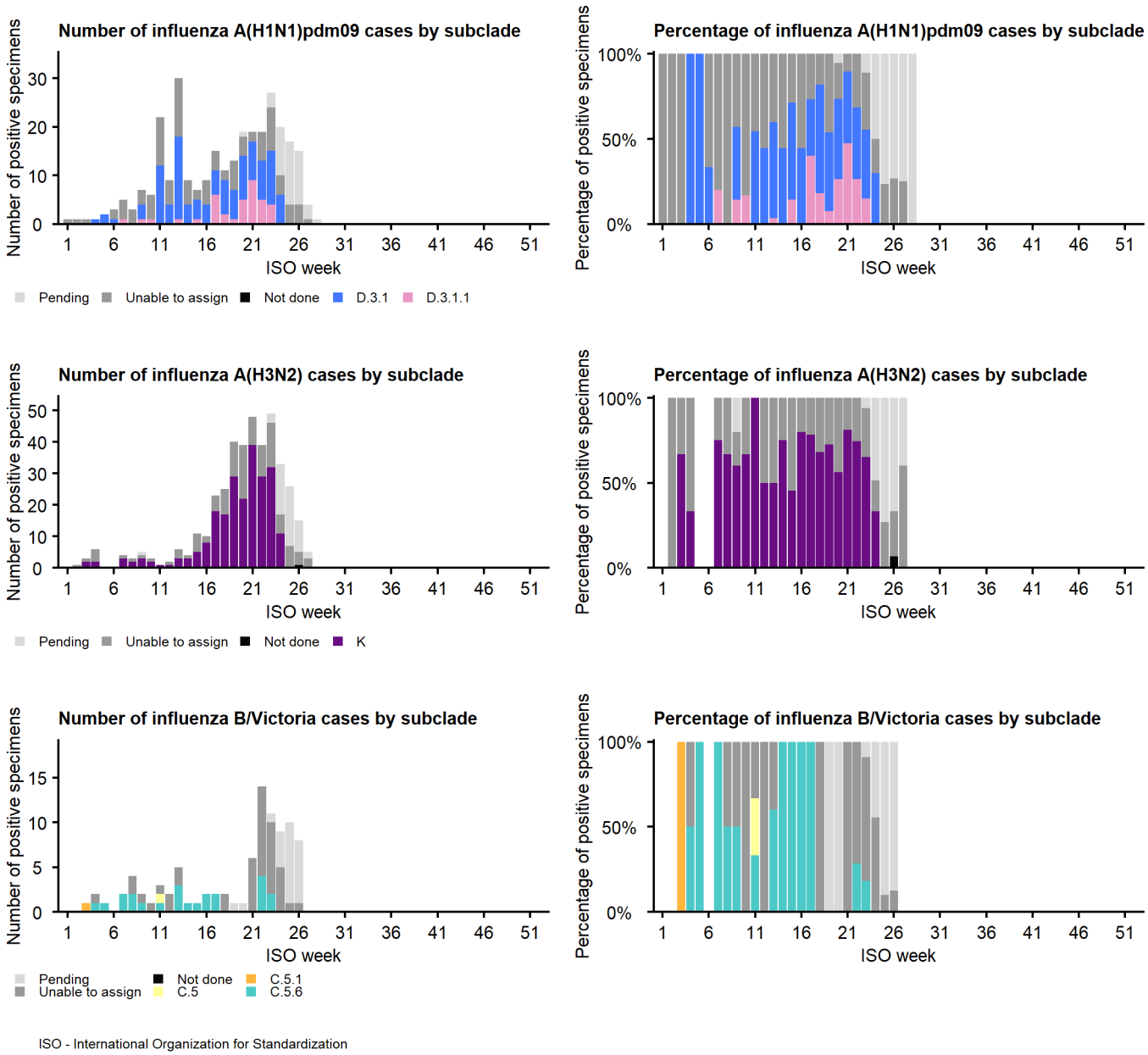

Figure 4: Combined number and percentage of influenza cases by subclade for all ages from three sentinel surveillance systems: outpatient influenza-like illness (ILI) surveillance in public primary health care clinics, outpatient ILI surveillance in private general practitioner practices, and inpatient pneumonia surveillance in public hospitals, South Africa, 29 December 2025 to 12 July 2026.

Table 1: Combined number of influenza cases by subclade and clade for 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, 29 December 2025 to 12 July 2026.

Subtype/Lineage	Clade	Subclade	Count
A(H1N1) pdm09	6B.1A.5a.2a.1	D.3.1	112
A(H1N1) pdm09	6B.1A.5a.2a.1	D.3.1.1	37
A(H3N2)	3C.2a1b.2a.2a.3a.1	K	232
B/Victoria	V1A.3a.2	C.5	1
B/Victoria	V1A.3a.2	C.5.1	1
B/Victoria	V1A.3a.2	C.5.6	23

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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 for all ages, outpatient ILI surveillance in public primary health care clinics, South Africa, 29 December 2025 to 12 July 2026.

Clinic (Province)	A(H1N1) pdm09	A(H3N2)	A subtype inconclusive	A subtype pending	B/ Victoria	B lineage inconclusive	B lineage pending	Co-infection	Total influenza	Total specimens
Edendale Gateway (KZN)	41	27	5	0	26	5	3	0	107	444
Agincourt (MP)	33	12	0	0	2	0	0	1	46	184
Jouberton (NW)	2	40	1	2	0	0	0	0	45	211
Eastridge (WC)	35	40	2	6	13	0	16	0	112	430
Mitchell's Plain (WC)	0	0	0	1	0	0	0	0	1	15
Total	111	119	8	9	41	5	19	1	311	1284

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 3: Number of laboratory-confirmed influenza cases by subtype and lineage and total number of samples tested by province for all ages, outpatient ILI surveillance in private general practitioner practices, South Africa, 29 December 2025 to 12 July 2026.

Province	A(H1N1) pdm09	A(H3N2)	A subtype inconclusive	A subtype pending	B/ Victoria	B lineage inconclusive	B lineage pending	Co-infection	Total influenza	Total specimens
Eastern Cape	0	4	0	0	1	0	0	0	5	7
Free State	2	4	0	0	0	0	0	0	6	21
Gauteng	47	81	1	2	2	0	1	0	134	532
KwaZulu-Natal	7	18	2	2	5	0	1	1	35	69
Limpopo	0	0	0	0	0	0	0	0	0	0
Mpumalanga	4	1	0	0	1	0	0	0	6	11
North West	0	0	0	0	0	0	0	0	0	0
Northern Cape	0	0	0	0	0	0	0	0	0	0
Western Cape	43	66	3	0	24	1	8	4	141	270
Total	103	174	6	4	33	1	10	5	327	910

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 for all ages, inpatient pneumonia surveillance in public hospitals, South Africa, 29 December 2025 to 12 July 2026.

Hospital (Province)	A(H1N1) pdm09	A(H3N2)	A subtype inconclusive	A subtype pending	B/ Victoria	B lineage inconclusive	B lineage pending	Co-infection	Total influenza	Total specimens
Helen Joseph-Rahima Moosa (GP)	20	17	0	2	0	0	1	0	40	461
Harry Gwala (KZN)	14	10	1	2	13	0	2	0	42	301
Mapulaneng (MP)	13	8	0	0	1	0	1	1	22	152
Klerksdorp-Tshepong (NW)	5	54	4	2	0	0	1	0	66	498
Mitchell's Plain (WC)	10	11	0	1	1	0	3	0	26	335
Red Cross (WC)	20	8	2	0	2	1	6	0	39	561
Total	82	108	7	7	17	1	14	1	235	2308

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 5: Description of cases with more than one influenza subtype or lineage detected (denoted as co-infection in plots and tables), by surveillance programme

Infection description	Outpatient ILI surveillance in public primary health care clinics	Outpatient ILI surveillance in private general practitioner practices	Inpatient pneumonia surveillance in public hospitals
A(H1N1) pdm09 + A(H3N2)	1	0	0
A subtype inconclusive + B/Victoria	0	2	0
A subtype results pending + B/Victoria	0	1	0
A(H1N1) pdm09 + B/Victoria	0	1	0
A(H3N2) + B lineage results pending	0	1	0
A(H1N1) pdm09 + B lineage results pending	0	0	1

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Respiratory syncytial virus (RSV)

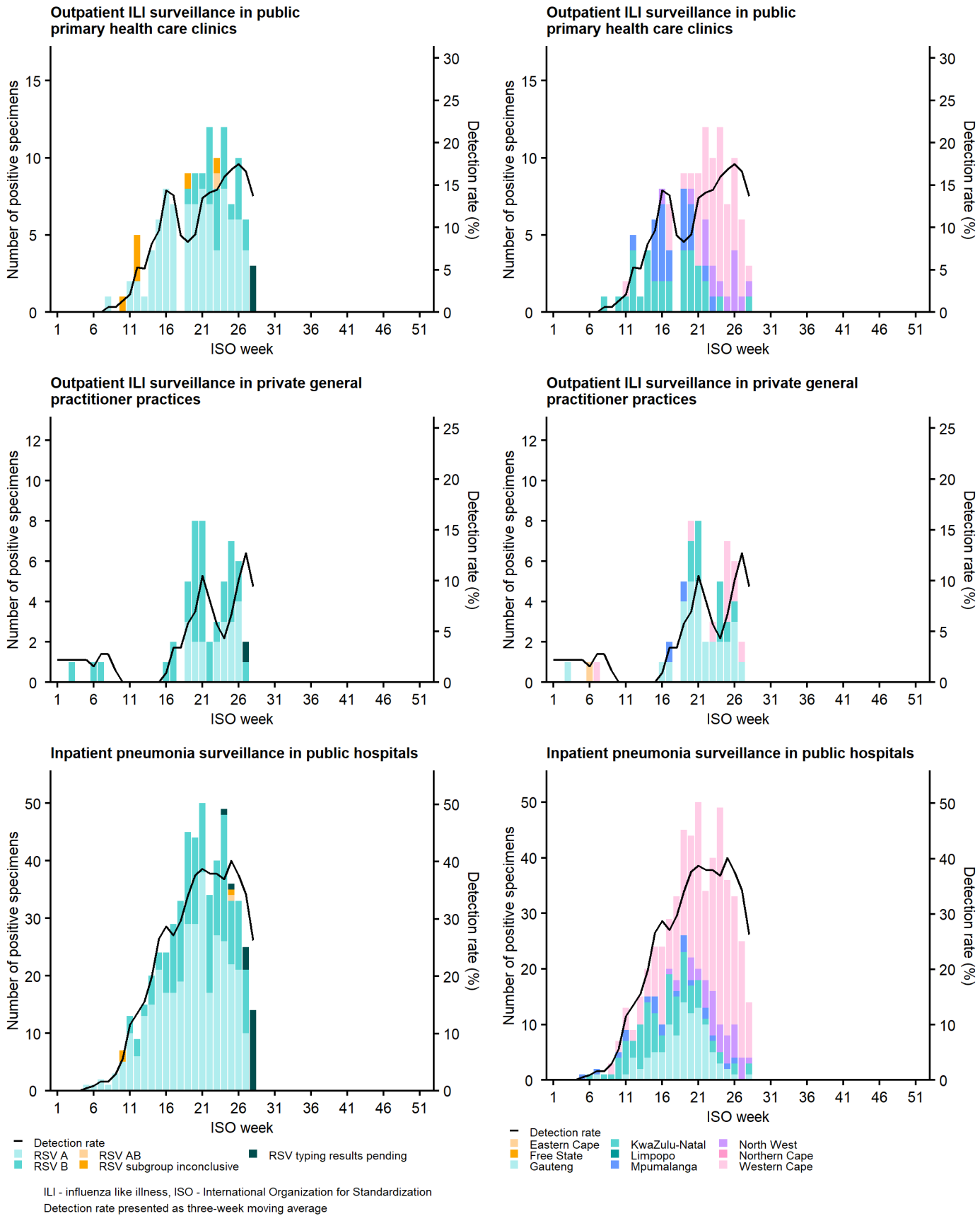

Figure 5: Number of laboratory-confirmed respiratory syncytial virus (RSV) cases and detection rate by type (left) and province (right) for all ages, sentinel surveillance, South Africa, 29 December 2025 to 12 July 2026.

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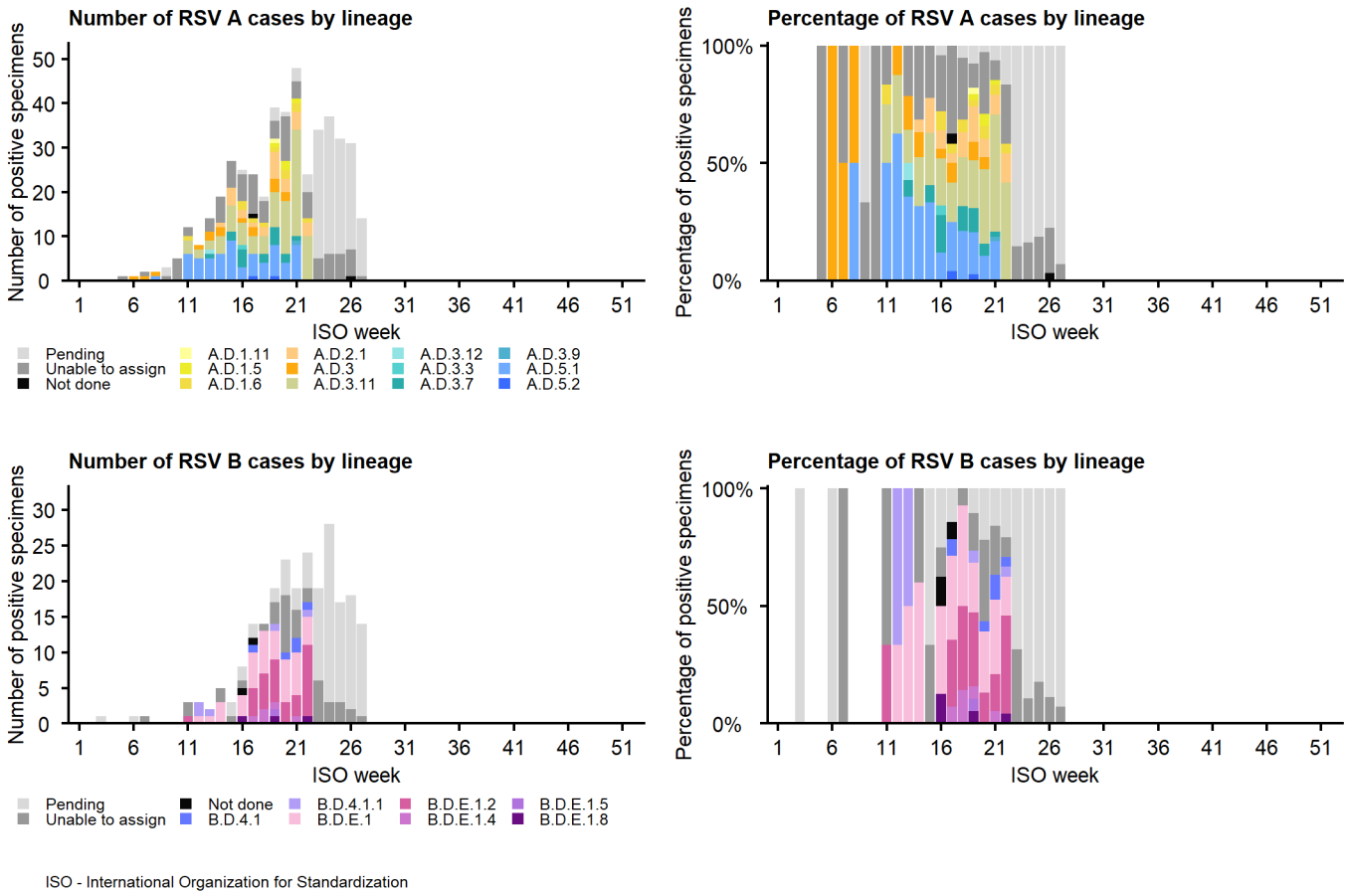

Figure 6: Combined number and percentage of RSV cases by lineage for all ages from three sentinel surveillance systems: outpatient influenza-like illness (ILI) surveillance in public primary health care clinics, outpatient ILI surveillance in private general practitioner practices, and inpatient pneumonia surveillance in public hospitals, South Africa, 29 December 2025 to 12 July 2026.

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Table 6: Number of laboratory-confirmed respiratory syncytial virus (RSV) cases by type and total number of samples tested by clinic and province for all ages, outpatient ILI surveillance in public primary health care clinics, South Africa, 29 December 2025 to 12 July 2026.

Clinic (Province)	RSV A	RSV B	RSV AB	RSV subgroup inconclusive	RSV typing results pending	Total RSV	Total specimens
Edendale Gateway (KZN)	27	0	0	5	1	33	444
Agincourt (MP)	20	1	0	0	0	21	184
Jouberton (NW)	3	11	0	0	1	15	211
Eastridge (WC)	38	11	1	1	1	52	430
Mitchell's Plain (WC)	0	1	0	0	0	1	15
Total	88	24	1	6	3	122	1284

Table 7: Number of laboratory-confirmed respiratory syncytial virus (RSV) cases by type and total number of samples tested by province for all ages, outpatient ILI surveillance in private general practitioner practices, South Africa, 29 December 2025 to 12 July 2026.

Province	RSV A	RSV B	RSV AB	RSV subgroup inconclusive	RSV typing results pending	Total RSV	Total specimens
Eastern Cape	0	1	0	0	0	1	7
Free State	0	0	0	0	0	0	21
Gauteng	4	24	0	0	1	29	532
KwaZulu-Natal	8	2	0	0	0	10	69
Limpopo	0	0	0	0	0	0	0
Mpumalanga	1	1	0	0	0	2	11
North West	0	0	0	0	0	0	0
Northern Cape	0	0	0	0	0	0	0
Western Cape	6	4	0	0	0	10	270
Total	19	32	0	0	1	52	910

Table 8: Number of laboratory-confirmed respiratory syncytial virus (RSV) cases by type and total number of samples tested by hospital and province for all ages, inpatient pneumonia surveillance in public hospitals, South Africa, 29 December 2025 to 12 July 2026.

Hospital (Province)	RSV A	RSV B	RSV AB	RSV subgroup inconclusive	RSV typing results pending	Total RSV	Total specimens
Helen Joseph-Rahima Moosa (GP)	42	58	0	0	1	101	461
Harry Gwala (KZN)	82	3	0	1	2	88	301
Mapulaneng (MP)	18	3	0	0	0	21	152
Klerksdorp-Tshepong (NW)	8	33	0	0	2	43	498
Mitchell's Plain (WC)	45	22	1	0	6	74	335
Red Cross (WC)	155	59	0	2	9	225	561
Total	350	178	1	3	20	552	2308

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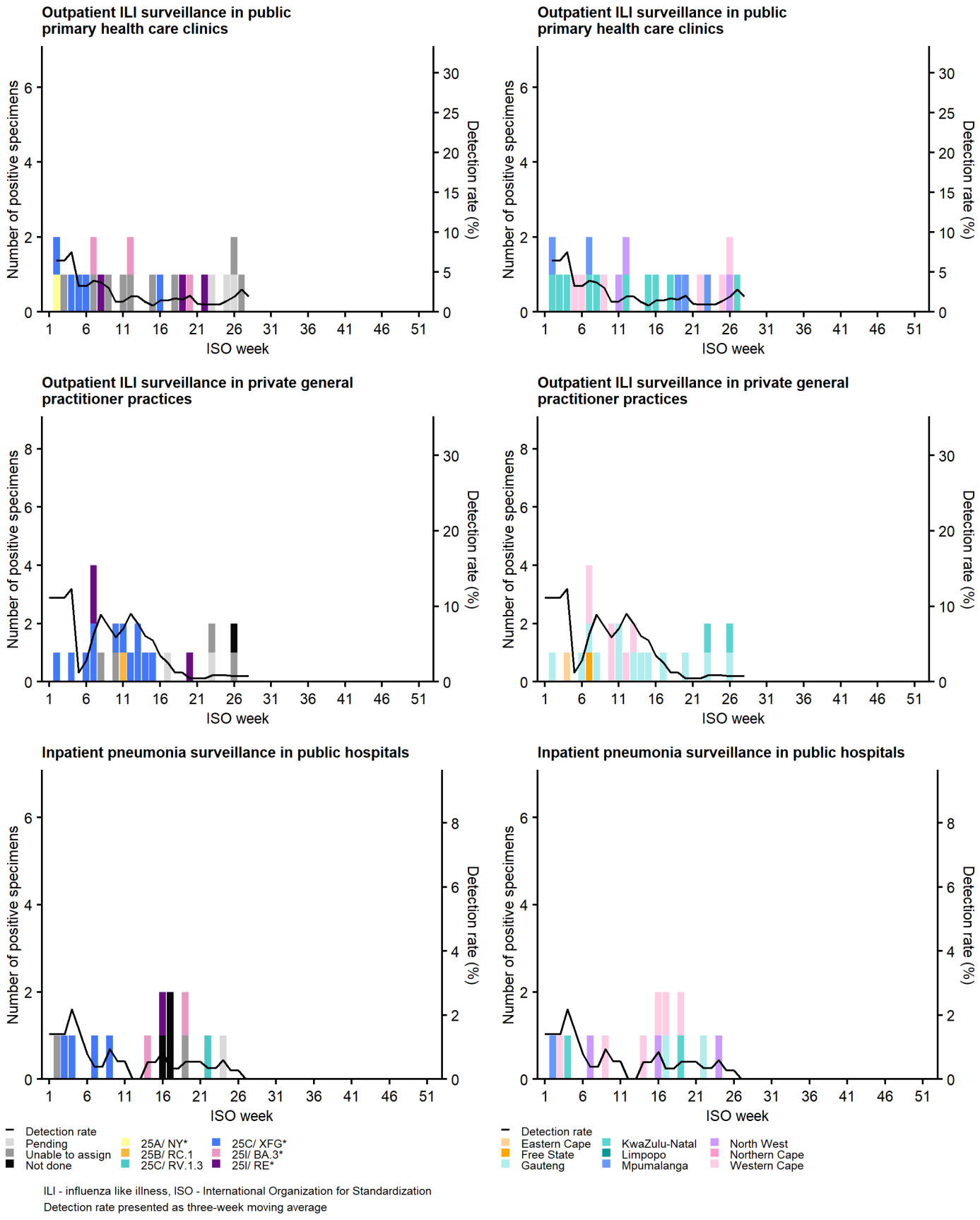

Figure 7: Number of laboratory-confirmed SARS-CoV-2 cases and detection rate by variant type (left) and province (right) for all ages, sentinel surveillance, South Africa, 29 December 2025 to 12 July 2026.

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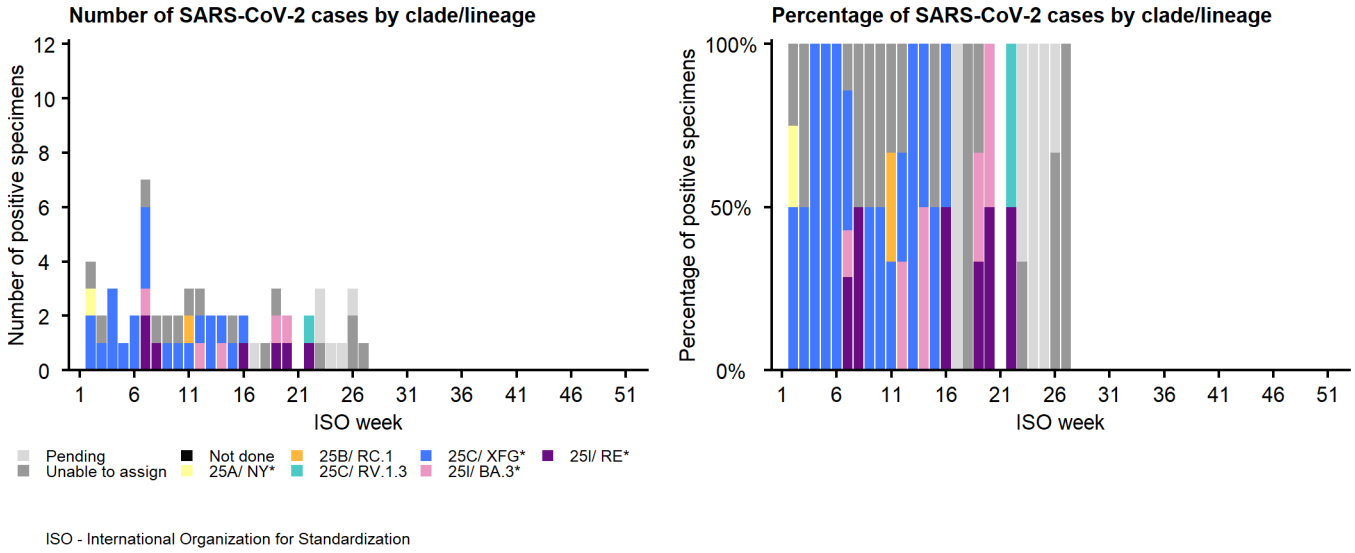

ISO - International Organization for Standardization

Figure 8: Combined number and percentage of SARS-CoV-2 variants for 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, 29 December 2025 to 12 July 2026.

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Table 9: Number of laboratory-confirmed SARS-CoV-2 cases by variant type and total number of samples tested by clinic and province for all ages, outpatient ILI surveillance in public primary health care clinics, South Africa, 29 December 2025 to 12 July 2026.

Clinic (Province)	25A/ NY*	25B/ RC.1	25C/ RV.1.3	25C/ XFG*	25I/ BA.3*	25I/ RE*	Pending	Unable to assign	Not done	Total SARS-CoV-2	Total specimens
Edendale Gateway (KZN)	0	0	0	3	2	1	0	4	0	10	444
Agincourt (MP)	1	0	0	0	1	1	1	1	0	5	184
Jouberton (NW)	0	0	0	0	0	0	0	3	0	3	211
Eastridge (WC)	0	0	0	2	0	1	2	1	0	6	430
Mitchell's Plain (WC)	0	0	0	0	0	0	0	0	0	0	15
Total	1	0	0	5	3	3	3	9	0	24	1284

*Including sub-lineages

Table 10: Number of laboratory-confirmed SARS-CoV-2 cases by variant type and total number of samples tested by province for all ages, outpatient ILI surveillance in private general practitioner practices, South Africa, 29 December 2025 to 12 July 2026.

Province	25A/ NY*	25B/ RC.1	25C/ RV.1.3	25C/ XFG*	25I/ BA.3*	25I/ RE*	Pending	Unable to assign	Not done	Total SARS-CoV-2	Total specimens
Eastern Cape	0	0	0	1	0	0	0	0	0	1	7
Free State	0	0	0	1	0	0	0	0	0	1	21
Gauteng	0	1	0	6	0	2	2	2	0	13	532
KwaZulu-Natal	0	0	0	0	0	0	0	1	0	1	69
Limpopo	0	0	0	0	0	0	0	0	0	0	0
Mpumalanga	0	0	0	0	0	0	0	0	0	0	11
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	0	0	0	4	0	1	0	1	0	6	270
Total	0	1	0	12	0	3	2	4	0	22	910

*Including sub-lineages

Table 11: Number of laboratory-confirmed SARS-CoV-2 cases by variant type and total number of samples tested by hospital and province for all ages, inpatient pneumonia surveillance in public hospitals, South Africa, 29 December 2025 to 12 July 2026.

Hospital (Province)	25A/ NY*	25B/ RC.1	25C/ RV.1.3	25C/ XFG*	25I/ BA.3*	25I/ RE*	Pending	Unable to assign	Not done	Total SARS-CoV-2	Total specimens
Helen Joseph-Rahima Moosa (GP)	0	0	1	0	0	0	0	0	0	1	461
Harry Gwala (KZN)	0	0	0	1	1	0	0	0	0	2	301
Mapulaneng (MP)	0	0	0	0	0	0	0	1	0	1	152
Klerksdorp-Tshepong (NW)	0	0	0	1	0	1	1	0	0	3	498
Mitchell's Plain (WC)	0	0	0	1	0	0	0	0	0	1	335
Red Cross (WC)	0	0	0	1	1	0	0	1	0	3	561
Total	0	0	1	4	2	1	1	2	0	11	2308

*Including sub-lineages

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Bordetella pertussis

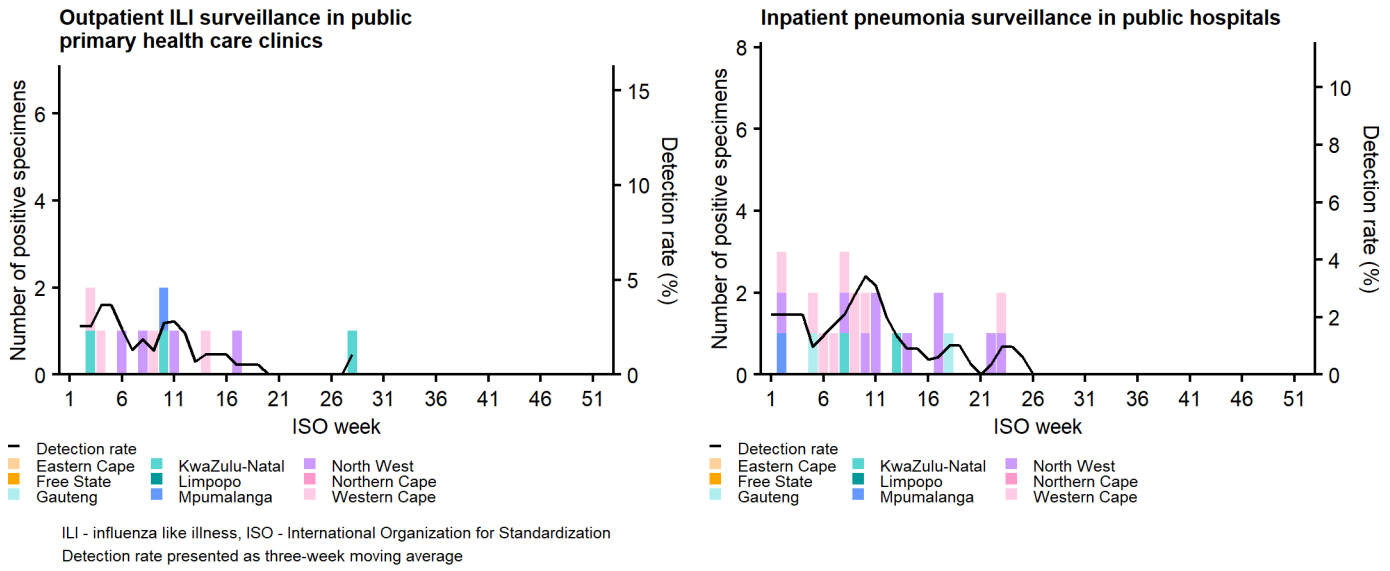

Figure 9: Number of laboratory-confirmed *Bordetella pertussis* cases and detection rate by province for all ages, sentinel surveillance, South Africa, 29 December 2025 to 12 July 2026.

Table 12: Number of laboratory-confirmed *Bordetella pertussis* cases and total number of samples tested by province for all ages, outpatient ILI surveillance in public primary health care clinics, South Africa, 29 December 2025 to 12 July 2026.

Clinic (Province)	Positive	Pending testing	Total specimens
Edendale Gateway (KZN)	3	2	444
Agincourt (MP)	1	0	184
Jouberton (NW)	4	3	211
Eastridge (WC)	4	2	430
Mitchell's Plain (WC)	0	0	15
Total	12	7	1284

Table 13: Number of laboratory-confirmed *Bordetella pertussis* cases and total number of samples tested by province for all ages, inpatient pneumonia surveillance in public hospitals, South Africa, 29 December 2025 to 12 July 2026.

Hospital (Province)	Positive	Pending testing	Total specimens
Helen Joseph-Rahima Moosa (GP)	2	5	461
Harry Gwala (KZN)	2	0	301
Mapulaneng (MP)	1	0	152
Klerksdorp-Tshepong (NW)	10	11	498
Mitchell's Plain (WC)	3	0	335
Red Cross (WC)	6	0	561
Total	24	16	2308

Additional respiratory viruses (human adenovirus, human metapneumovirus, human parainfluenza virus, and human rhinovirus)

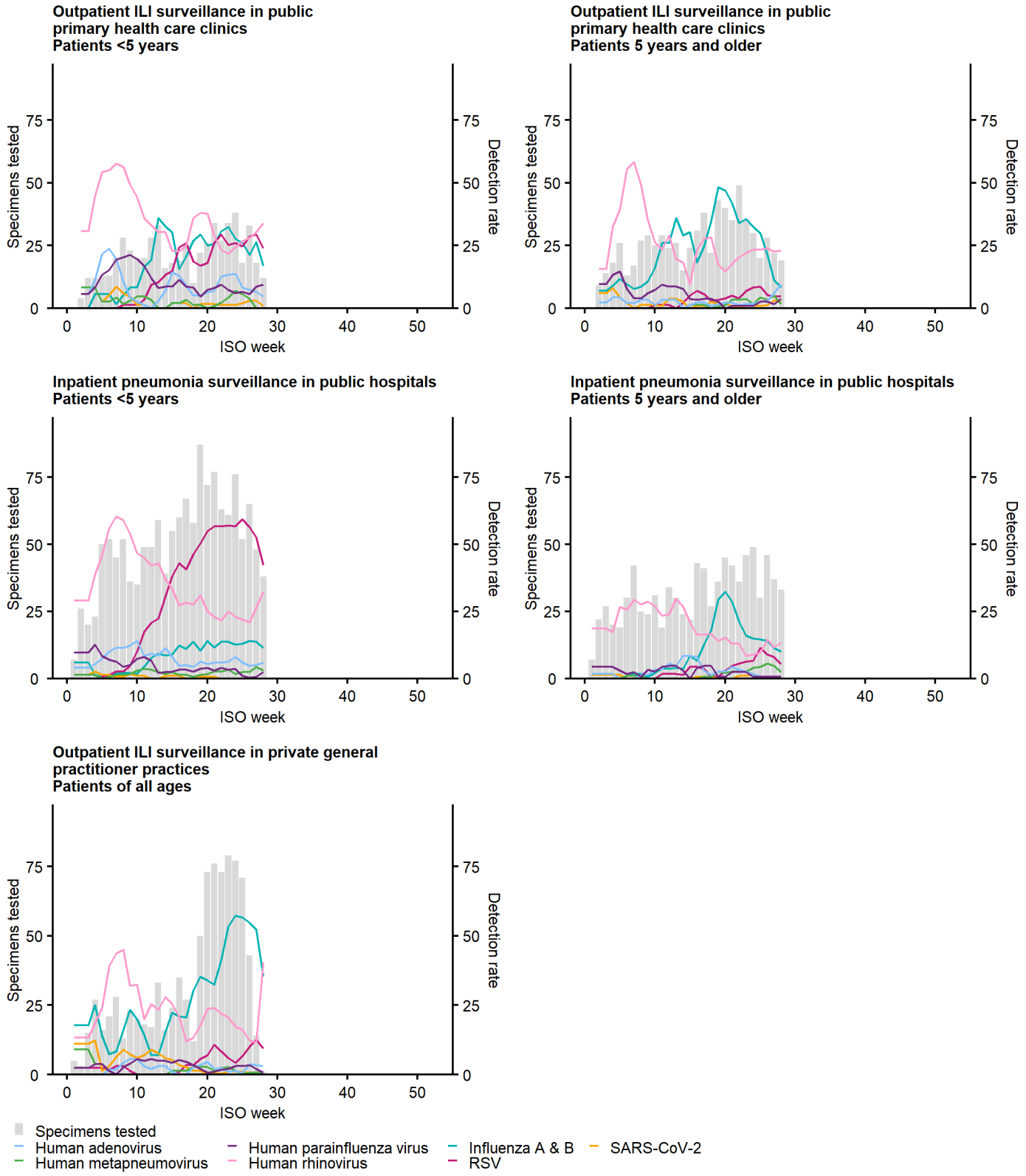

Figure 10: Number of specimens tested in light grey bars and virus detection rate (coloured lines) from outpatient ILI surveillance in public primary health care clinics by age (top); inpatient pneumonia surveillance in public hospitals by age (middle); and outpatient ILI surveillance in private general practitioner practices in all ages (bottom left), South Africa, 29 December 2025 to 12 July 2026.

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Table 14: Number of detections of human adenovirus, human metapneumovirus, human parainfluenza virus, and human rhinovirus, and total number of samples tested, clinic and province for all ages, outpatient ILI surveillance in public primary health care clinics, South Africa, 29 December 2025 to 12 July 2026.

Clinic (Province)	Human adenovirus	Human metapneumovirus	Human parainfluenza virus	Human rhinovirus	Total specimens tested
Edendale Gateway (KZN)	15	2	22	90	444
Agincourt (MP)	4	6	20	49	184
Jouberton (NW)	20	11	22	90	211
Eastridge (WC)	30	7	26	142	430
Mitchell's Plain (WC)	0	0	0	4	15
Total	69	26	90	375	1284

Table 15: Number of detections of human adenovirus, human metapneumovirus, human parainfluenza virus, and human rhinovirus, and total number of samples tested, by province for all ages, outpatient ILI surveillance in private general practitioner practices, South Africa, 29 December 2025 to 12 July 2026.

Province	Human adenovirus	Human metapneumovirus	Human parainfluenza virus	Human rhinovirus	Total specimens tested
Eastern Cape	0	0	0	1	7
Free State	0	1	1	6	21
Gauteng	10	9	16	114	532
KwaZulu-Natal	4	0	4	14	69
Limpopo	0	0	0	0	0
Mpumalanga	0	0	0	2	11
North West	0	0	0	0	0
Northern Cape	0	0	0	0	0
Western Cape	5	2	5	49	270
Total	19	12	26	186	910

Table 16: Number of detections of human adenovirus, human metapneumovirus, human parainfluenza virus, and human rhinovirus, and total number of samples tested, by hospital and province, all ages, inpatient pneumonia surveillance in public hospitals, South Africa, 29 December 2025 to 12 July 2026.

Hospital (Province)	Human adenovirus	Human metapneumovirus	Human parainfluenza virus	Human rhinovirus	Total specimens tested
Helen Joseph-Rahima Moosa (GP)	36	19	18	158	461
Harry Gwala (KZN)	14	2	14	63	301
Mapulaneng (MP)	6	1	4	23	152
Klerksdorp-Tshepong (NW)	24	15	22	116	498
Mitchell's Plain (WC)	10	1	5	89	335
Red Cross (WC)	38	8	14	193	561
Total	128	46	77	642	2308

Methods

Table 17: 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	KZN, NW, WC, MP	EC, FS, GP, KZN, LP, MP, NC, NW, WC	EC, GP, KZN, 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, human metapneumovirus, human adenovirus, human rhinovirus, human parainfluenza virus and B. pertussis	Influenza virus, RSV, SARS-CoV-2, human metapneumovirus, human adenovirus, human rhinovirus and human parainfluenza virus	Influenza virus, RSV, SARS-CoV-2, human metapneumovirus, human adenovirus, human rhinovirus, human parainfluenza virus and B. pertussis
Testing Methods	Respiratory viruses: Allplex™ RV Master Assay. B. pertussis: Multiplex real-time PCR (Tatti et al., 2011)	Respiratory viruses: Allplex™ RV Master Assay.	Respiratory viruses: Allplex™ RV Master Assay. 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
- KZN: KwaZulu-Natal
- LP: Limpopo
- 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)
- Not done (lineage/subclade): Sequencing not performed due to insufficient specimen volume.
- 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 are 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, influenza B, RSV (detects subgroup A and B), SARS-CoV-2, human metapneumovirus, human adenovirus (detects species A-F), human rhinovirus (detects species A-C) and human parainfluenza virus (detects types 1-4) were identified using a commercial multiplex RT-PCR assay (Allplex™ RV Master Assay,

Seegene Inc., Seoul, South Korea). A specimen was considered positive for human metapneumovirus if the Ct was ≤39 for the respective target; RSV at Ct ≤38, and for the remaining respiratory pathogens (adenovirus, SARS-CoV-2, influenza A, influenza B, rhinovirus, and parainfluenza) at Ct ≤42.
B.pertussis was tested throughout the period 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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