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: 01 January 2024 to 14 July 2024
Date of data extraction: 18 July 2024

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

Highlights

- The influenza season started in week 17 (week starting 22 April 2024) and peaked in week 23 (week starting 3 June 2024) when the influenza transmission and impact was at a high level, and has since been decreasing.
- The respiratory syncytial virus (RSV) season started in week 6 (week starting 05 February 2024) and peaked in week 16 (week starting 15 April 2024) at the moderate level, thereafter, declining. There was an increase in RSV in week 25 to 27 (17 June 2024 to 7 July 2024), which has started to decrease.
- SARS-CoV-2 has been increasing among outpatients in the Viral Watch programme, but remains constant among hospitalised patients.
- In the month of June, we detected 0 (0%, 0/539) cases of *B. pertussis*.
- From 01 January 2024 to 14 July 2024, we detected 818 cases of influenza, 793 cases of RSV, 191 cases of SARS-CoV-2 and 24 cases of *Bordetella pertussis*.
- In week 28 (08 July 2024 to 14 July 2024), we detected 6 (5.8%, 6/104) cases of influenza, 13 (12.5%, 13/104) cases of RSV and 7 (6.7%, 7/104) cases of SARS-CoV-2.

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Epidemic thresholds

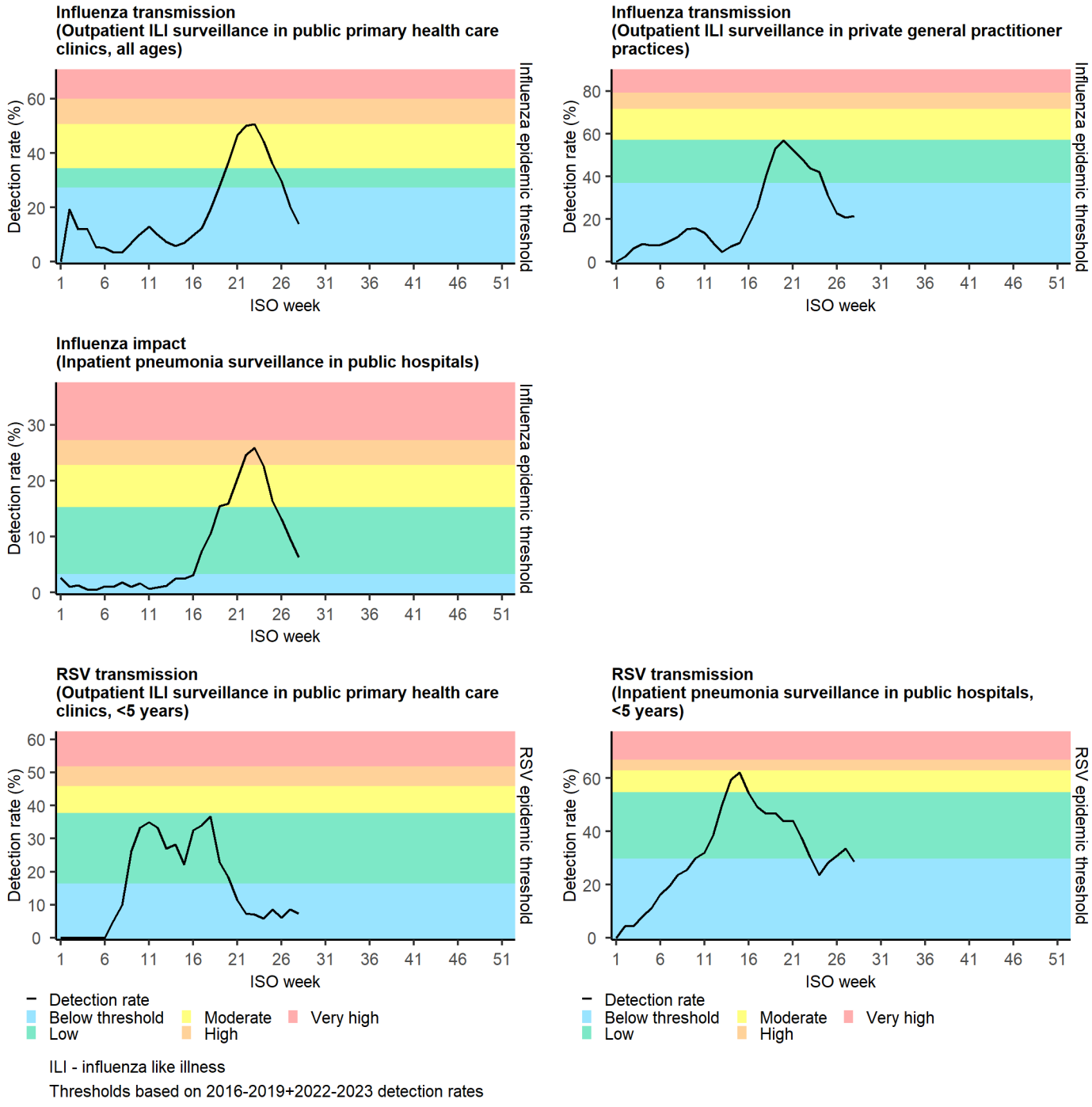

Figure 1: Influenza and respiratory syncytial virus (RSV) surveillance epidemic threshold summary, sentinel surveillance, South Africa, 01 January 2024 to 14 July 2024.

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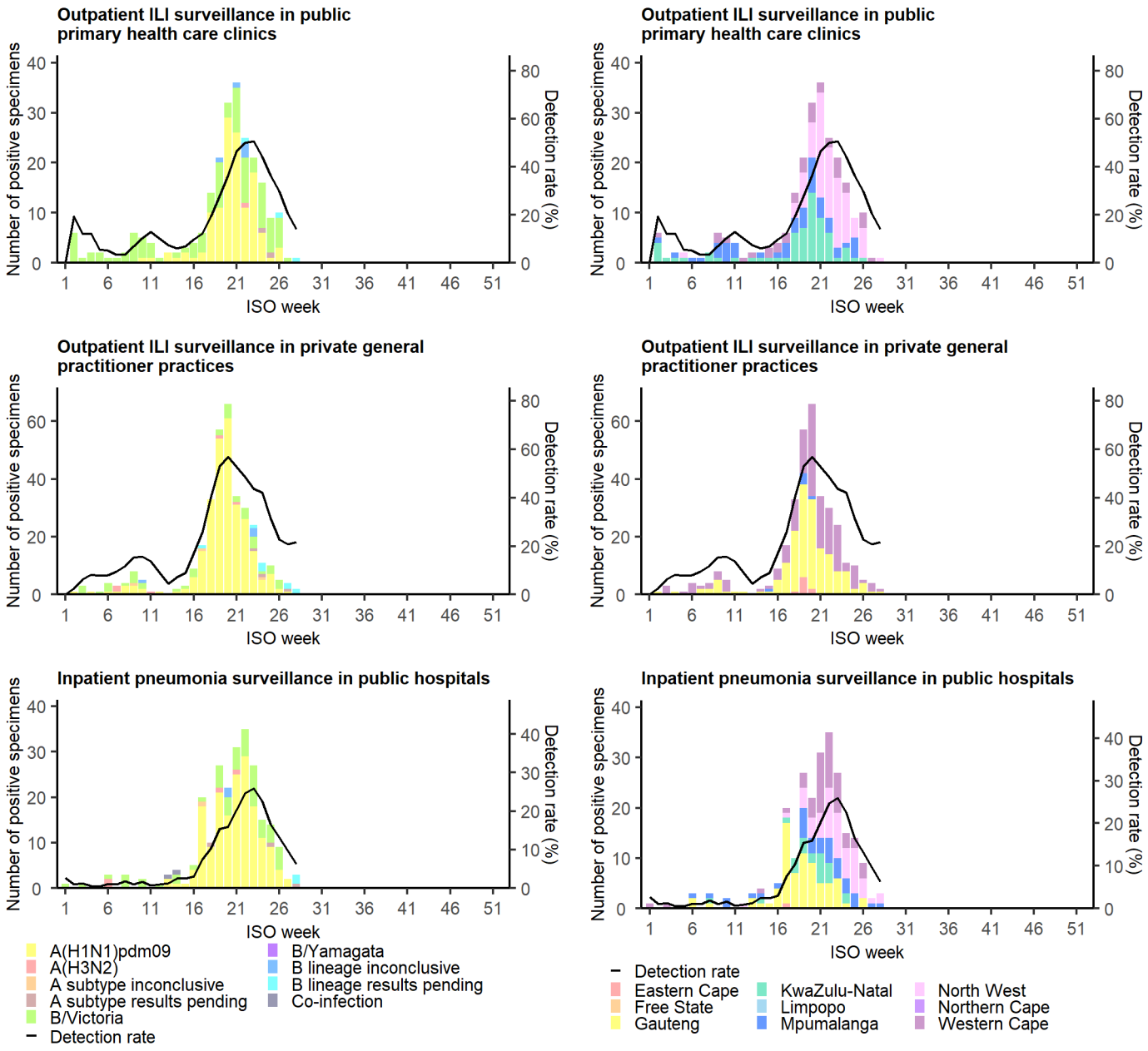
Influenza



ILI - influenza like illness, ISO - International Organization for Standardization

Detection rate presented as three-week moving average

Enrolment ended on the 15th of March 2024 for the following sites: Tambo Memorial Hospital, Tembisa Hospital and Tygerberg Hospital; and on 31 May 2024 for Livingstone Hospital

Figure 2: Number of laboratory-confirmed influenza cases and detection rate by subtype and lineage (left) and province (right) in all ages, sentinel surveillance, South Africa, 01 January 2024 to 14 July 2024.

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 1: 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, 01 January 2024 to 14 July 2024.

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)	46	0	0	0	18	0	0	0	0	66	303
Agincourt (MP)	17	1	0	1	27	0	1	0	0	49	150
Jouberton (NW)	41	0	0	1	40	0	4	2	0	88	385
Eastridge (WC)	17	0	0	0	13	0	0	1	0	31	157
Mitchell's Plain (WC)	4	0	0	0	0	0	0	0	0	4	92
Total	125	1	0	2	98	0	5	3	0	238	1087

Specimens where more than one influenza subtype or lineage was detected denoted as co-infection, and included in the counts for each separate type as well. Enrolment ended on the 15th of March 2024 for the following sites: Tambo Memorial Hospital, Tembisa Hospital and Tygerberg Hospital; and on 31 May 2024 for Livingstone Hospital.

Table 2: 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, 01 January 2024 to 14 July 2024.

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	8	1	0	0	0	0	0	0	0	9	15
Free State	0	0	0	0	0	0	0	0	0	0	0
Gauteng	152	3	3	2	8	0	0	2	0	170	707
Limpopo	0	0	0	0	0	0	0	0	0	0	0
Mpumalanga	6	0	0	0	0	0	0	0	0	6	10
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	2
Western Cape	106	1	0	1	35	0	4	7	0	155	339
Total	272	5	3	3	43	0	4	9	0	340	1073

Specimens where more than one influenza subtype or lineage was detected denoted as co-infection, and included in the counts for each separate type as well. Enrolment ended on the 15th of March 2024 for the following sites: Tambo Memorial Hospital, Tembisa Hospital and Tygerberg Hospital; and on 31 May 2024 for Livingstone Hospital.

Table 3: 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, 01 January 2024 to 14 July 2024.

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
Livingstone (EC)	1	0	0	0	0	0	0	0	0	1	41
Helen Joseph-Rahima Moosa (GP)	65	2	0	1	3	0	0	0	0	71	607
Tambo Memorial (GP)	0	0	0	0	0	0	0	0	0	0	28
Tembisa (GP)	2	0	0	0	0	0	0	0	0	2	113
Harry Gwala (KZ)	20	0	0	0	3	0	0	0	0	23	292
Mapulaneng-Matikwana (MP)	12	1	0	0	8	0	1	1	1	22	263
Tintswalo (MP)	8	0	0	0	6	0	0	0	0	14	93
Klerksdorp-Tshepong (NW)	37	0	1	1	15	0	1	1	0	56	325
Khayelitsha (WC)	0	0	0	0	0	0	0	0	0	0	106
Mitchell's Plain (WC)	10	1	0	0	5	0	0	0	0	16	169
Red Cross (WC)	17	0	0	1	16	0	1	0	1	35	714
Tygerberg (WC)	0	0	0	0	0	0	0	0	0	0	0
Total	172	4	1	3	56	0	3	2	2	240	2751

Specimens where more than one influenza subtype or lineage was detected denoted as co-infection, and included in the counts for each separate type as well. Enrolment ended on the 15th of March 2024 for the following sites: Tambo Memorial Hospital, Tembisa Hospital and Tygerberg Hospital; and on 31 May 2024 for Livingstone Hospital.

Table 4: 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 + B lineage inconclusive	0	0	1
A(H1N1)pdm09 + B/Victoria	0	0	1

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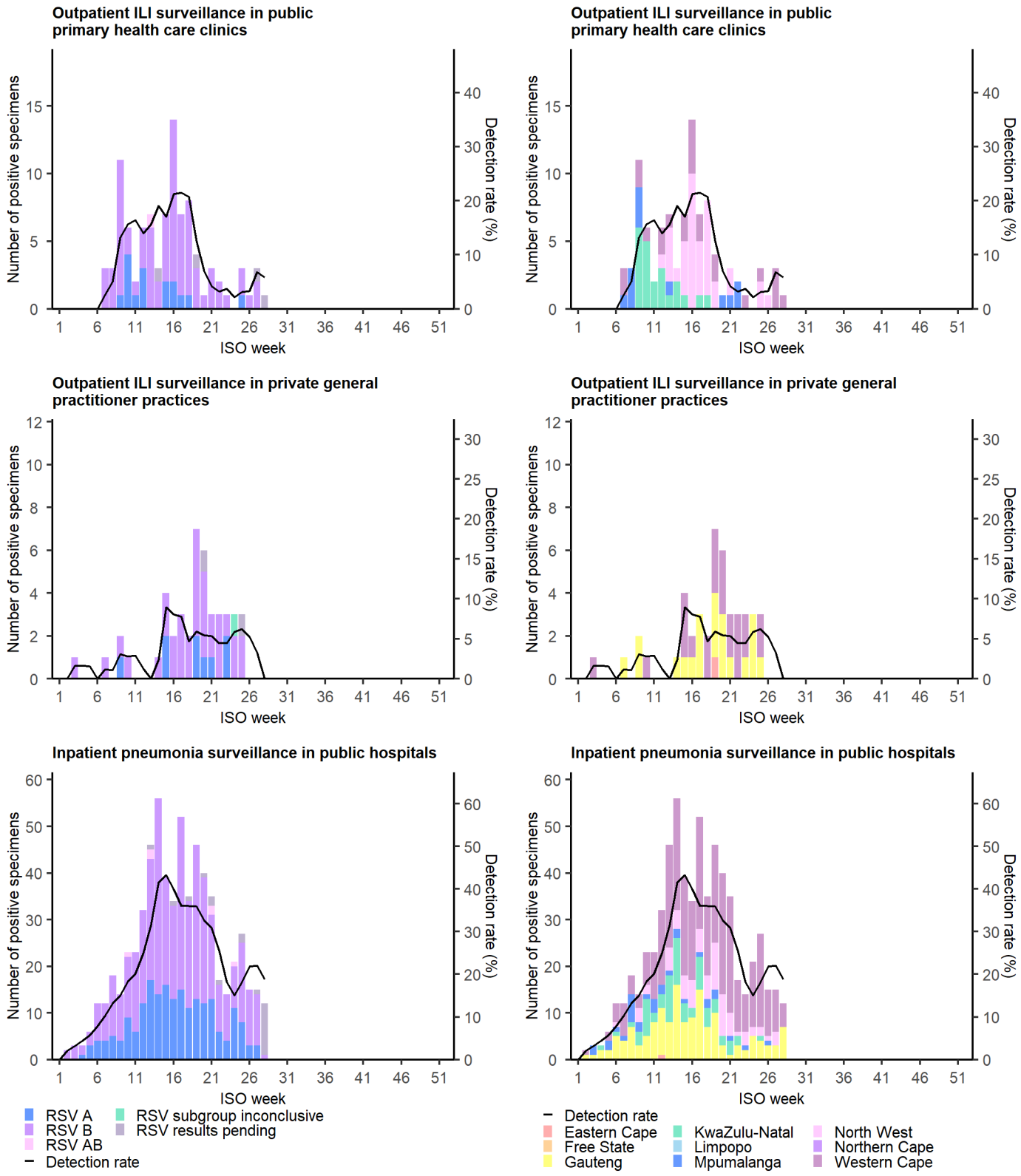
Respiratory syncytial virus (RSV)



ILI - influenza like illness, ISO - International Organization for Standardization

Detection rate presented as three-week moving average

Enrolment ended on the 15th of March 2024 for the following sites: Tambo Memorial Hospital, Tembisa Hospital and Tygerberg Hospital; and on 31 May 2024 for Livingstone Hospital

Figure 3: 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, 01 January 2024 to 14 July 2024.

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 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, 01 January 2024 to 14 July 2024.

Clinic (Province)	RSV A	RSV B	RSV AB	RSV subgroup inconclusive	RSV results pending	Total RSV	Total specimens
Edendale Gateway (KZ)	9	12	0	0	1	22	303
Agincourt (MP)	0	12	0	0	0	12	150
Jouberton (NW)	0	38	0	0	0	38	385
Eastridge (WC)	7	12	1	0	3	23	157
Mitchell's Plain (WC)	0	1	0	0	0	1	92
Total	16	75	1	0	4	96	1087

Enrolment ended on the 15th of March 2024 for the following sites: Tambo Memorial Hospital, Tembisa Hospital and Tygerberg Hospital; and on 31 May 2024 for Livingstone Hospital.

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, 01 January 2024 to 14 July 2024.

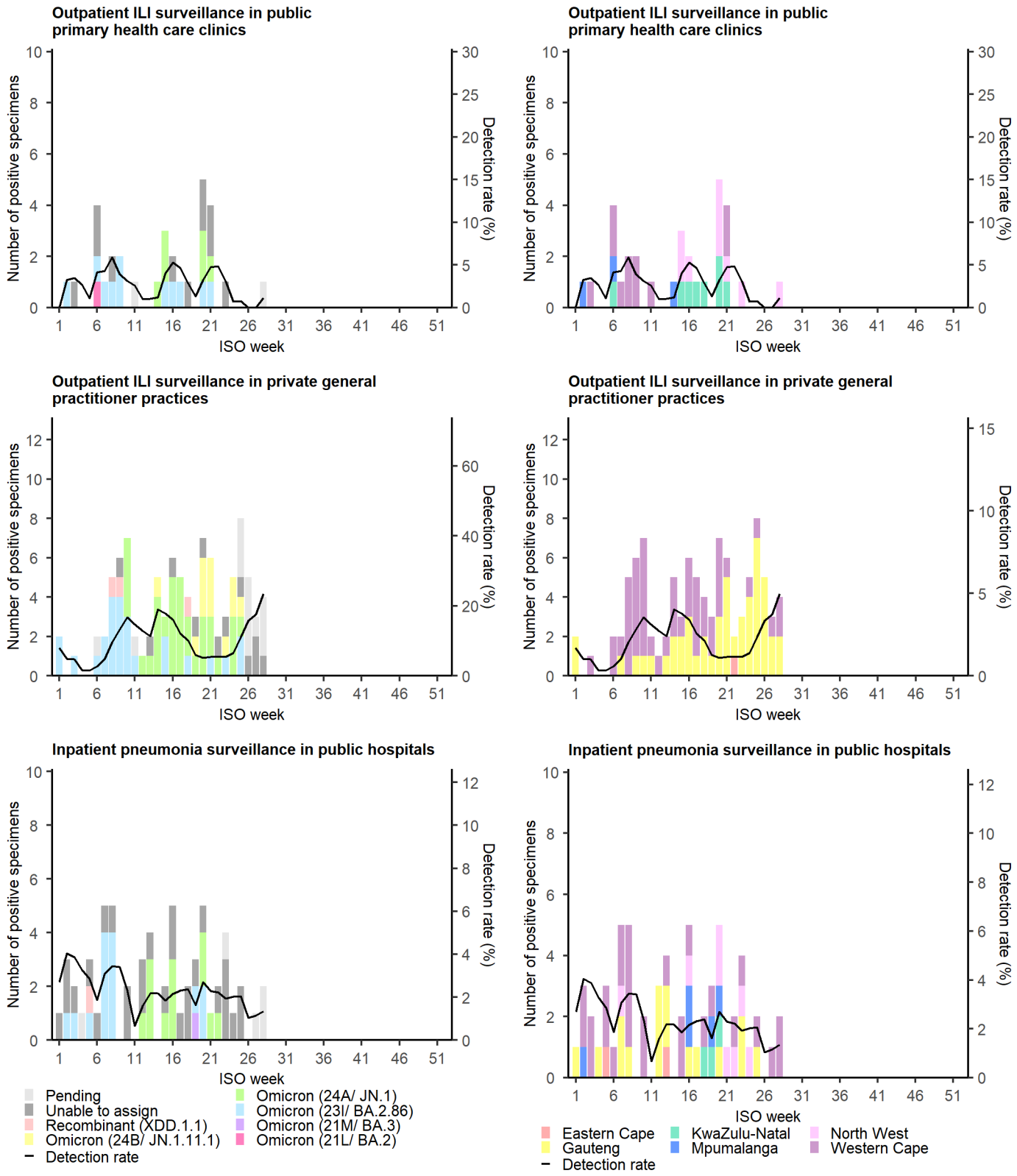
Province	RSV A	RSV B	RSV AB	RSV subgroup inconclusive	RSV results pending	Total RSV	Total specimens
Eastern Cape	0	1	0	0	0	1	15
Free State	0	0	0	0	0	0	0
Gauteng	1	18	0	1	1	21	707
Limpopo	0	0	0	0	0	0	0
Mpumalanga	0	0	0	0	0	0	10
North West	0	0	0	0	0	0	0
Northern Cape	0	0	0	0	0	0	2
Western Cape	8	14	0	0	1	23	339
Total	9	33	0	1	2	45	1073

Enrolment ended on the 15th of March 2024 for the following sites: Tambo Memorial Hospital, Tembisa Hospital and Tygerberg Hospital; and on 31 May 2024 for Livingstone Hospital.

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, 01 January 2024 to 14 July 2024.

Hospital (Province)	RSV A	RSV B	RSV AB	RSV subgroup inconclusive	RSV results pending	Total RSV	Total specimens
Livingstone (EC)	0	1	0	0	0	1	41
Helen Joseph-Rahima Moosa (GP)	25	117	0	0	8	150	607
Tambo Memorial (GP)	0	0	0	0	0	0	28
Tembisa (GP)	0	2	0	0	0	2	113
Harry Gwala (KZ)	40	25	1	0	1	67	292
Mapulaneng-Matikwana (MP)	1	22	0	0	0	23	263
Tintswalo (MP)	0	9	0	0	0	9	93
Klerksdorp-Tshepong (NW)	9	61	0	0	2	72	325
Khayelitsha (WC)	2	3	0	0	0	5	106
Mitchell's Plain (WC)	19	18	1	0	1	39	169
Red Cross (WC)	111	160	4	0	9	284	714
Tygerberg (WC)	0	0	0	0	0	0	0
Total	207	418	6	0	21	652	2751

Enrolment ended on the 15th of March 2024 for the following sites: Tambo Memorial Hospital, Tembisa Hospital and Tygerberg Hospital; and on 31 May 2024 for Livingstone Hospital.



ILI - influenza like illness, ISO - International Organization for Standardization

Detection rate presented as three-week moving average

Enrolment ended on the 15th of March 2024 for the following sites: Tambo Memorial Hospital, Tembisa Hospital and Tygerberg Hospital; and on 31 May 2024 for Livingstone Hospital

Figure 4: 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, 01 January 2024 to 14 July 2024.

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Table 8: Number of laboratory-confirmed SARS-CoV-2 cases by variant type and total number of samples tested by clinic and province in all ages, outpatient ILI surveillance in public primary health care clinics, South Africa, 01 January 2024 to 14 July 2024.

Clinic (Province)	Omicron (21L/ BA.2)	Omicron (21M/ BA.3)	Omicron (23I/ BA.2.86)	Omicron (24A/ JN.1)	Omicron (24B/ JN.1.11.1)	Recombinant (XDD.1.1)	Pending	Unable to assign	Total SARS-CoV-2	Total specimens
Edendale Gateway (KZ)	1	0	5	0	0	0	0	2	8	303
Agincourt (MP)	0	0	1	1	0	0	0	1	3	150
Jouberton (NW)	0	0	0	4	0	0	1	4	9	385
Eastridge (WC)	0	0	2	1	0	0	0	2	5	157
Mitchell's Plain (WC)	0	0	3	0	0	0	1	2	6	92
Total	1	0	11	6	0	0	2	11	31	1087

Enrolment ended on the 15th of March 2024 for the following sites: Tambo Memorial Hospital, Tembisa Hospital and Tygerberg Hospital; and on 31 May 2024 for Livingstone Hospital.

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, 01 January 2024 to 14 July 2024.

Province	Omicron (21L/ BA.2)	Omicron (21M/ BA.3)	Omicron (23I/ BA.2.86)	Omicron (24A/ JN.1)	Omicron (24B/ JN.1.11.1)	Recombinant (XDD.1.1)	Pending	Unable to assign	Total SARS-CoV-2	Total specimens
Eastern Cape	0	0	0	1	0	0	0	0	1	15
Free State	0	0	0	0	0	0	0	0	0	0
Gauteng	0	0	12	15	6	0	9	8	50	707
Limpopo	0	0	0	0	0	0	0	0	0	0
Mpumalanga	0	0	0	0	0	0	0	0	0	10
North West	0	0	0	0	0	0	0	0	0	0
Northern Cape	0	0	0	0	0	0	0	0	0	2
Western Cape	0	0	13	18	6	3	4	4	48	339
Total	0	0	25	34	12	3	13	12	99	1073

Enrolment ended on the 15th of March 2024 for the following sites: Tambo Memorial Hospital, Tembisa Hospital and Tygerberg Hospital; and on 31 May 2024 for Livingstone Hospital.

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, 01 January 2024 to 14 July 2024.

Hospital (Province)	Omicron (21L/ BA.2)	Omicron (21M/ BA.3)	Omicron (23I/ BA.2.86)	Omicron (24A/ JN.1)	Omicron (24B/ JN.1.11.1)	Recombinant (XDD.1.1)	Pending	Unable to assign	Total SARS-CoV-2	Total specimens
Livingstone (EC)	0	0	0	0	0	0	0	2	2	41
Helen Joseph-Rahima Moosa (GP)	0	0	2	3	0	0	1	7	13	607
Tambo Memorial (GP)	0	0	1	0	0	0	0	1	2	28
Tembisa (GP)	0	0	0	1	0	0	0	0	1	113
Harry Gwala (KZ)	0	0	2	0	0	0	0	1	3	292
Mapulaneng-Matikwana (MP)	0	1	1	2	0	0	0	1	5	263
Tintswalo (MP)	0	0	0	0	0	0	0	0	0	93
Klerksdorp-Tshepong (NW)	0	0	0	4	0	0	1	3	8	325
Khayelitsha (WC)	0	0	2	1	0	0	0	3	6	106
Mitchell's Plain (WC)	0	0	1	0	0	0	2	3	6	169
Red Cross (WC)	0	0	5	1	0	1	1	7	15	714
Tygerberg (WC)	0	0	0	0	0	0	0	0	0	0
Total	0	1	14	12	0	1	5	28	61	2751

Enrolment ended on the 15th of March 2024 for the following sites: Tambo Memorial Hospital, Tembisa Hospital and Tygerberg Hospital; and on 31 May 2024 for Livingstone Hospital.

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Bordetella pertussis

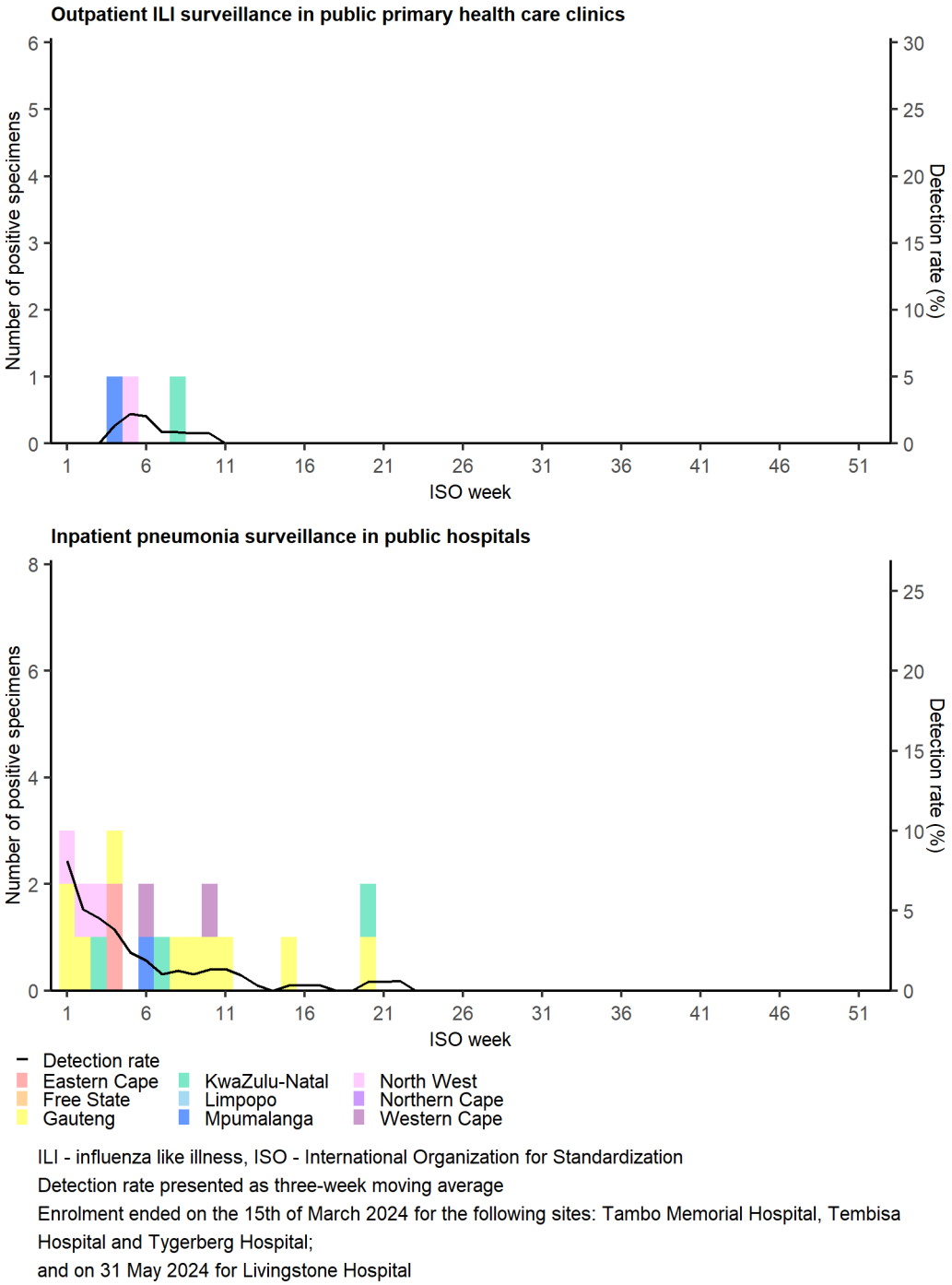

Figure 5: Number of laboratory-confirmed *Bordetella pertussis* cases and detection rate by province in all ages, sentinel surveillance, South Africa, 01 January 2024 to 14 July 2024.

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, 01 January 2024 to 14 July 2024.

Province	Positive	Pending testing	Total specimens
KwaZulu-Natal	1	23	303
Mpumalanga	1	12	150
North West	1	38	385
Western Cape	0	21	249
Total	3	94	1087

Enrolment ended on the 15th of March 2024 for the following sites: Tambo Memorial Hospital, Tembisa Hospital and Tygerberg Hospital; and on 31 May 2024 for Livingstone Hospital.

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, 01 January 2024 to 14 July 2024.

Province	Positive	Pending testing	Total specimens
Eastern Cape	2	0	41
Gauteng	10	58	748
KwaZulu-Natal	3	21	292
Mpumalanga	1	30	356
North West	3	28	325
Western Cape	2	73	989
Total	21	210	2751

Enrolment ended on the 15th of March 2024 for the following sites: Tambo Memorial Hospital, Tembisa Hospital and Tygerberg Hospital; and on 31 May 2024 for Livingstone Hospital.

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) 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) 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, *B. pertussis*.	Influenza virus, RSV, SARS-CoV-2.	Influenza virus, RSV, SARS-CoV-2, *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., J Clin Microbiol 2011) and culture.	Influenza virus, RSV, SARS-CoV-2: Allplex™ SARS-CoV-2/ FluA/FluB/RSV PCR kit.	Influenza virus, RSV, SARS-CoV-2: Allplex™ SARS-CoV-2/FluA/FluB/RSV PCR kit. *B. pertussis*: Multiplex real-time PCR (Tatti et al., J Clin Microbiol 2011) and culture.

Abbreviations and definitions:

- ILI: Influenza-like illness
- SRI: Severe respiratory infection
- EC: Eastern Cape
- FS: Free State
- GP: Gauteng
- KZ: KwaZulu-Natal
- LP: Limpopo Province
- MP: Mpumalanga
- NW: North West
- NC: Northern Cape
- WC: Western Cape
- Subtype/lineage/subgroup inconclusive: Insufficient viral load in sample and unable to characterize further
- Subtype/lineage/subgroup pending: Further characterization in progress
- Unable to assign SARS-CoV-2 lineage: No lineage assigned due to poor sequence quality OR low viral load (Ct≥35)
- Epidemic threshold: Thresholds are calculated using the Moving Epidemic Method (MEM), a sequential analysis using the R Language, available from: http://CRAN.R-project.org/web/package=mem) designed to calculate the duration, start and end of the annual influenza epidemic. MEM uses the 40th, 90th and 97.5th percentiles established from available years of historical data to calculate thresholds of activity. Thresholds of activity for influenza and RSV are defined as follows: Below seasonal threshold, low activity, moderate activity, high activity, very high activity. For influenza, thresholds from outpatient influenza like illness (ILI in primary health care clinics) are used as an indicator of disease transmission in the community and thresholds from pneumonia surveillance are used as an indicator of impact of disease. For 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 impact of disease. For RSV the start and end of the season is defined as once the three week moving average of the detection rate in children <5 years from inpatient pneumonia surveillance in public hospitals remains above or below 15% for two consecutive weeks, respectively.

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

Influenza A and B viruses, RSV and SARS-CoV-2 were tested using a commercial multiplex RT-PCR assay (Allpex SARS-CoV-2/FluA/FluB/RSV PCR kit, Seegene Inc., Seoul, South Korea). A specimen was considered positive for influenza A, B or RSV if the PCR cycle threshold (Ct) was <40 for the respective target, and considered positive for SARS-CoV-2 when the Ct was <40 for ≥1 of the S, N or RdRp gene targets. *B.pertussis* was tested using a previously described RT-PCR method (Tatti KM, et al. Journal of Clinical Microbiology. 2011;49(12):4059-4066). A specimen was considered positive when the IS481 and/or ptxS1 gene targets are detected with a Ct <45.

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

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

(Illumina, CA, USA).

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

RNA extraction: RNA was extracted either manually or automatically in batches, using the QIAamp viral RNA mini kit (QIAGEN, CA, USA) or the Chemagic 360 using the CMG-1049 kit (PerkinElmer, MA, USA). A modification was done on the manual extractions by adding 280 µl per sample, in order to increase yields. 300 µl of each sample was used for automated magnetic bead-based extraction using the Chemagic 360. RNA was eluted in 60 µl of the elution buffer. Isolated RNA was stored at -80 °C prior to use.

PCR and library preparation:

Sequencing was performed using the Illumina COVIDSeq protocol (Illumina Inc., CA, USA) or nCoV-2019 ARTIC network sequencing protocol v3 (https://artic.network/ncov-2019). These are amplicon-based next-generation sequencing approaches. Briefly, for the nCoV-2019 ARTIC network sequencing protocol, the first strand synthesis was carried out on extracted RNA samples using random hexamer primers from the SuperScript IV reverse transcriptase synthesis kit (Life Technologies, CA, USA) or LunaScript RT SuperMix Kit (New England Biolabs (NEB), MA , USA). The synthesized cDNA was amplified using multiplex polymerase chain reactions (PCRs) using ARTIC nCoV-2019 v3 primers. For the COVIDSeq protocol, the first strand synthesis was carried out using random hexamer primers from Illumina and the synthesized cDNA underwent two separate multiplex PCR reactions. For Illumina sequencing using the nCoV-2019 ARTIC network sequencing protocol, the pooled PCR products underwent bead-based tagmentation using the Nextera Flex DNA library preparation kit (Illumina Inc., CA, USA). The adapter-tagged amplicons were cleaned up using AmpureXP purification beads (Beckman Coulter, High Wycombe, UK) and amplified using one round of PCR. The PCRs were indexed using the Nextera CD indexes (Illumina Inc., CA, USA) according to the manufacturer's instructions. For COVIDSeq sequencing protocol, pooled PCR amplified products were processed for tagmentation and adapter ligation using IDT for Illumina Nextera UD Indexes. Further enrichment and clean-up was performed as per protocols provided by the manufacturer (Illumina Inc., CA, USA). Pooled samples from both COVIDSeq protocol and nCoV-2019 ARTIC network protocol were quantified using Qubit 3.0 or 4.0 fluorometer (Invitrogen Inc., MA, USA) using the Qubit dsDNA High Sensitivity assay according to manufacturer's instructions. The fragment sizes were analyzed using TapeStation 4200 (Invitrogen Inc., MA, USA). The pooled libraries were further normalized to 4nM concentration and 25 µl of each normalized pool containing unique index adapter sets were combined in a new tube. The final library pool was denatured and neutralized with 0.2 N sodium hydroxide and 200 mM Tris-HCL (pH7), respectively. 1.5 pM sample library was spiked with 2% PhiX. Libraries were loaded onto a 300-cycle NextSeq 500/550 HighOutput Kit v2 and run on the Illumina NextSeq 550 instrument (Illumina Inc., CA, USA).

Assembly, processing and quality control of genomic sequences:

Raw reads from Illumina sequencing were assembled using the Exatype NGS SARS-CoV-2 pipeline v1.6.1, (https://sars-cov-2.exatype.com/). The resulting consensus sequence was further manually polished by considering and correcting indels in homopolymer regions that break the open reading frame (probably sequencing errors) using Aliview v1.27, (http://ormbunkar.se/aliview/) (Larsson, 2014). Mutations resulting in mid-gene stop codons and frameshifts were reverted to wild type. All assemblies determined to have acceptable quality (defined as having at least 1 000 000 reads and at least 40 % 10 X coverage) were deposited on GISAID (https://www.gisaid.org/) (Elbe & Buckland-Merrett, 2017; Shu & McCauley, 2017).

Classification of lineage, clade and associated mutations:

Assembled genomes were assigned lineages using the 'Phylogenetic Assignment of Named Global Outbreak Lineages' (PANGOLIN) software suite (https://github.com/hCoV-2019/pangolin) (Rambaut et al., 2020), a tool used for dynamic SARS-CoV-2 lineage classification. The SARS-CoV-2 genomes in our dataset were also classified using the clade classification proposed by NextStrain (https://nextstrain.org/), a tool built for real-time tracking of the pathogen evolution (Hadfield et al., 2018).