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: 39
Reporting period: 29 December 2025 to 27 September 2026
Date of data extraction: 1 October 2026
Data are provisional as of the date of data 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 39 (21 September 2026 to 27 September 2026), from 79 samples tested, we detected 6 (7.6%) cases of influenza, 1 (1.3%) case of respiratory syncytial virus (RSV) and 0 (0.0%) cases of SARS-CoV-2.
- The influenza season has peaked, but circulation is ongoing, with an increase detected since week 35. In week 39, influenza transmission is below the seasonal threshold in outpatient surveillance from public clinics and remains at low level in outpatient surveillance from private general practitioner practices. Influenza morbidity and mortality from inpatient pneumonia surveillance from public hospitals remains in the low threshold. The recent increase was predominated by influenza B, 67% (37/55) of detections in weeks 35-39.
- The RSV season ended in week 34 (17 August 2026 to 23 August 2026), with the detection rate remaining below the seasonal threshold from week 35 onwards. The RSV season started in week 11 (week starting 9 March 2026) and peaked in week 25 (15 June 2026 to 21 June 2026).
- From 29 December 2025 to 27 September 2026, from 5961 samples tested, we detected 1023 (17.2%) cases of influenza, 809 (13.6%) cases of RSV, 75 (1.3%) cases of SARS-CoV-2, 268 (4.5%) cases of human adenovirus, 146 (2.4%) cases of human metapneumovirus, 252 (4.2%) cases of human parainfluenza virus, 1594 (26.7%) cases of human rhinovirus and 40 (0.9% of 4688 tested in public clinics and hospitals) cases of *B. pertussis*.

Table of contents

Monitoring potentially imported cases of respiratory viruses 1
Influenza & respiratory syncytial virus (RSV) epidemic thresholds 2
SARS-CoV-2 epidemic thresholds 3
Influenza 4
Respiratory syncytial virus (RSV) 7
SARS-CoV-2 10
Bordetella pertussis 13
Additional respiratory viruses (human adenovirus, human metapneumovirus, human parainfluenza virus, and human rhinovirus) 14
Methods 16

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

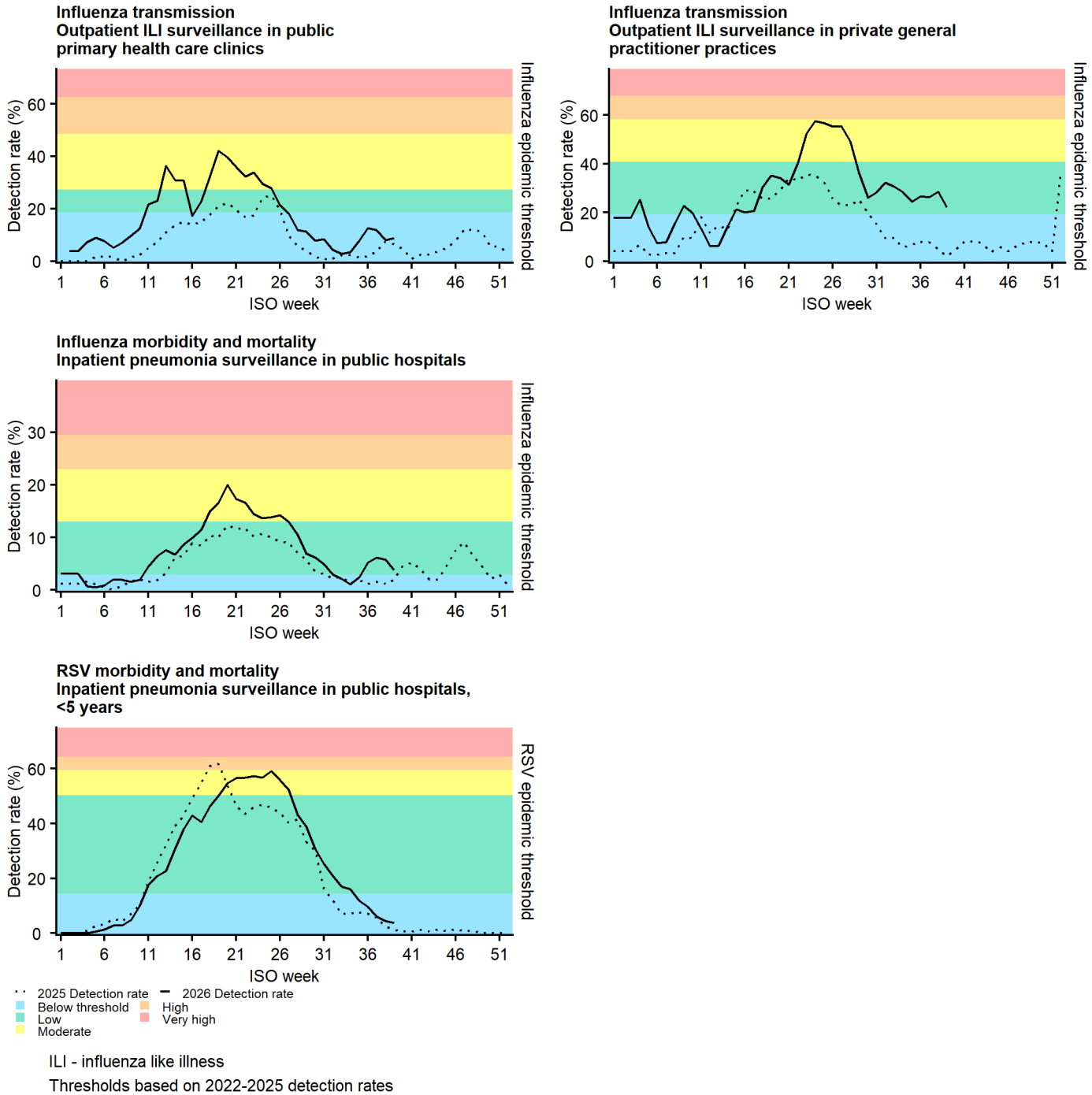


Figure 1: Influenza and respiratory syncytial virus (RSV) surveillance epidemic threshold summary, sentinel surveillance, South Africa, 29 December 2025 to 27 September 2026.

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.

SARS-CoV-2 epidemic thresholds

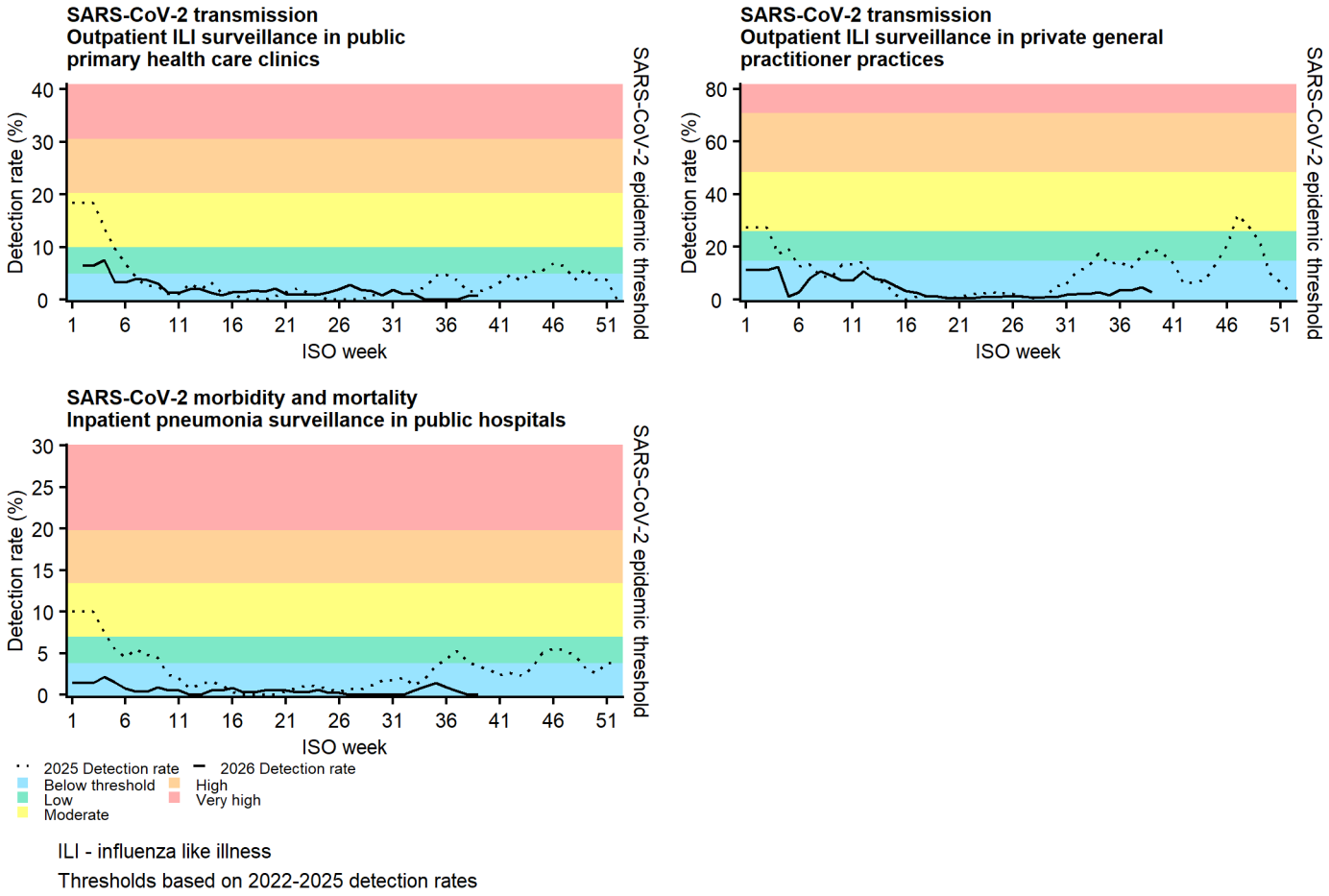


Figure 2: Severe acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) surveillance epidemic threshold summary, sentinel surveillance, South Africa, 29 December 2025 to 27 September 2026.

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.

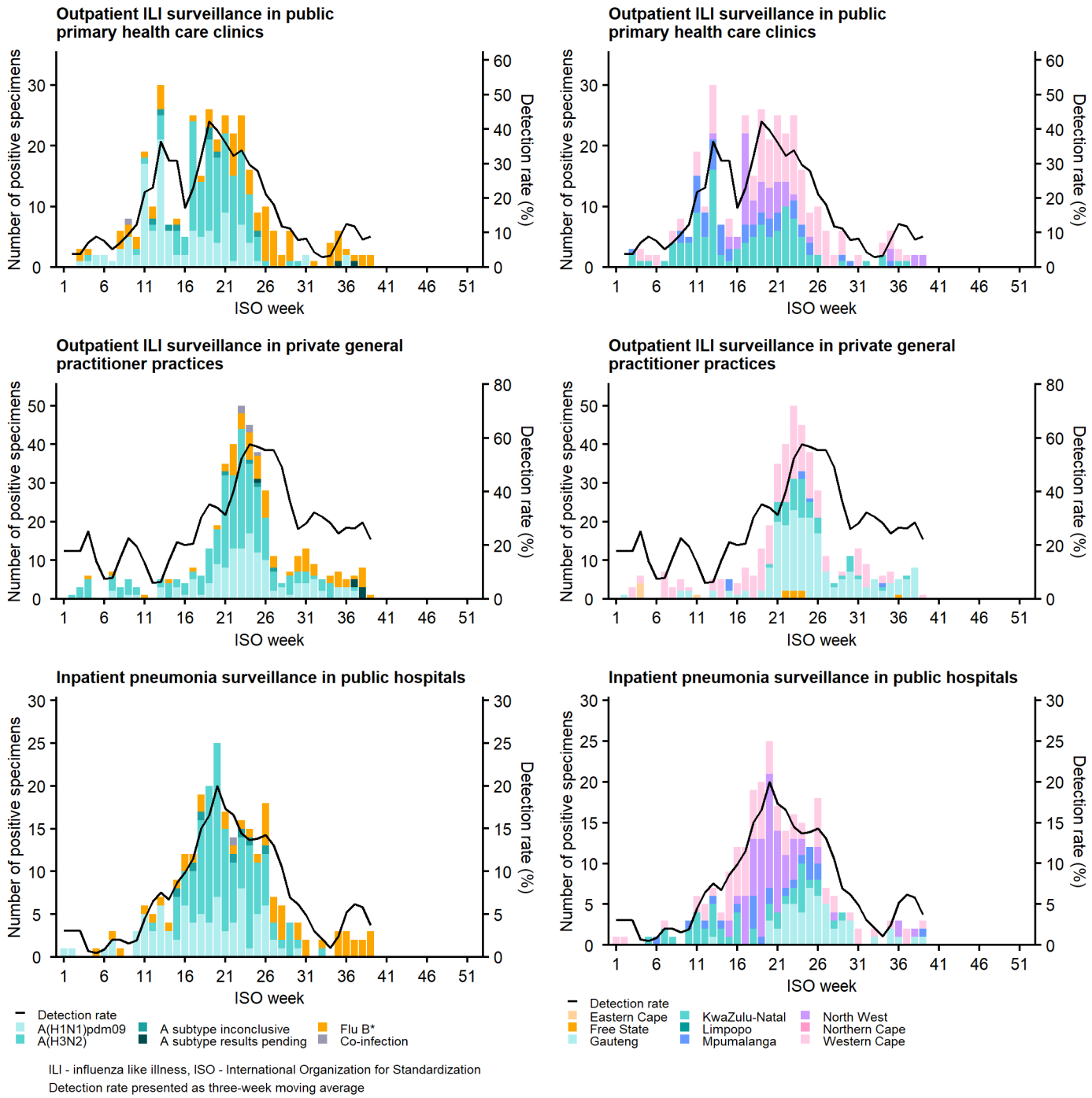


Figure 3: Number of laboratory-confirmed influenza cases and detection rate by subtype (left) and province (right) for all ages, sentinel surveillance, South Africa, 29 December 2025 to 27 September 2026.

*Subtyping of influenza B positive samples stopped in June 2026. From January to June 2026, 100% (N=111) of influenza B with lineage results were B/Victoria.

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.

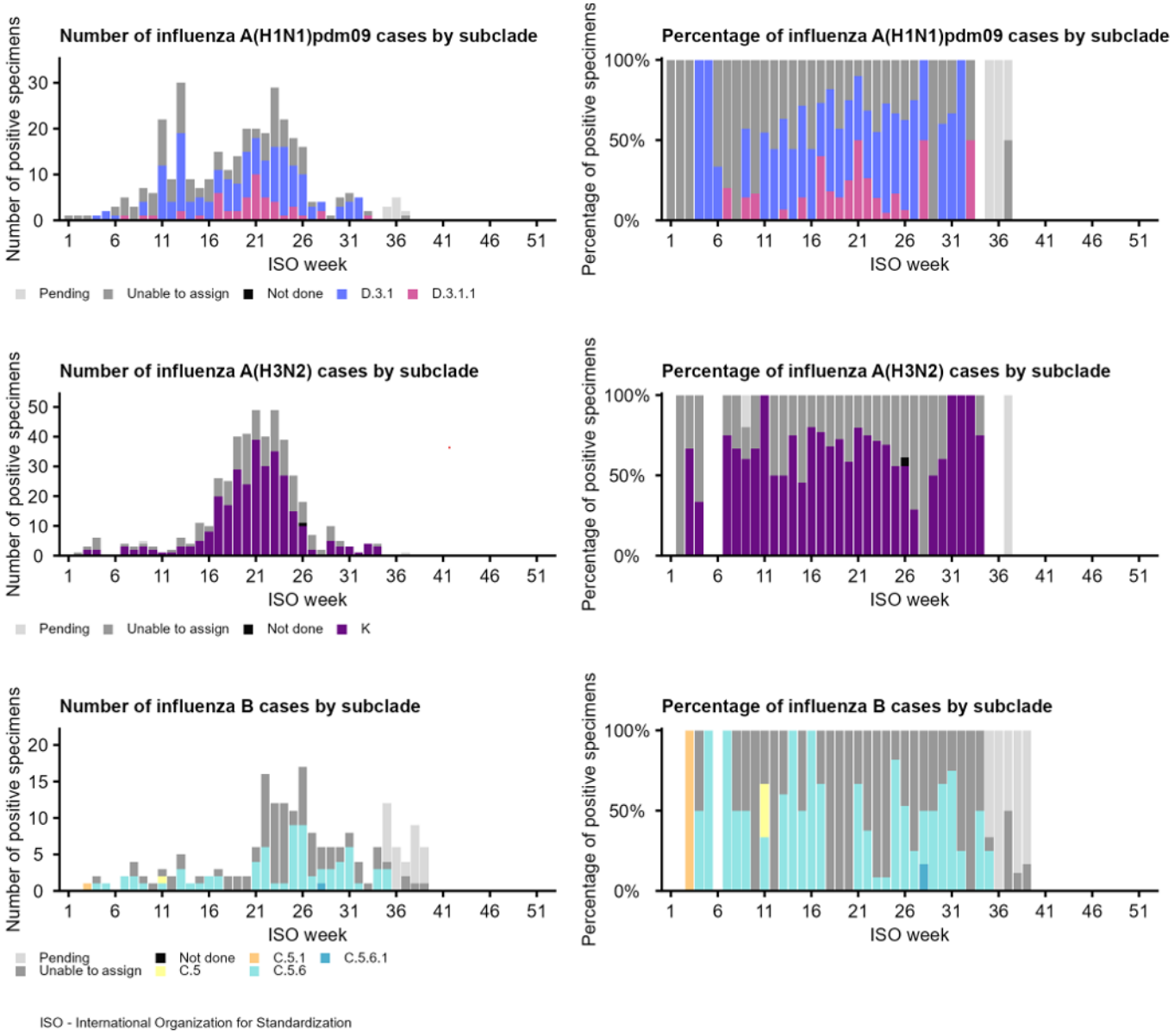


Figure 4: Combined number and percentage of influenza cases by subclade for all ages from three sentinel surveillance systems: outpatient influenza-like illness (ILI) surveillance in public primary health care clinics, outpatient ILI surveillance in private general practitioner practices, and inpatient pneumonia surveillance in public hospitals, South Africa, 29 December 2025 to 27 September 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 27 September 2026.

Subtype/Lineage	Clade	Subclade	Count
A(H1N1)pdm09	6B.1A.5a.2a.1	D.3.1	158
A(H1N1)pdm09	6B.1A.5a.2a.1	D.3.1.1	48
A(H3N2)	3C.2a1b.2a.2a.3a.1	K	302
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	71
B/Victoria	V1A.3a.2	C.5.6.1	1

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.

Table 2: Number of laboratory-confirmed influenza cases by subtype 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 27 September 2026.

Clinic (Province)	A(H1N1) pdm09	A(H3N2)	A subtype inconclusive	A subtype pending	Flu B*	Co-infection	Total influenza	Total specimens
Edendale Gateway (KZN)	41	27	5	1	39	0	113	576
Agincourt (MP)	33	14	0	1	2	1	49	237
Jouberton (NW)	2	42	1	0	6	0	51	264
Eastridge (WC)	42	43	2	0	39	0	126	568
Mitchell's Plain (WC)	1	0	0	0	0	0	1	32
Total	119	126	8	2	86	1	340	1677

Specimens where more than one influenza type or subtype was detected were denoted as co-infection and included in the counts for each separate type as well.
*Subtyping of influenza B positive samples stopped in June 2026. From January to June 2026, 100% (N=111) of influenza B with lineage results were B/Victoria.

Table 3: Number of laboratory-confirmed influenza cases by subtype 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 27 September 2026.

Province	A(H1N1) pdm09	A(H3N2)	A subtype inconclusive	A subtype pending	Flu B*	Co-infection	Total influenza	Total specimens
Eastern Cape	0	4	0	0	1	0	5	10
Free State	3	4	0	0	0	0	7	29
Gauteng	67	106	1	5	16	0	195	764
KwaZulu-Natal	12	21	3	1	11	1	47	89
Limpopo	0	0	0	0	0	0	0	0
Mpumalanga	4	1	0	0	2	0	7	12
North West	0	0	0	0	0	0	0	0
Northern Cape	0	0	0	0	0	0	0	0
Western Cape	47	69	3	0	47	4	162	354
Total	133	205	7	6	77	5	423	1258

Specimens where more than one influenza type or subtype was detected were denoted as co-infection and included in the counts for each separate type as well.
*Subtyping of influenza B positive samples stopped in June 2026. From January to June 2026, 100% (N=111) of influenza B with lineage results were B/Victoria.

Table 4: Number of laboratory-confirmed influenza cases by subtype 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 27 September 2026.

Hospital (Province)	A(H1N1) pdm09	A(H3N2)	A subtype inconclusive	A subtype pending	Flu B*	Co-infection	Total influenza	Total specimens
Helen Joseph-Rahima Moosa (GP)	22	23	0	0	7	0	52	591
Harry Gwala (KZN)	14	13	1	0	15	0	43	371
Mapulaneng (MP)	13	8	0	0	3	1	23	179
Klerksdorp-Tshepong (NW)	6	55	4	0	4	0	69	675
Mitchell's Plain (WC)	10	12	0	0	10	0	32	472
Red Cross (WC)	20	8	2	0	11	0	41	738
Total	85	119	7	0	50	1	260	3026

Specimens where more than one influenza type or subtype was detected were denoted as co-infection and included in the counts for each separate type as well.
*Subtyping of influenza B positive samples stopped in June 2026. From January to June 2026, 100% (N=111) of influenza B with lineage results were B/Victoria.

Table 5: Description of cases with more than one influenza type or subtype 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 + Flu B	0	3	0
A(H1N1)pdm09 + Flu B	0	1	1
A(H3N2) + Flu B	0	1	0

Respiratory syncytial virus (RSV)

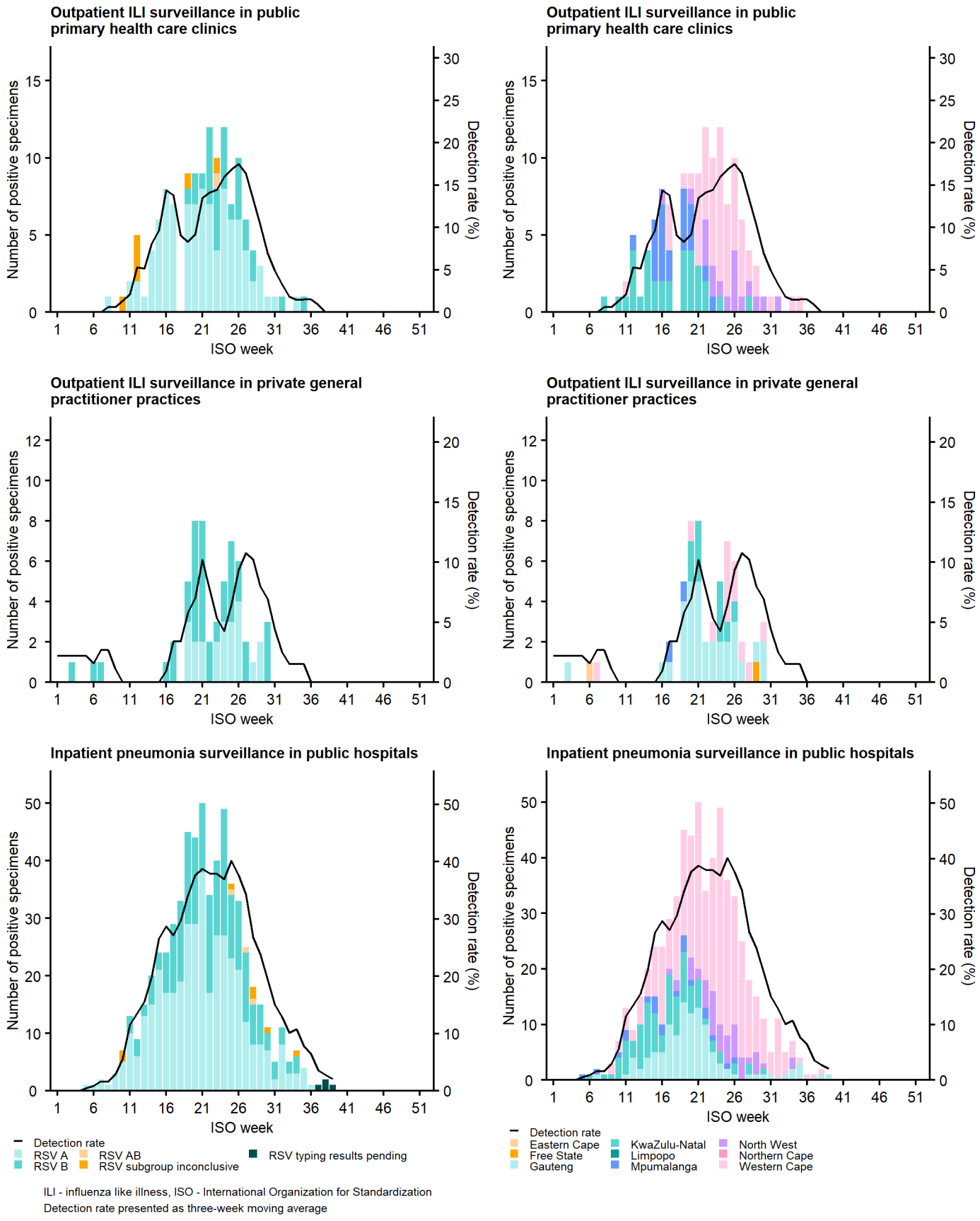


Figure 5: Number of laboratory-confirmed respiratory syncytial virus (RSV) cases and detection rate by type (left) and province (right) for all ages, sentinel surveillance, South Africa, 29 December 2025 to 27 September 2026.

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.

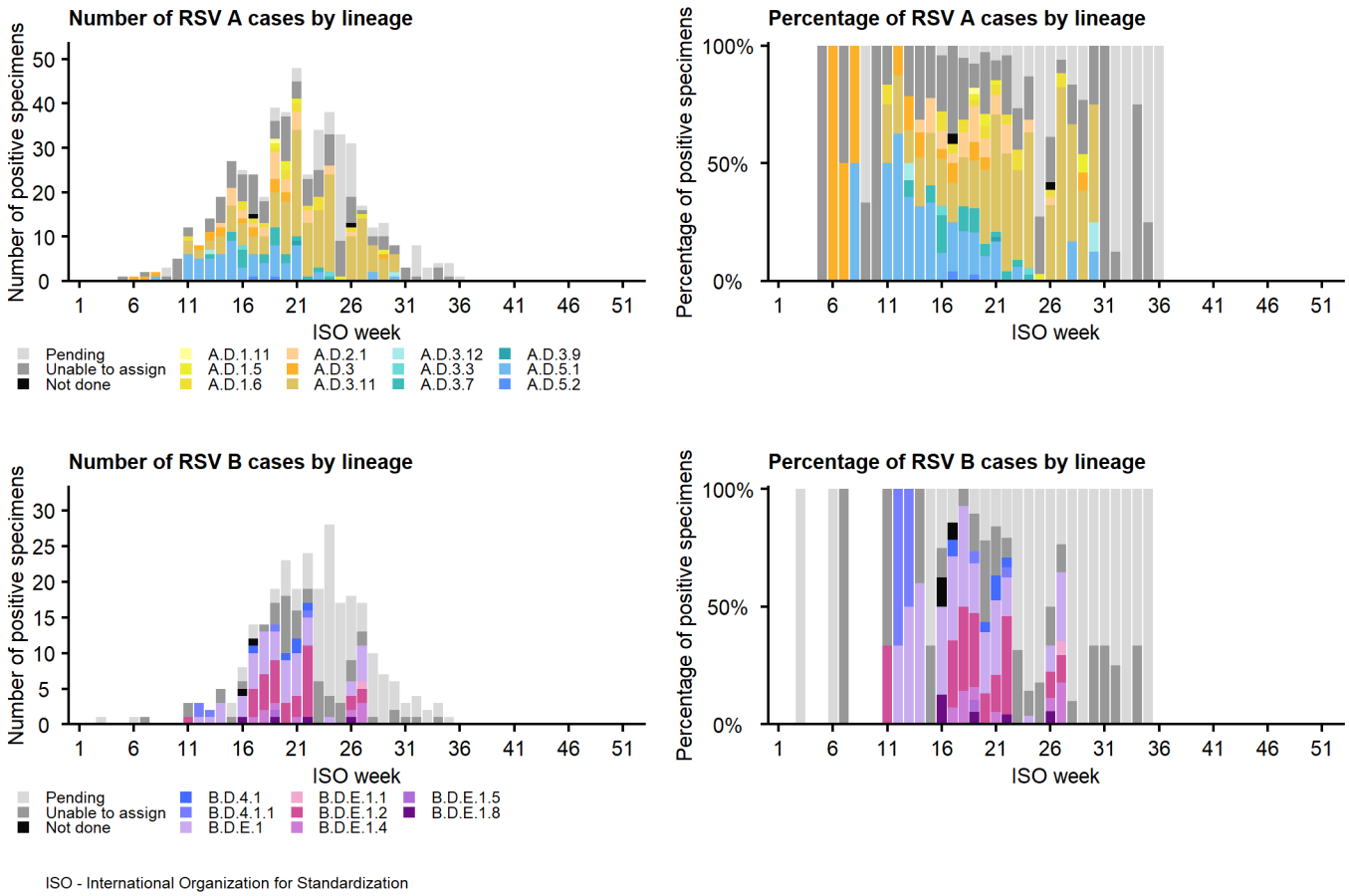


Figure 6: Combined number and percentage of RSV cases by lineage for all ages from three sentinel surveillance systems: outpatient influenza-like illness (ILI) surveillance in public primary health care clinics, outpatient ILI surveillance in private general practitioner practices, and inpatient pneumonia surveillance in public hospitals, South Africa, 29 December 2025 to 27 September 2026.

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.

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 27 September 2026.

Clinic (Province)	RSV A	RSV B	RSV AB	RSV subgroup inconclusive	RSV typing results pending	Total RSV	Total specimens
Edendale Gateway (KZN)	28	0	0	5	0	33	576
Agincourt (MP)	20	1	0	0	0	21	237
Jouberton (NW)	5	13	0	0	0	18	264
Eastridge (WC)	43	13	1	1	0	58	568
Mitchell's Plain (WC)	0	1	0	0	0	1	32
Total	96	28	1	6	0	131	1677

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 27 September 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	10
Free State	1	0	0	0	0	1	29
Gauteng	5	27	0	0	0	33	764
KwaZulu-Natal	8	2	0	0	0	10	89
Limpopo	0	0	0	0	0	0	0
Mpumalanga	1	1	0	0	0	2	12
North West	0	0	0	0	0	0	0
Northern Cape	0	0	0	0	0	0	0
Western Cape	7	5	0	0	0	12	354
Total	22	36	0	0	0	59	1258

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 27 September 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)	47	62	0	1	2	112	591
Harry Gwala (KZN)	84	3	0	2	0	89	371
Mapulaneng (MP)	18	3	0	0	0	21	179
Klerksdorp-Tshepong (NW)	9	41	0	0	0	50	675
Mitchell's Plain (WC)	56	28	3	1	0	88	472
Red Cross (WC)	184	70	0	3	2	259	738
Total	398	207	3	7	4	619	3026

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.

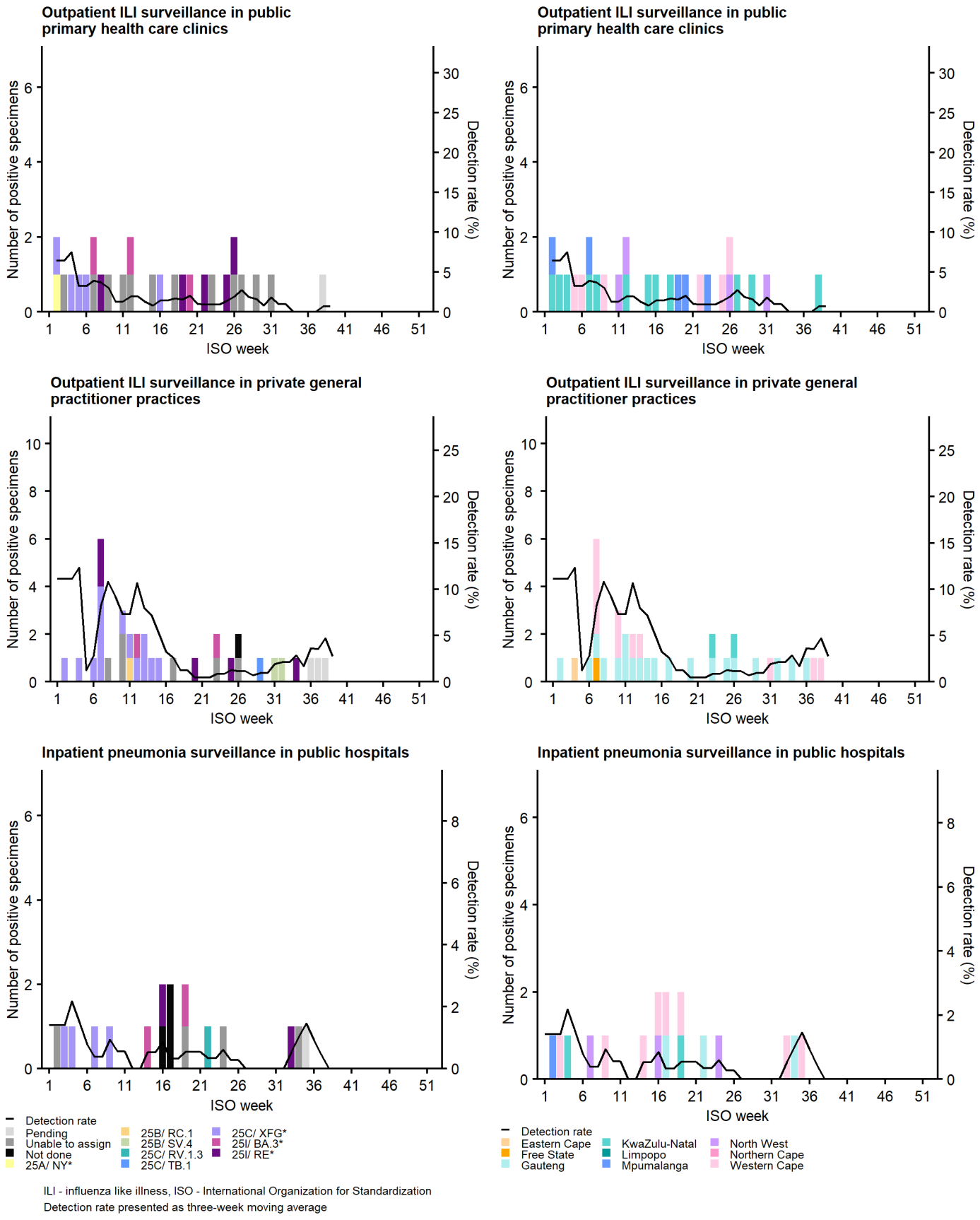
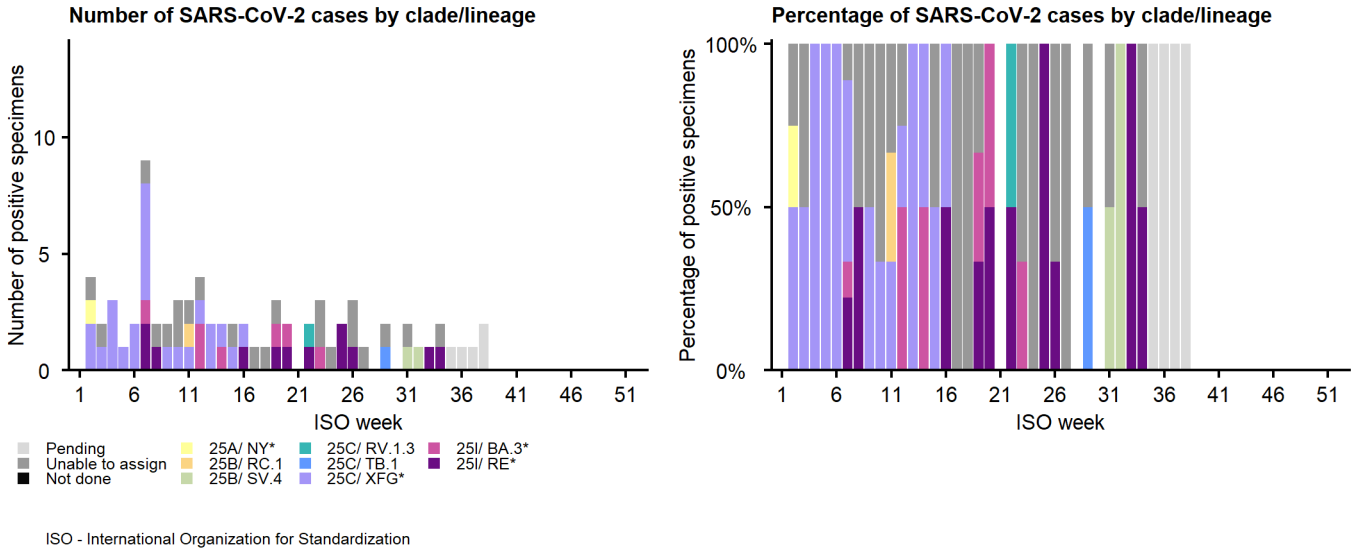


Figure 7: Number of laboratory-confirmed SARS-CoV-2 cases and detection rate by variant type (left) and province (right) for all ages, sentinel surveillance, South Africa, 29 December 2025 to 27 September 2026.

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.



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 27 September 2026.

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.

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 27 September 2026.

Clinic (Province)	25A/ NY*	25B/ RC.1	25B/ SV.4	25C/ RV.1.3	25C/ TB.1	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	0	0	3	2	1	1	5	0	12	576
Agincourt (MP)	1	0	0	0	0	0	1	1	0	2	0	5	237
Jouberton (NW)	0	0	0	0	0	0	0	0	0	4	0	4	264
Eastridge (WC)	0	0	0	0	0	2	0	3	0	1	0	6	568
Mitchell's Plain (WC)	0	0	0	0	0	0	0	0	0	0	0	0	32
Total	1	0	0	0	0	5	3	5	1	12	0	27	1677

*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 27 September 2026.

Province	25A/ NY*	25B/ RC.1	25B/ SV.4	25C/ RV.1.3	25C/ TB.1	25C/ XFG*	25I/ BA.3*	25I/ RE*	Pending	Unable to assign	Not done	Total SARS-CoV-2	Total specimens
Eastern Cape	0	0	0	0	0	1	0	0	0	0	0	1	10
Free State	0	0	0	0	0	1	0	0	0	0	0	1	29
Gauteng	0	1	1	0	1	6	2	4	1	4	0	20	764
KwaZulu-Natal	0	0	0	0	0	0	0	0	0	1	0	1	89
Limpopo	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	12
North West	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
Western Cape	0	0	1	0	0	6	0	1	2	1	0	11	354
Total	0	1	2	0	1	14	2	5	3	6	0	34	1258

*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 27 September 2026.

Hospital (Province)	25A/ NY*	25B/ RC.1	25B/ SV.4	25C/ RV.1.3	25C/ TB.1	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	0	1	0	0	0	0	0	1	0	2	591
Harry Gwala (KZN)	0	0	0	0	0	1	1	0	0	0	0	2	371
Mapulaneng (MP)	0	0	0	0	0	0	0	0	0	1	0	1	179
Klerksdorp-Tshepong (NW)	0	0	0	0	0	1	0	1	0	1	0	3	675
Mitchell's Plain (WC)	0	0	0	0	0	1	0	0	1	0	0	2	472
Red Cross (WC)	0	0	0	0	0	1	1	1	0	1	0	4	738
Total	0	0	0	1	0	4	2	2	1	4	0	14	3026

*Including sub-lineages

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.

Bordetella pertussis

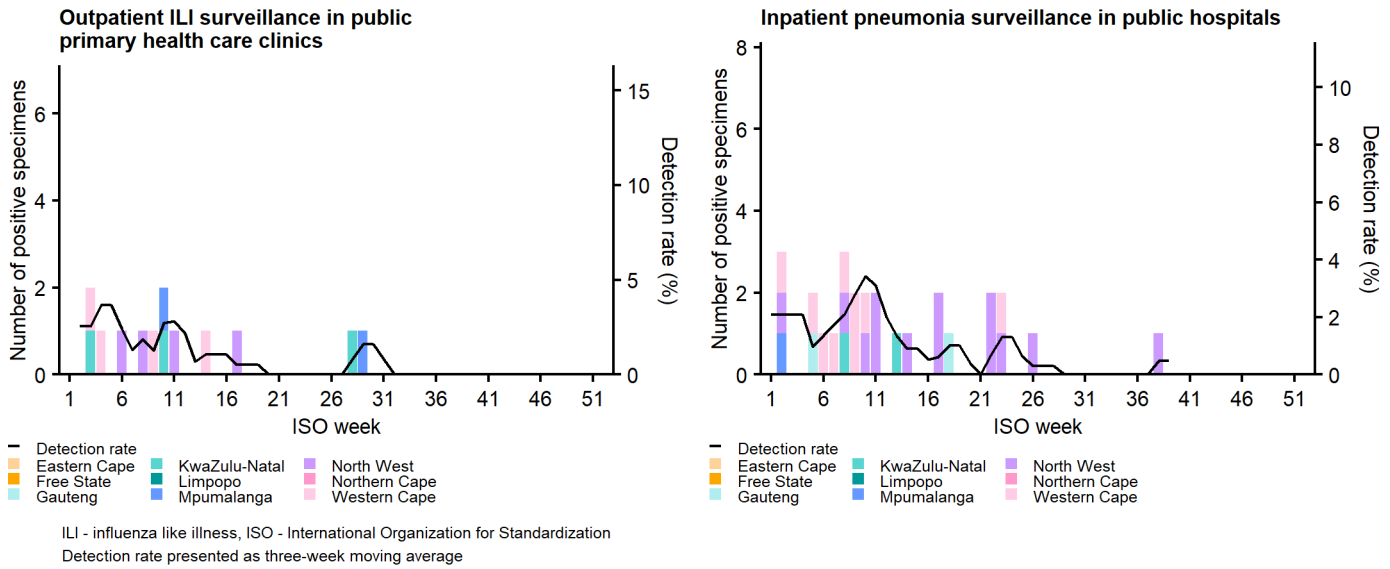


Figure 9: Number of laboratory-confirmed *Bordetella pertussis* cases and detection rate by province for all ages, sentinel surveillance, South Africa, 29 December 2025 to 27 September 2026.

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 27 September 2026.

Clinic (Province)	Positive	Pending testing	Total specimens
Edendale Gateway (KZN)	3	0	576
Agincourt (MP)	2	0	237
Jouberton (NW)	4	0	264
Eastridge (WC)	4	0	568
Mitchell's Plain (WC)	0	0	32
Total	13	0	1677

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 27 September 2026.

Hospital (Province)	Positive	Pending testing	Total specimens
Helen Joseph-Rahima Moosa (GP)	2	0	591
Harry Gwala (KZN)	2	0	371
Mapulaneng (MP)	1	3	179
Klerksdorp-Tshepong (NW)	13	1	675
Mitchell's Plain (WC)	3	11	472
Red Cross (WC)	6	0	738
Total	27	15	3026

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

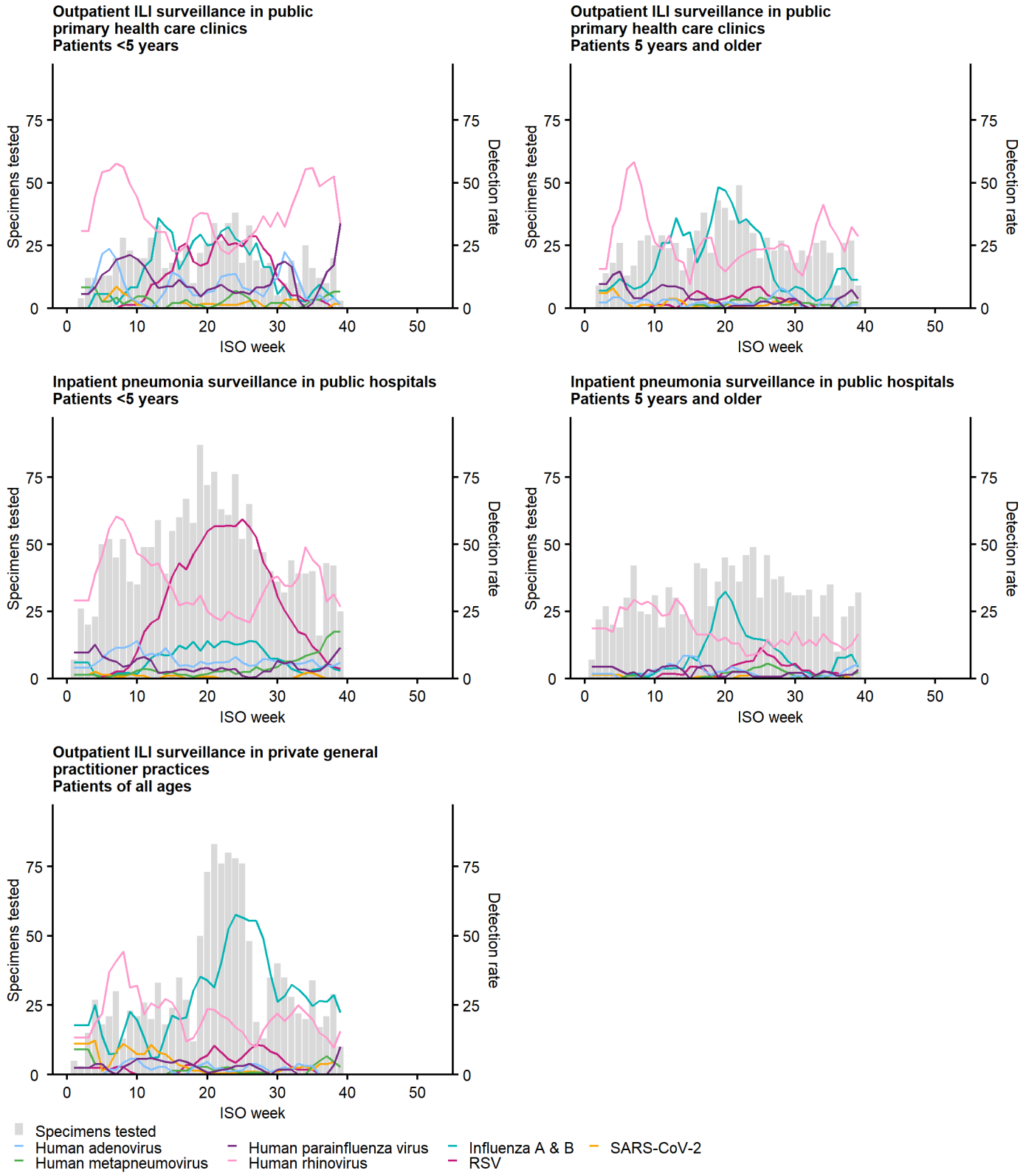


Figure 10: Number of specimens tested in light grey bars and virus detection rate (coloured lines) from outpatient ILI surveillance in public primary health care clinics by age (top); inpatient pneumonia surveillance in public hospitals by age (middle); and outpatient ILI surveillance in private general practitioner practices in all ages (bottom left), South Africa, 29 December 2025 to 27 September 2026.

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.

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 27 September 2026.

Clinic (Province)	Human adenovirus	Human metapneumovirus	Human parainfluenza virus	Human rhinovirus	Total specimens tested
Edendale Gateway (KZN)	17	3	24	134	576
Agincourt (MP)	6	7	20	76	237
Jouberton (NW)	24	11	26	111	264
Eastridge (WC)	37	15	39	186	568
Mitchell's Plain (WC)	1	0	0	6	32
Total	85	36	109	513	1677

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 27 September 2026.

Province	Human adenovirus	Human metapneumovirus	Human parainfluenza virus	Human rhinovirus	Total specimens tested
Eastern Cape	0	0	0	1	10
Free State	0	1	1	8	29
Gauteng	13	14	21	152	764
KwaZulu-Natal	6	0	5	19	89
Limpopo	0	0	0	0	0
Mpumalanga	0	0	0	2	12
North West	0	0	0	0	0
Northern Cape	0	0	0	0	0
Western Cape	6	3	8	64	354
Total	25	18	35	246	1258

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 27 September 2026.

Hospital (Province)	Human adenovirus	Human metapneumovirus	Human parainfluenza virus	Human rhinovirus	Total specimens tested
Helen Joseph-Rahima Moosa (GP)	45	23	19	190	591
Harry Gwala (KZN)	17	3	15	89	371
Mapulaneng (MP)	7	1	7	25	179
Klerksdorp-Tshepong (NW)	29	19	24	151	675
Mitchell's Plain (WC)	17	8	15	127	472
Red Cross (WC)	43	38	28	253	738
Total	158	92	108	835	3026

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 when 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 when the three-week moving average of the detection rate in children <5 years from inpatient pneumonia surveillance in public hospitals remains above or below the seasonal threshold 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 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/). Subtyping of influenza B stopped at the end of June 2026. 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.

References:

- García-Alcalde F, Okonechnikov K, Carbonell J, Cruz LM, Götz S, Tarazona S, Dopazo J, Meyer TF, Conesa A. Qualimap: evaluating next-generation sequencing alignment data. Bioinformatics. 2012 Oct 15;28(20):2678-9. doi: 10.1093/bioinformatics/bts503. Epub 2012 Aug 22. PMID: 22914218.
- Goya S, Ruis C, Neher RA, Meijer A, Aziz A, Hinrichs AS, von Gottberg A, Roemer C, Amoako DG, Acuña D, McBroome J, Otieno JR, Bhiman JN, Everatt J, Muñoz-Escalante JC, Ramaekers K, Duggan K, Presser LD, Urbanska L, Venter M, Wolter N, Peret TCT, Salimi V, Potdar V, Borges V, Viegas M. Standardized Phylogenetic Classification of Human respiratory syncytial virus below the Subgroup Level. Emerg Infect Dis. 2024 Aug;30(8):1631-1641. doi: 10.3201/eid3008.240209. PMID: 39043393; PMCID: PMC11286072.
- Hadfield J, Megill C, Bell SM, Huddleston J, Potter B, Callender C, Sagulenko P, Bedford T, Neher RA. Nextstrain: real-time tracking of pathogen evolution. Bioinformatics. 2018 Dec 1;34(23):4121-4123. doi: 10.1093/bioinformatics/bty407. PMID: 29790939; PMCID: PMC6247931. Rambaut A, Holmes EC, O'Toole Á, Hill V, McCrone JT, Ruis C, du Plessis L, Pybus OG. A dynamic nomenclature proposal for SARS-CoV-2 lineages to assist genomic epidemiology. Nat Microbiol. 2020 Nov;5(11):1403-1407. doi: 10.1038/s41564-020-0770-5. Epub 2020 Jul 15. PMID: 32669681; PMCID: PMC7610519.

- Shepard SS, Meno S, Bahl J, Wilson MM, Barnes J, Neuhaus E. Viral deep sequencing needs an adaptive approach: IRMA, the iterative refinement meta-assembler. BMC Genomics. 2016 Sep 5;17(1):708. doi: 10.1186/s12864-016-3030-6. Erratum in: BMC Genomics. 2016 Oct 13;17(1):801. doi: 10.1186/s12864-016-3138-8. PMID: 27595578; PMCID: PMC5011931.
- Sinnathamby MA, Bourouphael T, Boateng J, Collonnaz M, Quinot C, Aziz NA, Elgohari S, Green RE, Dabrera G, Lopez-Bernal J, Allen A. Setting thresholds to determine COVID-19 activity levels using the mean standard deviation (MSD) method, England, 2022-2024. Euro Surveill. 2024 Nov;29(45):2400696. doi: 10.2807/1560-7917.ES.2024.29.45.2400696. PMID: 39512168; PMCID: PMC11544723.
- Talts T, Mosscrop LG, Williams D, Tregoning JS, Paulo W, Kohli A, Williams TC, Hoschler K, Ellis J, Lusignan Sd, Zambon M. Robust and sensitive amplicon-based whole-genome sequencing assay of respiratory syncytial virus subtype A and B. Microbiol Spectr. 2024 Apr 2;12(4):e0306723. doi: 10.1128/spectrum.03067-23. Epub 2024 Feb 27. PMID: 38411056; PMCID: PMC10986592.
- Tatti KM, Sparks KN, Boney KO, Tondella ML. Novel multitarget real-time PCR assay for rapid detection of Bordetella species in clinical specimens. J Clin Microbiol. 2011 Dec;49(12):4059-66. doi: 10.1128/JCM.00601-11. Epub 2011 Sep 21. PMID: 21940464; PMCID: PMC3232951.
- Zhou B, Donnelly ME, Scholes DT, St George K, Hatta M, Kawaoka Y, Wentworth DE. Single-reaction genomic amplification accelerates sequencing and vaccine production for classical and Swine origin human influenza a viruses. J Virol. 2009 Oct;83(19):10309-13. doi: 10.1128/JVI.01109-09. Epub 2009 Jul 15. PMID: 19605485; PMCID: PMC2748056.
- Zhou B, Lin X, Wang W, Halpin RA, Bera J, Stockwell TB, Barr IG, Wentworth DE. Universal influenza B virus genomic amplification facilitates sequencing, diagnostics, and reverse genetics. J Clin Microbiol. 2014 May;52(5):1330-7. doi: 10.1128/JCM.03265-13. Epub 2014 Feb 5. PMID: 24501036; PMCID: PMC3993638.