Division of the National Health Laboratory Service

Weekly respiratory pathogens report Week 19 of 2023

<u>Highlights</u>

- The influenza season has started based on influenza detection exceeding the low threshold for two consecutive weeks in the pneumonia surveillance programme. The number and detection rate of influenza cases is increasing across all surveillance programmes.
- In 2023 to date, 179 influenza cases have been detected from all surveillance programmes, of which 53% (94/179) of those with typing information available were influenza A(H1N1)pdm09. The majority of cases were reported from Gauteng (n=81), followed by Western Cape (n=45), KwaZulu-Natal (n=20), Eastern Cape (n=17), North West (n=10), and Mpumalanga (n=6) sentinel surveillance sites.
- In 2023 to date, 593 respiratory syncytial virus (RSV) cases have been detected from all surveillance programmes. The RSV season started in week 6 when the detection rate (3-week moving average) among children aged <5 years in pneumonia surveillance crossed and remains above the seasonal threshold. Transmission levels among children aged < 5 years in pneumonia surveillance reached high level of activity in week 13 and currently, in week 19, are at low level.
- In 2023 to date, 104 cases of *Bordetella pertussis* were detected, of which, 21% (22/104) were from the Gauteng Province, 20% (21/104) from North West Province, 19% (20/104) were from Western Cape Province, 18% (19/104) from Mpumalanga Province, 16% (17/104) from KwaZulu-Natal Province and 5% (5/104) from Eastern Cape Province.
- In 2023 to date, 196 COVID-19 cases were detected from all surveillance programmes. Of the 111 specimens sequenced, variant could be assigned in 50% (55/111). Of these, 96% (53/55) was assigned Omicron variant, of which 47% (25/53) were Omicron (23A/XBB.1.5), 21% (11/53) were Omicron (22E/BQ.1.1), 17% (9/53) Omicron (22B/BA.5), 11% (6/53) Omicron (22F/BA.2.10.1), and 1% (1/53) each Omicron (21K/BA.1) and Omicron(22D/BM.1.1). One (1%, 1/106) was assigned XAY and XBL each, while for the remaining 50% (56/111), a variant could not be assigned due to a low viral load or insufficient sample.

Programme Descriptions

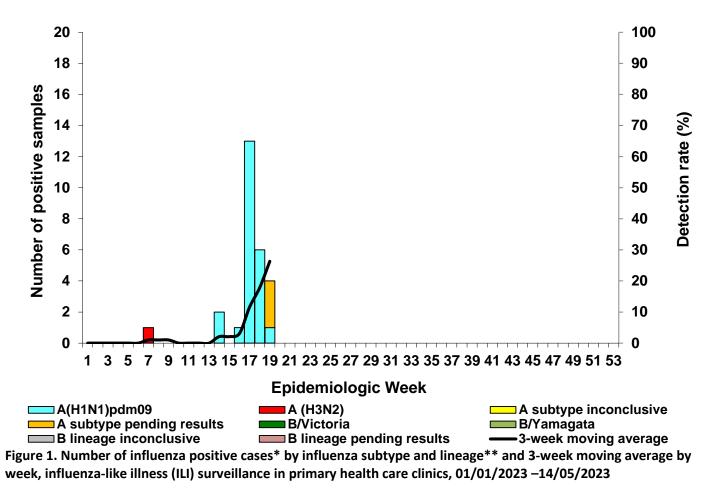
Programme	Influenza-like illness (ILI)	Viral Watch	National Syndromic Surveillance for Pneumonia
Start year	2012	1984	2009
Provinces*	KZ	EC	EC
	NW	FS	GP
	WC	GP	KZ
	MP	LP	MP
		MP	NW
		NC	WC
		NW	
		WC	
Type of site	Primary health care clinics	General practitioners	Public hospitals
Case definition	ILI: An acute respiratory illness with a	ILI: An acute respiratory illness with a	SRI : Acute (symptom onset≤10 days) or
cuse demition	temperature (\geq 38°C) and cough, & onset	temperature (\geq 38°C) and cough, & onset	chronic (symptom onset >10) lower
	≤10 days	≤10 days	respiratory tract illness
	510 days	510 days	respiratory tract miless
	Suspected pertussis		Suspected pertussis
	Any person with an acute cough illness		Any person with an acute cough illness
	lasting \geq 14 days (or cough illness of any		lasting ≥ 14 days (or cough illness of any
	duration for children <1 year), without a		duration for children <1 year), without a
	more likely diagnosis AND one or more of		more likely diagnosis AND one or more o
	the following signs or symptoms:		the following signs or symptoms:
	 paroxysms of coughing, 		 paroxysms of coughing,
	 or inspiratory "whoop", 		 or inspiratory "whoop",
	 or post-tussive vomiting 		 or post-tussive vomiting
	 or apnoea in children <1 year; OR 		 or apnoea in children <1 year; OR
	Any person in whom a clinician suspects pertussis		Any person in whom a clinician suspects pertussis.
	Suspected SARS-CoV-2		Suspected SARS-CoV-2
	Any person presenting with an acute	Suspected SARS-CoV-2	Any person admitted with a physician-
	(≤14 days) respiratory tract infection or	Any person presenting with an acute	diagnosis of suspected COVID-19 and
	other clinical illness compatible with	(≤14 days) respiratory tract infection or	not meeting SRI case definition.
	COVID-19**	other clinical illness compatible with	not meeting ski case definition.
		COVID-19**	
Specimens collected	Oropharyngeal & nasopharyngeal swabs	Throat and/or nasal swabs or Nasopharyngeal swabs	Oropharyngeal & nasopharyngeal swabs
Main pathogens	INF	INF	INF
tested***	RSV	RSV	RSV
	BP	SARS-CoV-2	BP
	SARS-CoV-2		SARS-CoV-2
Testing Methods	INF and RSV	INF and RSV	INF and RSV
•	- Fast-Track Diagnostics multiplex real-	- Fast-Track Diagnostics multiplex real-	- Fast Track Diagnostics multiplex real-
	time reverse transcription polymerase	time reverse transcription polymerase	time reverse transcription polymerase
	chain reaction (until 31 March 2021)	chain reaction (until 31 March 2021)	chain reaction (until 31 March 2021)
	B. pertussis	B. pertussis	B. pertussis
	Multiplex real-time PCR (Tatti <i>et al., J Clin</i>	Multiplex real-time PCR (Tatti <i>et al., J Clin</i>	Multiplex real-time PCR (Tatti <i>et al., J Clir</i>
	Microbiol 2011) and culture (if PCR cycle	Microbiol 2011) and culture (if PCR cycle	Microbiol 2011) and culture (if PCR cycle
	threshold ≤25)	threshold <25)	threshold <25)
	SARS-CoV-2	SARS-CoV-2	SARS-CoV-2
	1 April 2020 – 31 March 2021: Roche E		
	-	1 April 2020 – 31 March 2021: Roche E	1 April 2020 – 31 March 2021: Roche E
	gene real-time PCR essay (Corman <i>et al.,</i>	gene real-time PCR essay Corman <i>et al.,</i>	gene real-time PCR essay (Corman <i>et al.,</i>
	Euro Surv 2020)	Euro Surv 2020)	Euro Surv 2020)
	1 April 2021 to date: Allplex [™] SARS-CoV-	1 April 2021 to date: Allplex [™] SARS-CoV-	1 April 2021 to date: Allplex [™] SARS-CoV-
	2/FluA/FluB/RSV PCR kit	2/FluA/FluB/RSV PCR kit	2/FluA/FluB/RSV PCR kit
	 positivity assigned if PCR cycle 	 positivity assigned if PCR cycle 	 positivity assigned if PCR cycle
	threshold is <40 for ≥1 gene targets	threshold is <40 for ≥1 gene targets	threshold is <40 for ≥1 gene targets

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 RSV, thresholds from pneumonia surveillance, using data from children aged < 5 years are used to define the start and end of the season.

* EC: Eastern Cape; FS: Free State; GP: Gauteng; KZ: KwaZulu-Natal; LP: Limpopo; MP: Mpumalanga: NC: Northern Cape; NW: North West; WC: Western Cape **Symptoms include ANV of the following respiratory symptoms: cough, sore throat, shortness of breath, anosmia (loss of sense of smell) or dysgeusia (alteration of the sense of taste), with or without other symptoms (which may include fever, weakness, myalgia, or diarrhoea). Testing for SARS-CoV-2 was initiated in all three surveillance programmes in week 10 of 2020 (week starting 2 March 2020).***INF: influenza virus; RSV: respiratory syncytial virus; BP: Bordetella pertusis; SARS-CoV-2: severe acute respiratory syndrome coronavirus 2

Page 2 of 20



*Specimens from patients with influenza-like illnesses at 5 sentinel sites in 4 provinces ** Influenza was detected in 1 of 6 specimens from patients who met suspected SARS-CoV-2 or *B. pertussis* case definition but did not meet influenza-like illness (ILI) case definition.

Inconclusive: insufficient viral load in sample and unable to characterise further

Table 1. Number of laboratory-confirmed influenza* cases by subtype and lineage and total number of samples tested by clinic and province, influenza-like illness (ILI) surveillance in primary health care clinics, 01/01/2023 – 14/05/2023

Clinic (Province)	A(H1N1) pdm09	A(H3N2)	A subtype in- conclusive* *	A subtype pending results** *	B/ Victoria	B/ Yamagat a	B lineag e in- conclu sive*	B lineage pending results* **	Total sample s
Agincourt (MP)	2	0	0	0	0	0	0	0	85
Eastridge (WC)	6	1	0	0	0	0	0	0	129
Edendale Gateway (KZ)	14	0	0	0	0	0	0	0	194
Jouberton (NW)	1	0	0	3	0	0	0	0	106
Mitchell's Plain (WC)	0	0	0	0	0	0	0	0	56
Total:	23	1	0	3	0	0	0	0	570

KZ: KwaZulu-Natal; NW: North West; WC: Western Cape; MP: Mpumalanga

* Influenza was detected in 1 of 6 specimens from patients who met suspected SARS-CoV-2 or *B. pertussis* case definition but did not meet influenza-like illness (ILI) case definition.

**Inconclusive: insufficient viral load in sample and unable to characterise further

***Influenza A subtype or B lineage results are pending

Page **3** of **20**

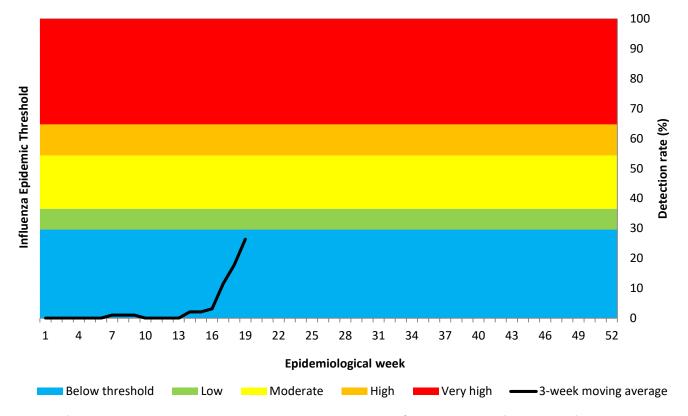


Figure 2. Influenza percentage detections and epidemic thresholds* among cases of all ages, influenza-like illness (ILI) surveillance in primary health care clinics, 01/01/2023 –14/05/2023 *Thresholds based on 2012-2019 data

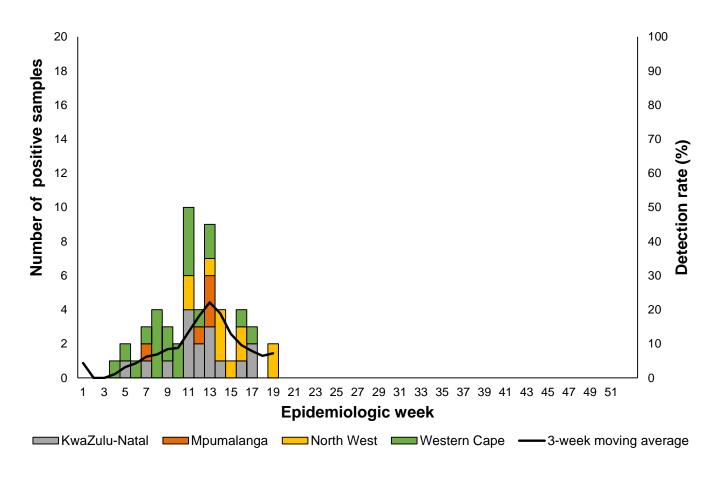


Figure 3. Number of patients testing positive for respiratory syncytial virus* by province and 3-week moving average by week, influenza-like illness (ILI) surveillance in primary health care clinics, 01/01/2023 –14/05/2023

*RSV was detected from 1 of 6 specimens of patients who met suspected SARS-CoV-2 or *B. pertussis* case definition but did not meet influenza-like illness (ILI) case definition.

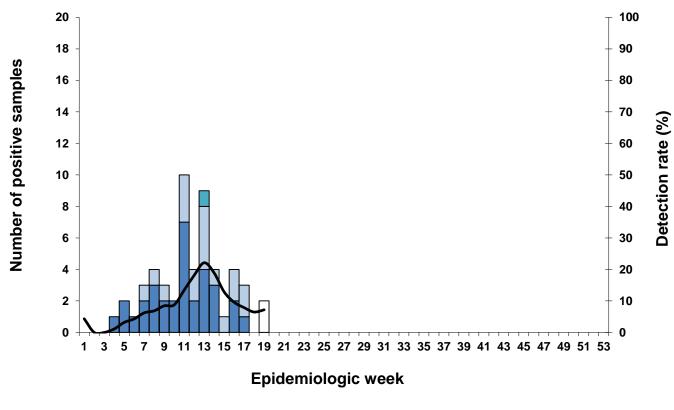


Figure 4. Number of patients testing positive for respiratory syncytial virus* by subgroup and 3-week moving average by week, influenza-like illness (ILI) surveillance in primary health care clinics, 01/01/2023 –14/05/2023

🗆 RSVA 📖 RSVB 📖 RSVAB 🛲 RSV subgroup inconclusive 📖 RSV subgroup pending — 3-week moving average

*RSV was detected in one of six specimens of patients who met suspected SARS-CoV-2 or *B. pertussis* case definition but did not meet influenza-like illness (ILI) case definition.

RSV AB: Both RSV A and B subgroups identified.

Inconclusive: insufficient viral load in sample and unable to characterise further

Table 2. Number of patients testing positive for respiratory syncytial virus (RSV)* by subgroups identified and total number of samples tested by clinic and province, influenza-like illness (ILI) surveillance in primary health care clinics, 01/01/2023 –14/05/2023

Clinic (Province)	RSVA	RSVB	RSVAB**	RSV subgroup inconclusive* **	RSV subgroup pending** **	Total samples
Agincourt (MP)	2	2	1	0	0	85
Eastridge (WC)	18	2	0	0	0	129
Edendale Gateway (KZ)	6	10	0	0	0	194
Jouberton (NW)	5	4	0	0	2	106
Mitchell's Plain (WC)	1	0	0	0	0	56
Total	32	18	1	0	2	570

KZ: KwaZulu-Natal; NW: North West; WC: Western Cape; MP: Mpumalanga

* RSV was detected in one of six specimens of patients who met suspected SARS-CoV-2 or *B. pertussis* case definition but did not meet influenza-like illness (ILI) case definition.

**RSV AB: Both RSV A and B subgroups identified

***Inconclusive: insufficient viral load in sample and unable to characterise further

****RSV results for subgroups are pending

Page 5 of 20

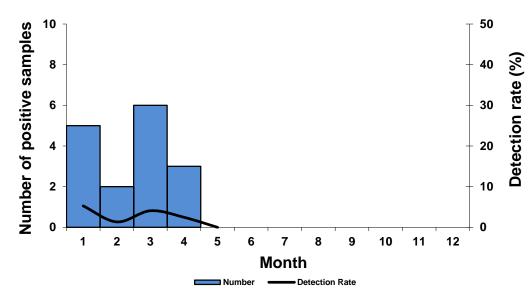


Figure 5. Number of patients testing positive for *B. pertussis** and detection rate by month, influenza-like illness (ILI) surveillance primary health care clinics**, 01/01/2023 –14/05/2023

**B. pertussis* was detected in 1 of 6 specimens of patients who met the suspected SARS-CoV-2 or *B. pertussis* case definition but did not meet influenza-like illness (ILI) case definition. These are not included in the epidemiological curve.

** Specimens from patients with influenza-like illnesses at 5 sentinel sites in 4 provinces

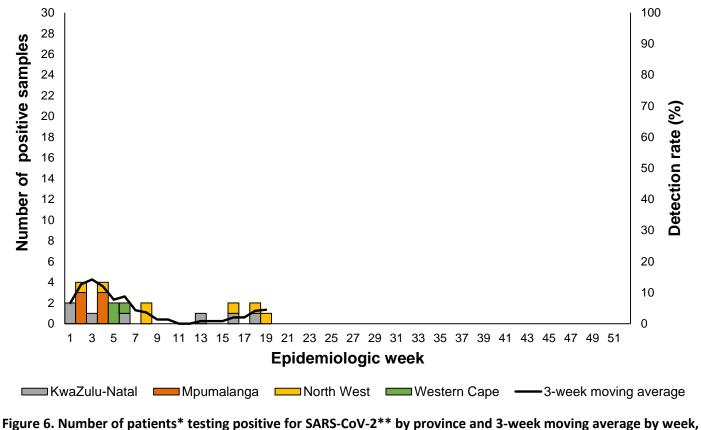
Table 3. Number of patients testing positive for *B. pertussis** identified and total number of samples tested by province, influenza-like illness (ILI) surveillance primary health care clinics, 01/01/2023 –14/05/2023

Clinic (Province)	<i>B. pertussis</i> Positive	Total samples
Agincourt (MP)	4	85
Eastridge (WC)	2	124
Edendale Gateway (KZ)	4	186
Jouberton (NW)	6	94
Mitchell's Plain (WC)	0	55
Total:	16	544

KZ: KwaZulu-Natal; NW: North West; WC: Western Cape; MP: Mpumalanga

*B. pertussis was detected in 1 of 6 specimens of patients who met the suspected SARS-CoV-2 or B. pertussis case definition but did not meet influenza-like illness (ILI) case definition. These are not included in the epidemiological curve.

NB: Results pending for 13 samples.



influenza-like illness (ILI) surveillance in primary health care clinics, 01/01/2023 –14/05/2023

*Specimens from patients with influenza-like illnesses at 5 sentinel sites in 4 provinces **SARS-CoV-2 was not detected in 7 specimens from patients who met suspected SARS-CoV-2 or *B. pertussis* case definition but did not meet influenza-like illness (ILI) case definition. These are not included in the epidemiological curve.

Table 4. Number of patients positive for SARS-CoV-2* identified and total number of samples tested by clinic and province, influenza-like illness (ILI) surveillance primary health care clinics, 01/01/2023 –14/05/2023

Clinic (Province)	SARS-CoV-2 positive	Total samples tested		
Agincourt (MP)	6	85		
Eastridge (WC)	1	129		
Edendale Gateway (KZ)	7	194		
Jouberton (NW)	7	106		
Mitchell's Plain (WC)	2	56		
Total:	23	570		

KZ: KwaZulu-Natal; NW: North West; WCP: Western Cape; MP: Mpumalanga

*SARS-CoV-2 was not detected in 7 specimens from patients who met suspected SARS-CoV-2 or *B. pertussis* case definition but did not meet influenza-like illness (ILI) case definition. These are not included in the epidemiological curve.

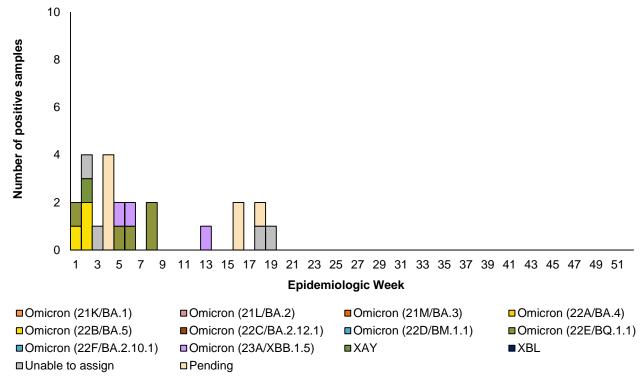


Figure 7. Number of laboratory-confirmed SARS-CoV-2* cases by variant type (variant PCR/sequencing) and week, influenza-like illness (ILI) surveillance in primary health care clinics, 01/01/2023-14/05/2023

*Specimens are from patients with influenza-like illness at 5 sentinel sites in 4 provinces who met influenza-like illness (ILI), suspected SARS-CoV-2 or *B. pertussis* case definition

Unable to assign: no lineage assigned due to poor- sequence quality OR low viral load (C₁≥35) OR variant PCR could not assign variant and no sequencing result Pending: outstanding variant results

Table 5. Number of cases positive for SARS-CoV-2* by variant** (variant PCR and/or sequencing) identified
and total number of samples tested by clinic and province, influenza-like illness (ILI) surveillance primary
health care clinics, 01/01/2023-14/05/2023

Province	Omicron (21L/BA.2)	Omicron (21M/ BA.3)	Omicron (22A/BA.4)	Omicron (22B/ BA.5)	Omicron (22C/BA.2.1 2.1)	Omicron (22D/BM.1.1)	Omicron (22E/BQ.1.1)	Omicron (22F/BA.2.1 0.1)	Omicron (23A/XBB.1. 5)	ХАҮ	Unable to assign**	Pending***	SARS-CoV-2 positive	Total samples tested
Agincourt	0	0	0	2	0	0	0	0	0	1	0	3	6	85
Clinic (MP)														
Eastridge	0	0	0	0	0	0	0	0	1	0	0	0	1	129
Clinic (WC)														
Edendale	0	0	0	1	0	0	2	0	1	0	1	2	7	194
Clinic (KZ)														
Jouberton	0	0	0	0	0	0	2	0	0	0	3	2	7	106
Clinic (NW)														
Mitchell's	0	0	0	0	0	0	1	0	1	0	0	0	2	56
Plain Clinic														
(WC)														
Total:	0	0	0	3	0	0	5	0	3	1	4	7	23	570

KZ: KwaZulu-Natal; NW: North West; WCP: Western Cape; MP: Mpumalanga

*Specimens are from patients with influenza-like illness at 5 sentinel sites in 4 provinces who met influenza-like illness (ILI), suspected SARS-CoV-2 or *B. pertussis* case definition

Unable to assign: no lineage assigned due to poor- sequence quality **OR** low viral load ($C_t \ge 35$) **OR** variant PCR could not assign variant and no sequencing result **Pending**: outstanding variant results

Page **8** of **20**

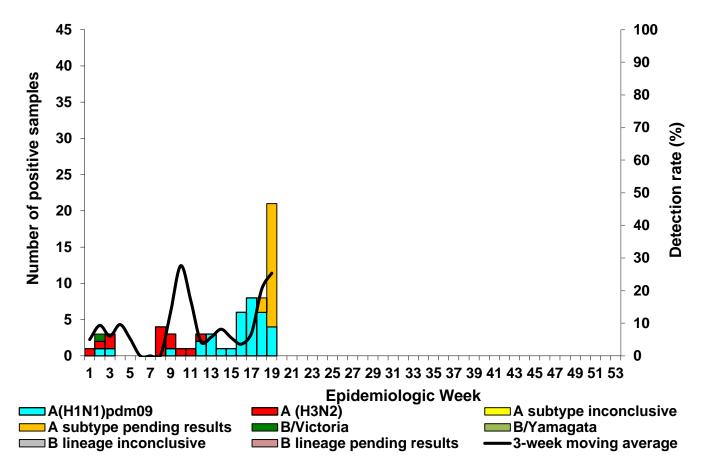


Figure 8. Number of positive patients* by influenza subtype and lineage and 3-week moving average by week, ILI surveillance - Viral Watch, 01/01/2023-14/05/2023

*Specimens from patients with influenza-like illnesses at 92 sentinel sites in 8 provinces Inconclusive: insufficient viral load in sample and unable to characterise further

Table 6. Number of laboratory-confirmed influenza cases by influenza subtype and lineage and total number of samples tested by province, ILI surveillance - Viral Watch, 01/01/2023-14/05/2023

Province	A(H1N1) pdm09	A(H3N2)	A subtype inconclusiv e	A subtype pending results*	B/Victor ia	B/Yamag ata	B lineage inconclus ive	B lineage pending results*	Total samples
Eastern Cape	4	0	0	0	0	0	0	0	13
Free State	0	0	0	0	0	0	0	0	0
Gauteng	18	8	0	7	1	0	0	0	290
Limpopo	0	0	0	0	0	0	0	0	0
Mpumalanga	0	0	0	3	0	0	0	0	9
North West	1	0	0	1	0	0	0	0	2
Northern Cape	0	0	0	0	0	0	0	0	0
Western Cape	11	5	0	8	0	0	0	0	69
Total:	34	13	0	19	1	0	0	0	383

*Inconclusive: insufficient viral load in sample and unable to characterise further **Influenza A subtype or B lineage results are pending

Page **9** of **20**

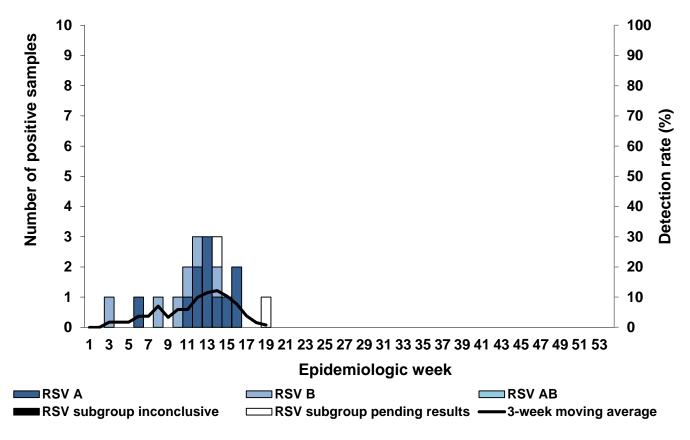


Figure 9. Number of RSV positive cases testing positive for respiratory syncytial virus (RSV)* by subgroup and 3week moving average by week, ILI surveillance - Viral Watch, 01/01/2023-14/05/2023 *Specimens from patients with Influenza-like illnesses at 92 sentinel sites in 8 provinces

Table 7. Number of RSV positive cases identified and total number of samples tested by province, ILIsurveillance - Viral Watch, 01/01/2023-14/05/2023

Province	RSV A	RSV B	RSV AB*	RSV subgroup inconclusive **	RSV subgroup pending results***	Total samples tested
Eastern Cape	0	1	0	0	0	13
Free State	0	0	0	0	0	0
Gauteng	9	4	0	0	1	290
Limpopo	0	0	0	0	0	0
Mpumalanga	0	0	0	0	0	9
North West	0	0	0	0	0	2
Northern Cape	0	0	0	0	0	0
Western Cape	2	1	0	0	1	69
Total:	11	6	0	0	2	383

*RSV AB: Both RSV A and B subgroup identified

**Inconclusive: insufficient viral load in sample and unable to characterise further

***RSV results for subgroups are pending

Page 10 of 20

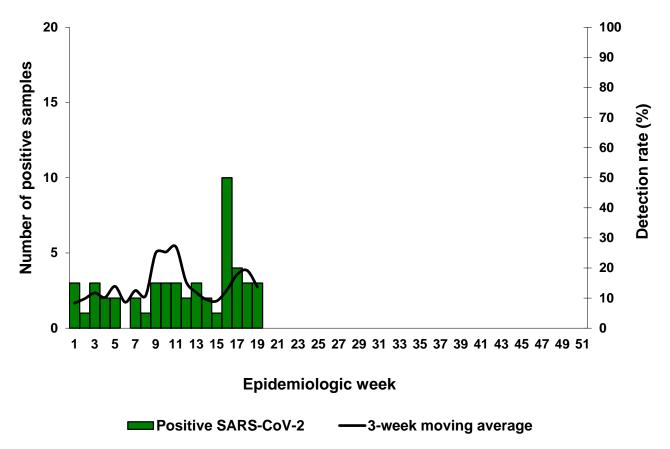


Figure 10. Number of patients testing positive for SARS-CoV-2*, by site and 3-week moving average** by week, ILI surveillance - Viral Watch, 01/01/2023-14/05/2023

*Specimens from patients with influenza-like illnesses at 92 sentinel sites in 8 provinces

Table 8. Number of SARS-CoV-2 positive cases identified and total number tested by province, ILI surveillance - Viral Watch, 01/01/2023-14/05/2023

Province	SARS-CoV-2 positive	Total samples tested
Eastern Cape	2	13
Free State	0	0
Gauteng	35	290
Limpopo	0	0
Mpumalanga	2	9
North West	0	2
Northern Cape	0	0
Western Cape	12	69
Total:	51	383

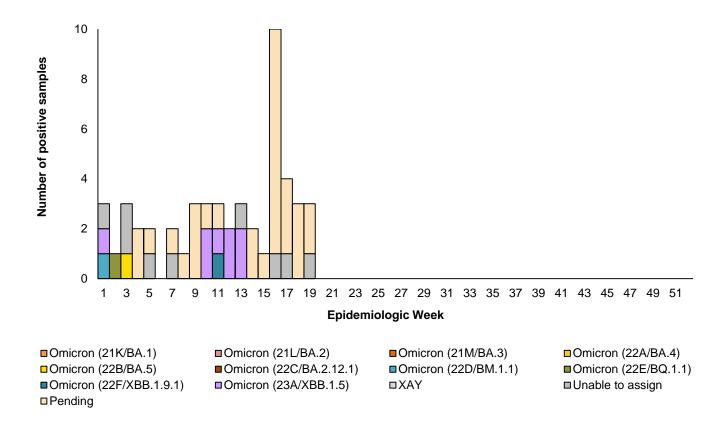


Figure 11. Number of laboratory confirmed SARS-CoV-2* cases by variant type (variant PCR/sequencing) and week, ILI surveillance - Viral Watch, 01/01/2023-14/05/2023

*Specimens from patients with influenza-like illnesses at 92 sentinel sites in 8 provinces **Unable to assign**: no lineage assigned due to poor- sequence quality **OR** low viral load ($C_t \ge 35$) **OR** variant PCR could not assign variant and no sequencing result **Pending**: outstanding variant results

Table 9. Number of SARS-CoV-2^{*} positive cases by variant (variant PCR and/or sequencing) identified and total number of samples tested by province, ILI surveillance - Viral Watch, 01/01/2023-14/05/2023

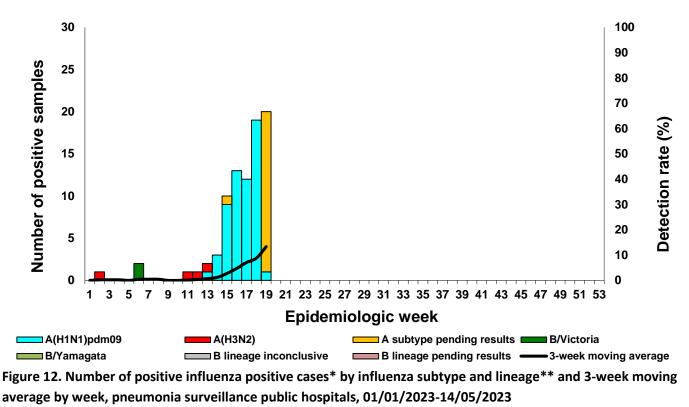
Clinic (Province)	Omicron (21L/BA.2)	Omicron (21M/BA.3)	Omicron (22A/BA.4)	Omicron (22B/BA.5)	Omicron (22C/ BA.2.12.1)	Omicron (22D/BM.1.1)	Omicron (22E/BQ.1.1)	Omicron (22F/XBB.1.9.1)	Omicron (23A/XBB.1.5)	ХАҮ	Unable to assign**	Pending***	Total SARS-CoV- 2 positive	Total samples tested
Eastern	0	0	0	0	0	0	0	0	0	0	0	2	2	13
Cape														
Free State	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Gauteng	0	0	0	1	0	1	1	1	7	0	4	20	35	290
Limpopo	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Mpumalan	0	0	0	0	0	0	0	0	0	0	0	2	2	9
ga														
North West	0	0	0	0	0	0	0	0	0	0	0	0	0	2
Northern	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cape														
Western	0	0	0	0	0	0	0	0	1	0	5	6	12	69
Саре														
Total:	0	0	0	1	0	1	1	1	8	0	9	30	51	383

*Specimens from patients with influenza-like illnesses at 92 sentinel sites in 8 provinces

**No cases of Alpha, Beta or 20D (C.1.2) variants detected.

Unable to assign: no lineage assigned due to poor- sequence quality OR low viral load (Ct≥35) OR variant PCR could not assign variant and no sequencing result Pending: outstanding variant results

Page **12** of **20**



Inconclusive: insufficient viral load in sample and unable to characterise further

*Specimens from patients hospitalised with pneumonia at 15 sentinel sites in 6 provinces

**No cases who met suspected the SARS-CoV-2 or *B. pertussis* case definition but did not meet pneumonia (SRI) case definition.

Table 10. Number of laboratory confirmed influenza cases by subtype and lineage* and total number of samples tested by hospital, pneumonia surveillance public hospitals, 01/01/2023-14/05/2023

Hospital (Province)	A(H1N1)pd m09	A(H3N2)	A subtype inconclusive	A subtype pending results***	B/Victoria	B/Yamagat a	B lineage inconclusive	B lineage pending results***	Total samples
Edendale (KZ)	5	0	0	0	1	0	0	0	310
Helen Joseph-Rahima Moosa (GP)	23	1	0	1	0	0	0	0	510
Khayelitsha (WC)	2	0	0	7	1	0	0	0	266
Klerksdorp-Tshepong (NW)	2	0	0	1	0	0	0	0	187
Livingstone (EC)	10	2	0	1	0	0	0	0	292
Mapulaneng- Matikwana (MP)	0	0	0	0	0	0	0	0	188
Mitchell's Plain (WC)	0	0	0	0	0	0	0	0	204
Red Cross (WC)	3	1	0	0	0	0	0	0	448
Tambo Memorial (GP)	5	0	0	5	0	0	0	0	185
Tembisa (GP)	8	0	0	4	0	0	0	0	192
Tintswalo (MP)	0	0	0	1	0	0	0	0	129
Tygerberg (WC)	0	0	0	0	0	0	0	0	64
Total:	58	4	0	20	2	0	0	0	2975

* No cases who met suspected SARS-CoV-2 or B. pertussis case definition but did not meet pneumonia (SRI) case definition.

**Inconclusive: insufficient viral load in sample and unable to characterise further

***Influenza A subtype or B lineage results are pending

Page 13 of 20

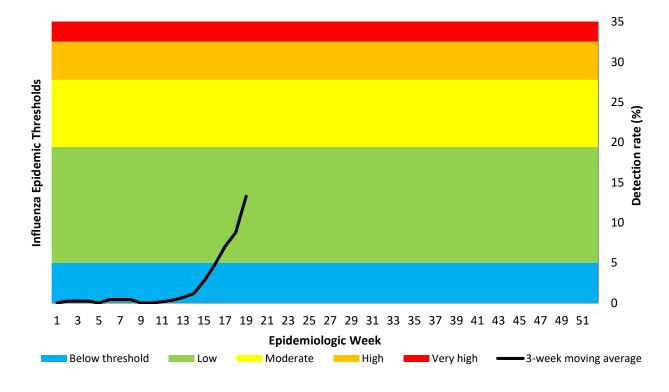
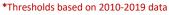


Figure 13. Influenza percentage detections and epidemic thresholds* among cases of all ages, pneumonia surveillance public hospitals, 01/01/2023-14/05/2023



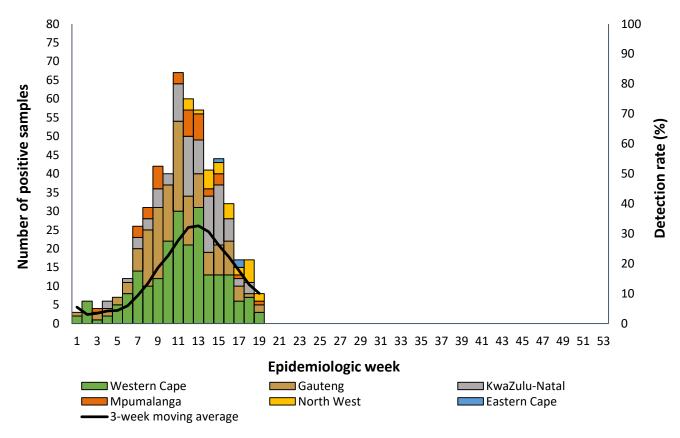


Figure 14. Number of patients (all ages) testing positive for respiratory syncytial virus* by province and 3-week moving average by week, pneumonia surveillance public hospitals, 01/01/2023-14/05/2023

Specimens from patients hospitalised with pneumonia at 15 sentinel sites in 6 provinces.

*No cases who met suspected SARS-CoV-2 or *B. pertussis* case definition but did not meet pneumonia (SRI) case definition.

Page 14 of 20

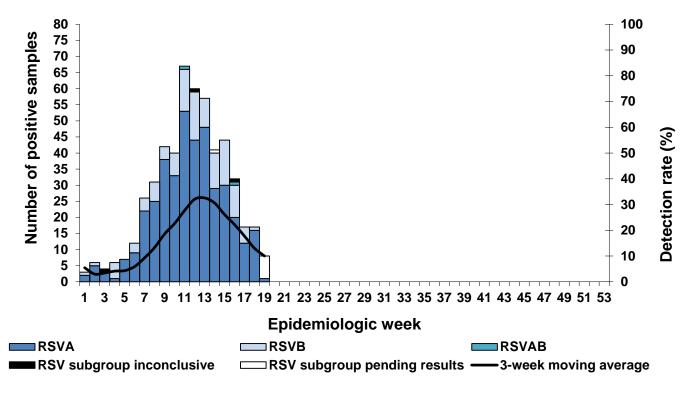


Figure 15. Number of patients (<u>all ages</u>) testing positive for respiratory syncytial virus* by subgroup and 3-week moving average by week, pneumonia surveillance public hospitals, 01/01/2023-14/05/2023

Specimens from patients hospitalised with pneumonia at 15 sentinel sites in 6 provinces.

Inconclusive: insufficient viral load in sample and unable to characterise further

RSV AB: Both RSV A and B subgroup identified

RSV subgroup pending: RSV results for subgroups are pending

* No cases who met suspected SARS-CoV-2 or *B. pertussis* case definition but did not meet pneumonia (SRI) case definition.

Table 11. Number of patients (all ages) positive for respiratory syncytial virus subgroups* by subgroups identified and total number of samples tested by hospital, pneumonia surveillance public hospitals, 01/01/2023-14/05/2023

Hospital (Province)	RSVA RSVB RSVAB**		RSV subgroup inconclusive** *	RSV subgroup pending** **	Total samples	
Edendale (KZ)	31	61	1	1	0	310
Helen Joseph-Rahima Moosa (GP)	128	8	0	1	1	510
Khayelitsha (WC)	4	2	0	0	0	266
Klerksdorp-Tshepong (NW)	24	0	0	0	2	187
Livingstone (EC)	1	2	0	0	0	292
Mapulaneng-Matikwana (MP)	7	4	1	0	1	188
Mitchell's Plain (WC)	55	8	0	0	0	204
Red Cross (WC)	122	22	0	1	3	448
Tambo Memorial (GP)	0	0	0	0	1	185
Tembisa (GP)	1	1	0	0	0	192
Tintswalo (MP)	24	0	0	0	0	129
Tygerberg (WC)	1	1	0	0	0	64
Total:	398	109	2	3	8	2975

*No cases who met suspected SARS-CoV-2 or B. pertussis case definition but did not meet pneumonia (SRI) case definition.

**RSV AB: Both RSV A and B subgroup identified

***Inconclusive: insufficient viral load in sample and unable to characterise further

****RSV results for subgroups are pending

Page 15 of 20

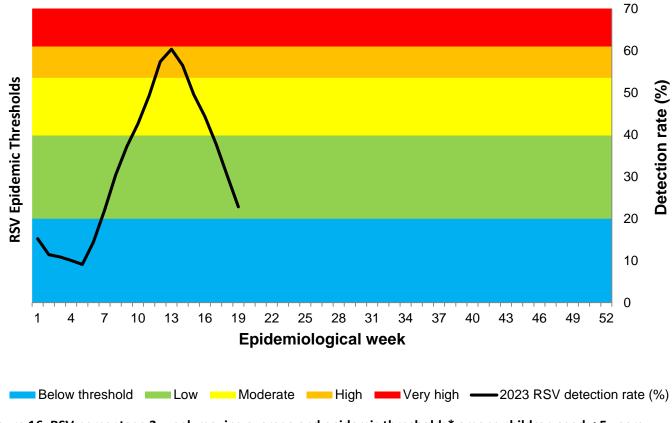


Figure 16. RSV percentage 3-week moving average and epidemic thresholds* among children aged < 5 years, pneumonia surveillance public hospitals, 01/01/2023-14/05/2023 *Thresholds based on 2010-2019 data

Page 16 of 20

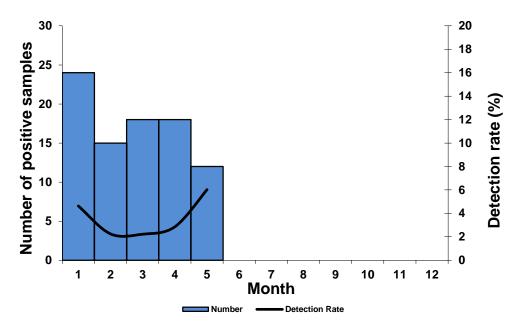


Figure 17. Number of patients testing positive for *B. pertussis** and 3-week moving average by month, pneumonia surveillance public hospitals**, 01/01/2023-14/05/2023

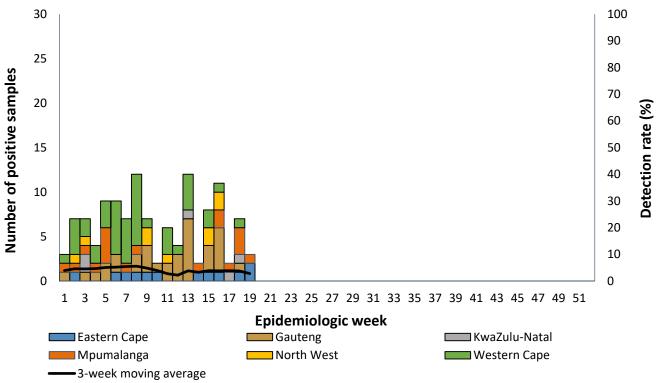
*No cases who met the suspected SARS-CoV-2 or *B. pertussis* case definition but did not meet Pneumonia Surveillance case definition. *Specimens from patients hospitalised with pneumonia at 15 sentinel sites in 6 provinces.

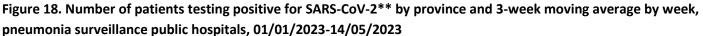
Table 12. Number of patients testing positive for *B. pertussis** identified and total number of samples tested by hospital and province, pneumonia surveillance public hospitals, 01/01/2023-14/05/2023

Hospital (Province)	<i>B. pertussis</i> Positive	Total samples		
Edendale (KZ)	13	299		
Helen Joseph-Rahima Moosa (GP)	14	500		
Khayelitsha (WC)	3	237		
Klerksdorp-Tshepong(NW)	14	178		
Livingstone (EC)	5	269		
Mapulaneng-Matikwana (MP)	12	181		
Mitchell's Plain (WC)	2	203		
Red Cross (WC)	11	424		
Tambo Memorial (GP)	4	165		
Tembisa (GP)	4	181		
Tintswalo (MP)	3	124		
Tygerberg (WC)	2	64		
Total:	87	2825		

*No cases who met the suspected SARS-CoV-2 or *B. pertussis* case definition but did not meet the pneumonia (SRI) case definition. These are not included in the table.

Page 17 of 20





*Specimens from patients hospitalized with pneumonia at 15 sentinel sites in 6 provinces.

**No cases met suspected SARS-CoV-2 or *B. pertussis* case definition but did not meet pneumonia (SRI) case definition.

Table 13. Number of patients positive for SARS-CoV-2* and total number of samples tested by hospital, pneumonia surveillance public hospitals, 01/01/2023-14/05/2023

Hospital (Province)	SARS-CoV-2 positive	Total samples tested		
Edendale (KZ)	5	310		
Helen Joseph-Rahima Moosa (GP)	14	510		
Khayelitsha (WC)	15	266		
Klerksdorp-Tshepong (NW)	9	187		
Livingstone (EC)	12	292		
Mapulaneng-Matikwana (MP)	13	188		
Mitchell's Plain (WC)	14	204		
Red Cross (WC)	12	448		
Tambo Memorial (GP)	13	185		
Tembisa (GP)	7	192		
Tintswalo (MP)	5	129		
Tygerberg (WC)	3	64		
Total:	122	2975		

* No cases who met suspected SARS-CoV-2 or B. pertussis case definition but did not meet pneumonia (SRI) case definition.

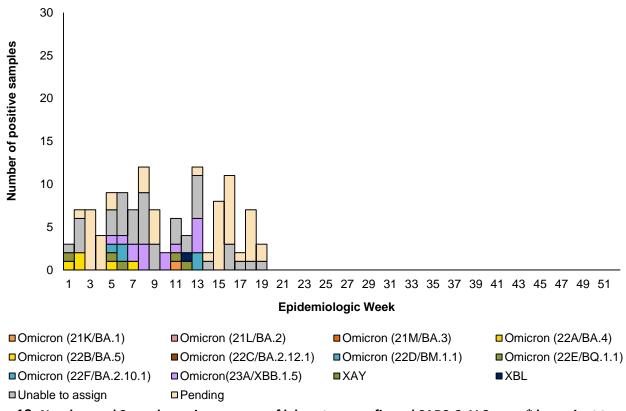


Figure 19. Number and 3-week moving average of laboratory-confirmed SARS-CoV-2 cases* by variant type (variant PCR/sequencing), pneumonia surveillance public hospitals, 01/01/2023-14/05/2023

*Specimens are from hospitalized patients at 15 sentinel sites in 6 provinces who met the pneumonia (SRI), suspected SARS-CoV-2 or *B. pertussis* case definition Unable to assign: no lineage assigned due to poor- sequence quality OR low viral load (Ct≥35) OR variant PCR could not assign variant and no sequencing result Pending: outstanding variant results

Table 14. Number of SARS-CoV-2 positive cases* by variant (variant PCR and/or sequencing) identified and total number of samples tested by hospital, pneumonia surveillance public hospitals, 01/01/2023-14/05/2023

Hospital (Province)	Omicron (21K/BA.1))	Omicron (22A/BA.4)	Omicron (22B/BA.5)	Omicron (22C/ BA.2.12.1)	Omicron (22D/BM.1.1)	Omicron(22E/BQ.1 .1)	Omicron (22F/BA.2.10.1)	Omicron(23A/XBB. 1.5)	ХАҮ	XBL	Unable to assign**	Pending***	Total SARS-CoV-2 positive	Total samples tested
Edendale (KZ)	0	0	0	0	0	0	0	0	0	0	1	4	5	310
Helen Joseph-Rahima Moosa (GP)	1	0	0	0	0	1	2	3	0	0	1	6	14	510
Khayelitsha (WC)	0	0	0	0	0	0	0	4	0	0	8	3	15	266
Klerksdorp-Tshepong (NW)	0	0	1	0	0	0	0	0	0	0	2	6	9	187
Livingstone (EC)	0	0	1	0	0	0	0	1	0	0	6	4	12	292
Mapulaneng- Matikwana (MP)	0	0	0	0	0	0	0	0	0	0	6	7	13	188
Mitchell's Plain (WC)	0	0	1	0	0	0	2	1	0	0	5	5	14	204
Red Cross (WC)	0	0	2	0	0	0	1	1	0	1	4	3	12	448
Tambo Memorial (GP)	0	0	0	0	0	3	0	3	0	0	2	5	13	185
Tembisa (GP)	0	0	0	0	0	1	0	0	0	0	4	2	7	192
Tintswalo (MP)	0	0	0	0	0	0	0	0	0	0	3	2	5	129
Tygerberg (WC)	0	0	0	0	0	0	0	1	0	0	1	1	3	64
Total:	1	0	5	0	0	5	5	14	0	1	43	48	122	2975

*Specimens are from hospitalized patients at 15 sentinel sites in 6 provinces who met the pneumonia (SRI), suspected SARS-CoV-2 or *B. pertussis* case definition Unable to assign: no lineage assigned due to poor- sequence quality OR low viral load (Ct≥35) OR variant PCR could not assign variant and no sequencing result Pending: outstanding variant results

Page **19** of **20**

Methods

SARS-CoV-2 Testing

March 2020 – March 2021: SARS-CoV-2 was detected using the Roche E gene real-time PCR assay (Corman et al. *Euro Surveillance* 2020) with cycle threshold (C_t) <40 interpreted as positive for SARS-CoV-2. From April 2021 to date the laboratory changed to the AllplexTM SARS-CoV-2/FluA/FluB/RSV kit (Seegene Inc., Seoul, South Korea), with positivity assigned if the PCR cycle threshold (C_t) was <40 for ≥1 gene targets (N, S or RdRp).

A confirmed SARS-CoV-2 case is a person of any age enrolled in surveillance with laboratory confirmation of SARS-CoV-2 infection by PCR. Only positive SARS-CoV-2 specimens on PCR are further tested to determine variant/lineage type by variant PCR or genomic sequencing. Variant PCR

AllplexTM SARS-CoV-2 Variants I PCR detects Alpha and Beta/Gamma variants. The assay was conducted on all SARS-CoV-2-positive samples from 1 March 2020 – 30 June 2021.

AllplexTM SARS-CoV-2 Variants II PCR detects Delta variant and distinguishes Beta from Gamma. The assay was conducted on SARS-CoV-2-positive samples from 1 Jan to 30 June 2021.

Extraction: Total nucleic acids were extracted from 200µl NP/OP samples in universal or viral transport medium using a MagNA Pure 96 automated extractor and DNA/Viral NA Small Volume v2.0 extraction kit (Roche Diagnostics, Mannheim, Germany).

SARS-CoV-2 genomic surveillance

SARS-CoV-2 Whole-Genome Sequencing and Genome Assembly

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, (<u>https://sars-cov-2.exatype.com/</u>). 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, (<u>http://ormbunkar.se/aliview/</u>) (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 (<u>https://www.gisaid.org/</u>) (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 (<u>https://github.com/hCoV-2019/pangolin</u>) (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 (<u>https://nextstrain.org/</u>), a tool built for real-time tracking of the pathogen evolution (Hadfield et al., 2018).