Division of the National Health Laboratory Service

Weekly respiratory pathogens report Week 34 of 2022

<u>Highlights</u>

- The 2022 influenza season started in week 17 (week starting 25 April 2022) when the influenza detection rate among patients in pneumonia surveillance breached the epidemic threshold as determined by the Moving Epidemic Method (MEM).
- In 2022 to date, 725 influenza cases have been detected from all surveillance programmes. Majority of cases were reported from Western Cape (n=227) and Gauteng (n=192), followed by KwaZulu-Natal (n=97), Mpumalanga (n=83), North West (n=60), Eastern Cape (n=53), Free State (n=7), and Limpopo (n=6) sentinel surveillance sites. We had two dual infections and they are influenza A(H1N1)pdm09 and A(H3N2) and influenza A(H1N1)pdm09 and influenza B(lineage inconclusive) from Gauteng.
- The 2022 RSV season which started in week 7 (week starting 14 February 2022) when RSV detection rate among children under five years of age in pneumonia surveillance rose above the seasonal threshold, ended in week 26. In 2022 to date, 849 respiratory syncytial virus (RSV) cases have been detected from all surveillance programmes.
- In 2022 to date, a total of 651 COVID-19 cases were detected from all surveillance programmes. Of the 312 hospitalised COVID-19 cases reported with available data on outcome, 21 (7%) died.
- Of the 596/651 (92%) SARS-CoV-2 specimens sequenced, 37% (221/596) sequences could not be assigned a variant. Of the 375 with assigned variants, Omicron was the dominant variant (99%, 370/375); of which 22% (83/370) was Omicron (21K/BA.1), 20% (73/370) was Omicron (21L/BA.2), 1% (2/370) was Omicron (21M/BA.3), 31% (114/370) was Omicron (22A/BA.4), 26% (97/370) was Omicron (22B/BA.5) and <1% (1/370) was Omicron (22C/BA.2.12.1). Alpha, Delta and C.1.2 (20D) variants contributed <1% each.
- A lower number of specimens was submitted in week 30 (31 July 6 August 2022) due to staff training this likely affected numbers and proportions of viruses detected, therefore trends should be regarded with caution.

Programme Descriptions

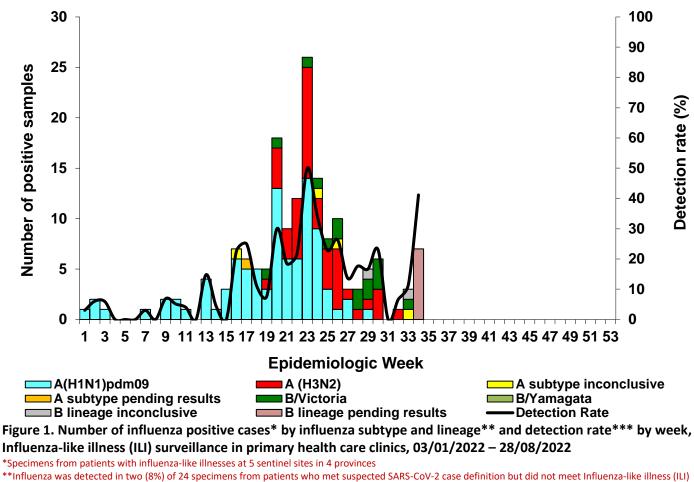
Programme	Influenza-like illness (ILI)	Viral Watch	National syndromic surveillance for pneumonia		
Start year	2012	1984	2009		
Provinces*	KΖ	EC	EC		
	NW	FS	GP		
	WC	GP	KZ		
	MP	LP	MP		
		MP	NW		
		NC	WC		
		NW	we		
		WC			
Type of site	Primary health care clinics	General practitioners	Public hospitals		
Case definition	ILI: An acute respiratory illness with a	ILI: An acute respiratory illness with a	SRI: Acute (symptom onset≤10 days) or		
case definition	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 infection		
	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 \geq 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	Suspected SARS-CoV-2		
	Any person presenting with an acute	Any person presenting with an acute	Any person admitted with a physician-		
	(≤14 days) respiratory tract infection or	(≤14 days) respiratory tract infection or	diagnosis of suspected COVID-19 and		
	other clinical illness compatible with	other clinical illness compatible with	not meeting SRI case definition.		
	COVID-19**	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	BP	BP		
	SARS-CoV-2	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 Clin</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 et al.,	gene real-time PCR essay Corman et al.,	gene real-time PCR essay (Corman et al.,		
	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 		
	 positivity assigned if PCR cycle threshold is <40 for ≥1 gene targets 	 positivity assigned if PCR cycle threshold is <40 for ≥1 gene targets 	 positivity assigned if PCR cycle 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 **19**



***Only reported for weeks with >10 specimens submitted

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 samplestested by clinic and province, Influenza-like illness (ILI) surveillance in primary health care clinics, 03/01/2022 –28/08/2022

Clinic (Province)	A(H1N1) pdm09	A(H3N2)	A subtype in- conclusive**	A subtype pending results** *	B/ Victoria	B/ Yamagata	B lineag e in- conclu sive*	B lineage pending results* **	Total sample s
Agincourt (MP)	20	0	0	1	11	0	1	0	174
Eastridge (WC)	11	11	0	0	2	0	0	4	188
Edendale Gateway (KZ)	23	26	0	0	2	0	0	0	312
Jouberton (NW)	24	0	1	0	0	0	0	2	226
Mitchell's Plain (WC)	15	8	3	0	0	0	1	1	195
Total:	93	45	4	1	15	0	2	7	1095

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

*Influenza was detected in two (8%) of 24 specimens from patients who met suspected SARS-CoV-2 case definition but did not meet Influenza-like illness (ILI) case definition. Of which one (50%) was influenza A(H3N2) and another (50%) was influenza B(Victoria). These are not included in the epidemiological curve. **Inconclusive: insufficient viral load in sample and unable to characterise further

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

Page **3** of **19**

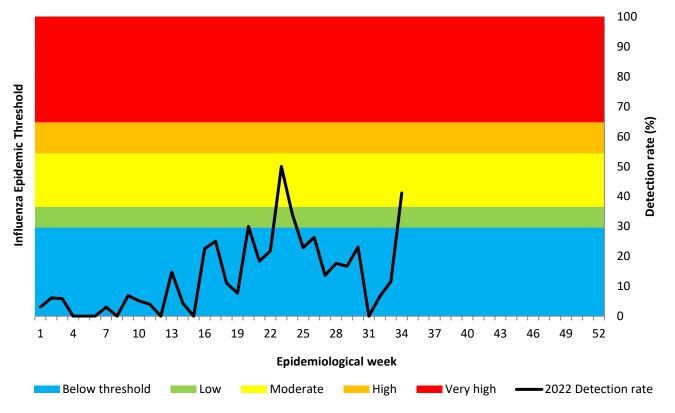


Figure 2. Influenza percentage detections and epidemic thresholds* among cases of all ages, Influenza-like illness (ILI) surveillance in primary health care clinics, 03/01/2022 – 28/08/2022 *Thresholds based on 2012-2019 data

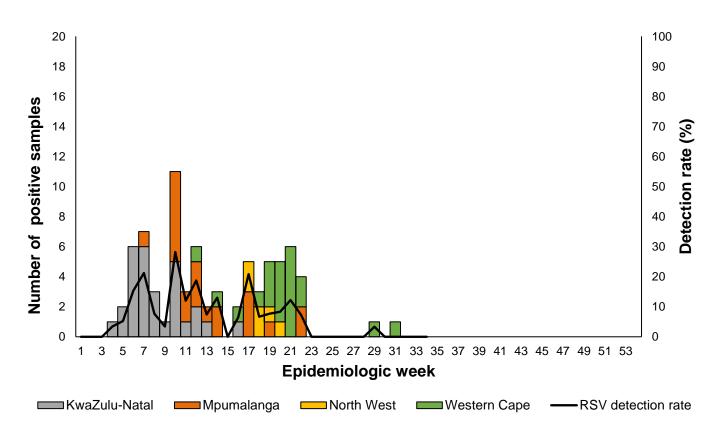


Figure 3. Number of patients testing positive for respiratory syncytial virus* by province and detection rate by week, Influenza-like illness (ILI) surveillance in primary health care clinics, 03/01/2022 – 28/08/2022

*RSV was not detected from 24 specimens of patients who met suspected SARS-CoV-2 case definition but did not meet influenza-like illness (ILI) case definition. **Only reported for weeks with >10 specimens submitted

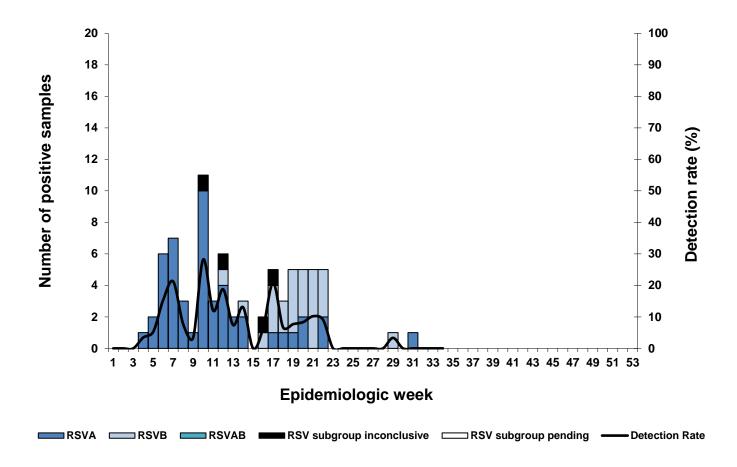


Figure 4. Number of patients testing positive for respiratory syncytial virus* by subgroup and detection rate by week, Influenza-like illness (ILI) surveillance in primary health care clinics, 03/01/2022 – 28/08/2022

*RSV was not detected from 24 specimens of patients who met suspected SARS-CoV-2 case definition but did not meet influenza-like illness (ILI) case definition. These are not included in the epidemiological curve.

**Only reported for weeks with >10 specimens submitted

RSV AB: Both RSV A and B subgroup 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, 03/01/2022 – 28/08/2022

Clinic (Province)	RSVA	RSVB	RSVAB**	RSV subgroup inconclusive* **	RSV subgroup pending** **	Total samples
Agincourt (MP)	18	2	0	1	0	174
Eastridge (WC)	2	9	0	0	0	188
Edendale Gateway (KZ)	26	0	0	3	0	312
Jouberton (NW)	3	3	0	0	0	226
Mitchell's Plain (WC)	0	10	0	0	0	195
Total	49	24	0	4	0	1095

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

*RSV was not detected from 24 specimens of patients who met suspected SARS-CoV-2 case definition but did not meet influenza-like illness (ILI) case definition. These are not included in the table.

**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 5 of 19

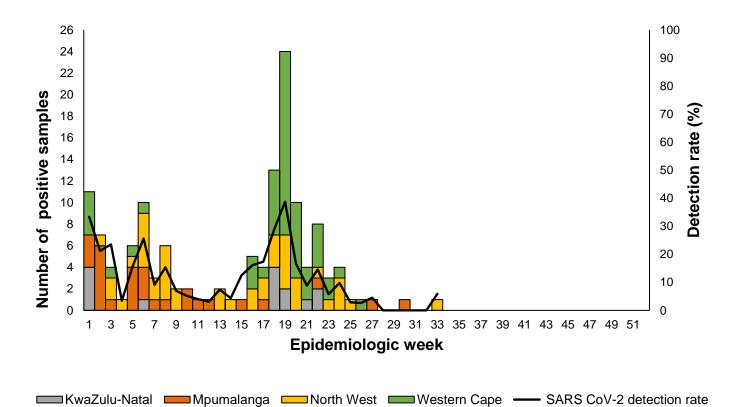


Figure 5. Number of patients* testing positive for SARS-CoV-2** by province and detection rate*** by week, Influenza-like illness (ILI) surveillance in primary health care clinics, 03/01/2022 – 28/08/2022

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

**SARS-CoV-2 was detected in 5 of 24 (21%) specimens from patients who met suspected SARS-CoV-2 case definition but did not meet influenza-like illness (ILI) case definition. These are not included in the epidemiological curve.

***Only reported for weeks with >10 specimens submitted

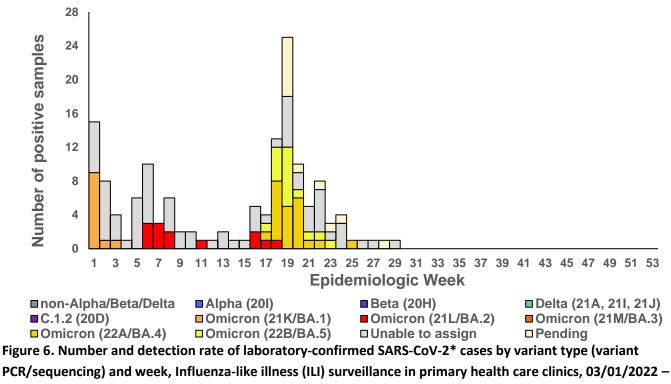
Clinic (Province)	SARS-CoV-2 positive	Total samples tested
Agincourt (MP)	28	174
Eastridge (WC)	9	188
Edendale Gateway (KZ)	15	312
Jouberton (NW)	45	226
Mitchell's Plain (WC)	43	195
Total:	140	1095

Table 3. Number of patients positive for SARS-CoV-2* identified and total number of samples tested by clinicand province, Influenza-like illness (ILI) surveillance primary health care clinics, 03/01/2022 – 28/08/2022

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

*SARS-CoV-2 was detected in 5 of 24 (21%) specimens from patients who met suspected SARS-CoV-2 case definition but did not meet influenza-like illness (ILI) case definition. These are not included in the table.

Page 6 of 19



28/08/2022

*Specimens are from patients with influenza-like illness at 5 sentinel sites in 4 provinces who met suspected SARS-CoV-2 case definition or met ILI 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 4. 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, 03/01/2022 – 28/08/2022

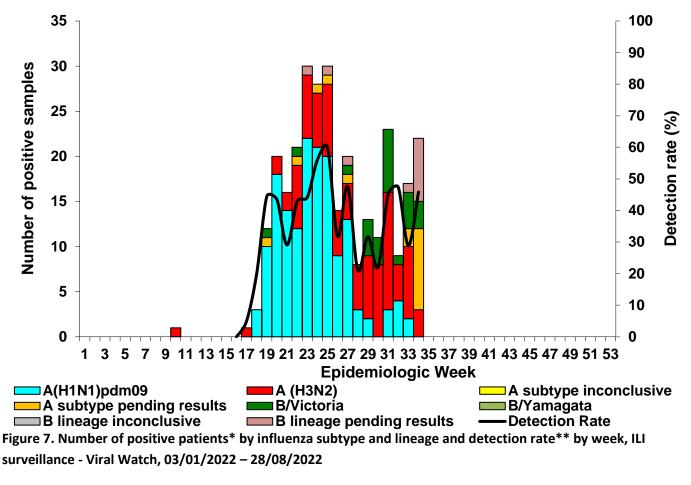
Clinic (Province)	Delta (21A, 21I, 21J)	Omicron (21K/BA.1)	Omicron (21L/BA.2)	Omicron (21M/BA.3)	Omicron (22A/BA.4)	Omicron (22B/BA.5)	Omicron (22C/ BA.2.12.1)	Unable to assign	Pending	Total SARS- CoV-2 positive	Total samples tested
Agincourt (MP)	0	4	3	0	0	0	0	22	1	30	179
Eastridge (WC)	0	2	0	0	0	0	0	4	3	9	188
Edendale Gateway (KZ)	0	2	1	0	0	6	0	7	1	17	325
Jouberton (NW)	0	1	5	0	6	6	0	25	3	46	232
Mitchell's Plain (WC)	0	2	4	0	16	4	0	13	4	43	195
Total:	0	11	13	0	22	16	0	71	12	145	1119

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 suspected SARS-CoV-2 case definition or met ILI case definition **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 7 of 19



*Specimens from patients with Influenza-like illnesses at 90 sentinel sites in 8 provinces **Only reported for weeks with >10 specimens submitted. Inconclusive: insufficient viral load in sample and unable to characterise further

Table 5. Number of laboratory confirmed influenza cases by influenza subtype and lineage and total number of samples tested by province, ILI surveillance - Viral Watch, 03/01/2022 – 28/08/2022

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	20	6	0	1	4	0	0	3	52
Free State	7	0	0	0	0	0	0	0	8
Gauteng	80	28	0	4	13	0	0	7	534
Limpopo	2	2	0	1	1	0	0	0	8
Mpumalanga	7	0	0	1	1	0	0	0	21
North West	3	0	0	0	0	0	0	0	6
Northern Cape	0	0	0	0	0	0	0	0	0
Western Cape	37	55	0	9	6	0	0	1	215
Total:	156	91	0	16	25	0	0	11	844

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

**Influenza A subtype or B lineage results are pending

Two cases were dual infections:

1. Influenza A(H1N1)pdm09 and A(H3N2)

2. Influenza A(H1N1)pdm09 and influenza B(lineage inconclusive)

Page 8 of 19

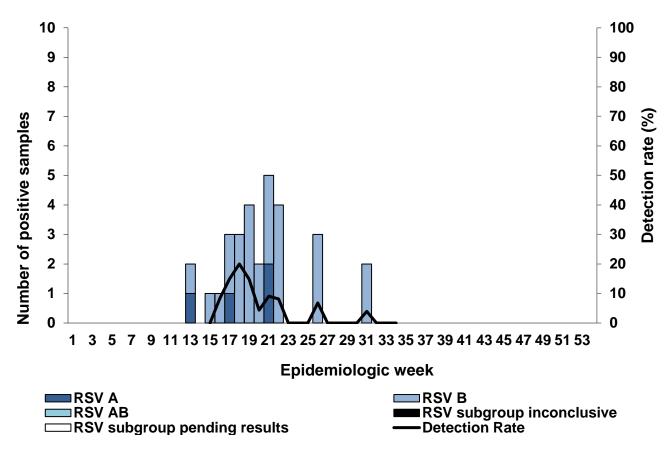


Figure 8. Number of RSV positive cases testing positive for respiratory syncytial virus (RSV)* by subgroup and detection rate** by week, ILI surveillance - Viral Watch, 03/01/2022 – 28/08/2022

*Specimens from patients with Influenza-like illnesses at 92 sentinel sites in 8 provinces **Only reported for weeks with >10 specimens submitted.

Table 6. Number of RSV positive cases identified and total number of samples tested by province, ILI surveillance - Viral Watch, 03/01/2022 – 28/08/2022

Province	RSV A	RSV B	RSV AB*	RSV subgroup inconclusive **	RSV subgroup pending results***	Total samples tested
Eastern Cape	0	1	0	0	0	52
Free State	0	0	0	0	0	8
Gauteng	4	13	0	0	0	534
Limpopo	0	0	0	0	0	8
Mpumalanga	0	0	0	0	0	21
North West	0	0	0	0	0	6
Northern Cape	0	0	0	0	0	0
Western Cape	0	12	0	0	0	215
Total:	4	26	0	0	0	844

*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 **9** of **19**

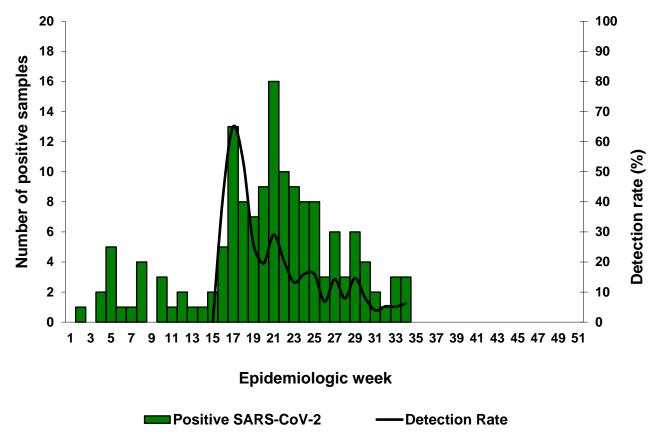


Figure 9. Number of patients testing positive for SARS-CoV-2*, by site and detection rate** by week, ILI surveillance - Viral Watch, 03/01/2022 – 28/08/2022

*Specimens from patients with Influenza-like illnesses at 92 sentinel sites in 8 provinces **Only reported for weeks with >10 specimens submitted.

 Table 7. Number of SARS-CoV-2 positive cases identified and total number tested by province, ILI surveillance

 - Viral Watch, 03/01/2022 – 28/08/2022

Province	SARS-CoV-2 positive	Total samples tested
Eastern Cape	3	52
Free State	0	8
Gauteng	108	534
Limpopo	1	8
Mpumalanga	2	21
North West	0	6
Northern Cape	0	0
Western Cape	34	215
Total:	148	844

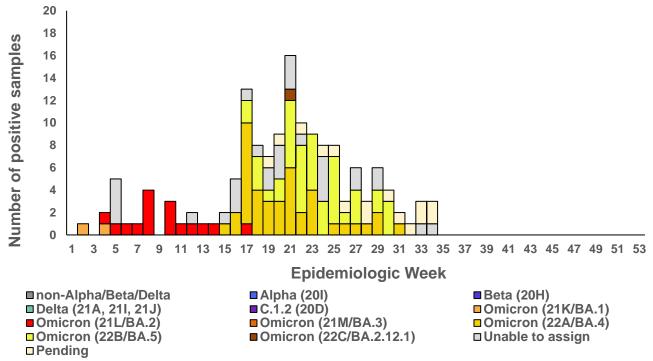


Figure 10. Number and detection rate of laboratory confirmed SARS-CoV-2* cases by variant type (variant PCR/sequencing) and week, ILI surveillance - Viral Watch, 03/01/2022 – 28/08/2022

*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

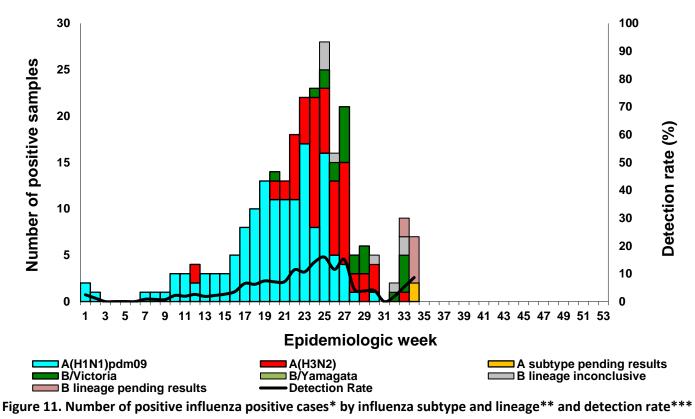
Table 8. Number of SARS-CoV-2^{*} positive cases by variant (variant PCR and/or sequencing) identified and total number of samples tested by province, ILI surveillance - Viral Watch, 03/01/2022 – 28/08/2022

Clinic (Province)	Delta (21A,21 I, 21J)	Omicron (21K/BA. 1)	Omicron (21L/BA. 2)	Omicron (21M/BA .3)	Omicron (22A/BA. 4)	Omicron (22B/BA. 5)	Omicr on (22C/ BA.2. 12.1)	Unabl e to assign	Pendin g	Total SARS- CoV-2 positiv e	Total sample s tested
Eastern Cape	0	0	1	0	1	0	0	0	1	3	52
Free State	0	0	0	0	0	0	0	0	0	0	8
Gauteng	0	2	8	0	34	32	1	22	9	108	534
Limpopo	0	0	0	0	0	0	0	1	0	1	8
Mpumalanga	0	0	0	0	1	1	0	0	0	2	21
North West	0	0	0	0	0	0	0	0	0	0	6
Northern Cape	0	0	0	0	0	0	0	0	0	0	0
Western Cape	0	0	7	0	5	10	0	7	5	34	215
Total:	0	2	16	0	41	43	1	30	15	148	844

*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



by week, pneumonia surveillance public hospitals, 03/01/2022 – 28/08/2022

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

*Specimens from patients hospitalised with pneumonia at 10 sentinel sites in 6 provinces

**Influenza was not detected in 16 specimens from patients who met suspected SARS-CoV-2 case definition but did not meet pneumonia (SRI) case definition. These are not included in the epidemiological curve.

***Only reported for weeks with >10 specimens submitted

Table 9. Number of laboratory confirmed influenza cases by subtype and lineage* and total number ofsamples tested by hospital, pneumonia surveillance public hospitals, 03/01/2022 – 28/08/2022

Hospital (Province)	A(H1N1)p dm09	A(H3N2)	A subtype inconclusive	A subtype pending results***	B/Victoria	B/Yamagata	B lineage inconclusive	B lineage pending results***	Total samples
Edendale (KZ)	26	15	1	0	2	0	0	0	664
Helen Joseph-Rahima Moosa (GP)	30	11	1	0	5	0	2	0	1016
Klerksdorp-Tshepong (NW)	27	1	0	0	0	0	2	0	389
Livingstone (EC)	8	1	1	1	6	0	2	0	256
Mapulaneng- Matikwana (MP)	11	0	0	0	5	0	0	1	380
Red Cross (WC)	5	12	1	0	2	0	1	1	516
Mitchell's Plain (WC)	9	18	2	1	1	0	0	4	924
Tembisa (GP)	7	2	0	0	0	0	1	1	158
Tintswalo (MP)	18	4	1	0	1	0	0	0	253
Tygerberg (WC)	3	3	1	0	0	0	0	0	94
Total:	144	67	8	2	22	0	8	7	4650

EC: Eastern Cape (Livingstone started enrolling on the 3rd of May 2022); GP: Gauteng (Tembisa started enrolling on the 10th March 2022); KZ: KwaZulu-Natal; NW: North West; MP: Mpumalanga; WC: Western Cape (Tygerberg started enrolling on the 20th April 2022)

*Influenza was not detected in 16 specimens from patients who met suspected SARS-CoV-2 case definition but did not meet pneumonia (SRI) case definition.

These are not included in the table.

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

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

Page 12 of 19

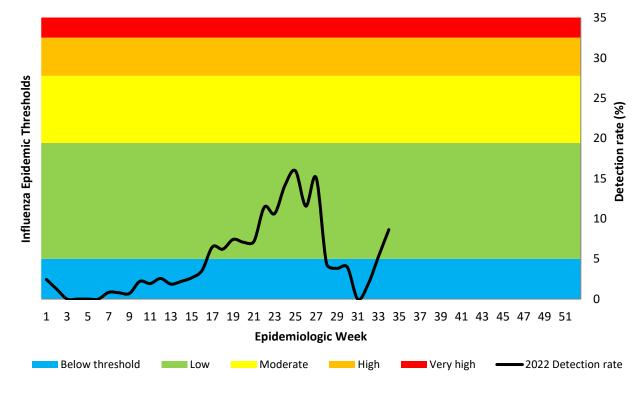


Figure 12. Influenza percentage detections and epidemic thresholds* among cases of all ages, pneumonia surveillance public hospitals, 03/01/2022 – 28/08/2022



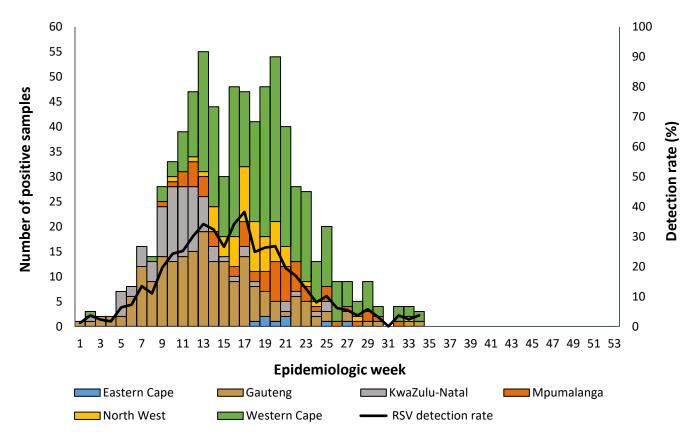


Figure 13. Number of patients (all ages) testing positive for respiratory syncytial virus* by province and detection rate by week, pneumonia surveillance public hospitals, 03/01/2022 – 28/08/2022

*RSV was not detected in 16 specimens from patients who met suspected SARS-CoV-2 case definition but did not meet pneumonia (SRI) case definition.

Page **13** of **19**

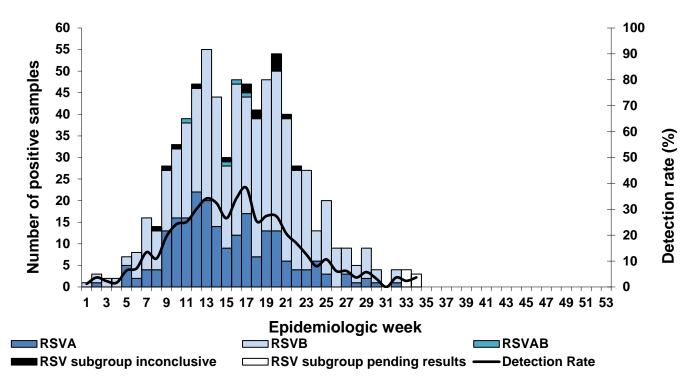


Figure 14. Number of patients (all ages) testing positive for respiratory syncytial virus* by subgroup and detection rate by week, pneumonia surveillance public hospitals, 03/01/2022 – 28/08/2022

RSV AB: Both RSV A and B subgroup identified

RSV subgroup pending: RSV results for subgroups are pending

*RSV was not detected in 16 specimens from patients who met suspected SARS-CoV-2 case definition but did not meet pneumonia (SRI) case definition. These are not included in the epidemiological curve.

Table 10. 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, 03/01/2022 – 28/08/2022

Hospital (Province)	RSVA	RSVB	RSVAB**	RSV subgroup inconclusive** *	RSV subgroup pending** **	Total samples
Edendale (KZ)	86	1	0	2	0	664
Helen Joseph-Rahima Moosa (GP)	39	151	3	1	2	1016
Klerksdorp-Tshepong (NW)	29	31	1	0	1	389
Livingstone (EC)	1	6	0	1	0	256
Mapulaneng-Matikwana (MP)	18	25	0	0	0	380
Red Cross (WC)	6	63	0	0	1	516
Mitchell's Plain (WC)	36	200	0	8	3	924
Tembisa (GP)	0	1	0	0	0	158
Tintswalo (MP)	4	15	0	3	0	253
Tygerberg (WC)	0	4	0	0	0	94
Total:	219	497	4	15	7	4650

EC: Eastern Cape (Livingstone started enrolling on the 3rd of May 2022); GP: Gauteng (Tembisa started enrolling on the 10th March 2022); KZ: KwaZulu-Natal; NW: North West; MP: Mpumalanga; WC: Western Cape (Tygerberg started enrolling on the 20th April 2022)

*RSV was not detected in 16 specimens from patients who met suspected SARS-CoV-2 case definition but did not meet pneumonia (SRI) case definition. These are not included in the table.

**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 14 of 19

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

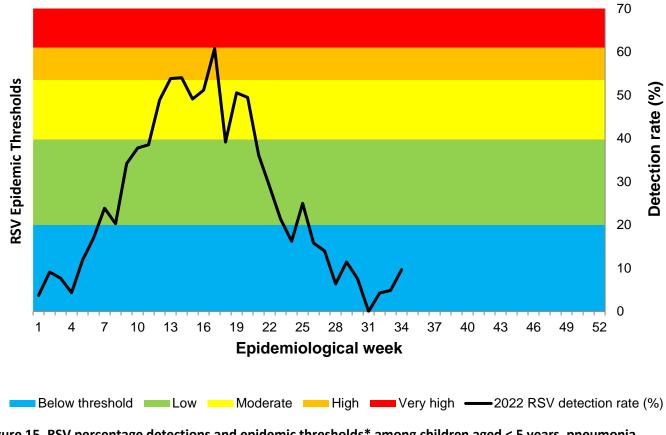


Figure 15. RSV percentage detections and epidemic thresholds* among children aged < 5 years, pneumonia surveillance public hospitals, 03/01/2022 – 28/08/2022 *Thresholds based on 2010-2019 data

Page **15** of **19**

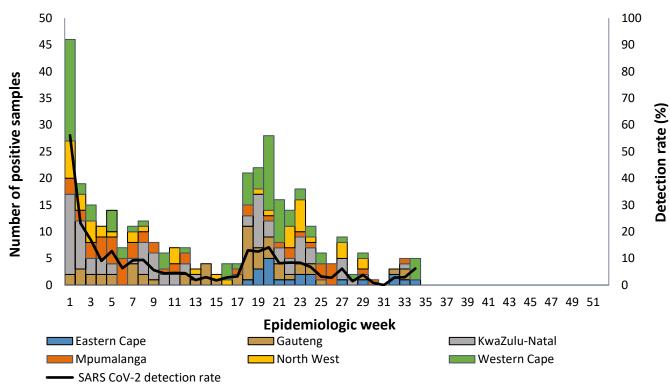


Figure 16. Number of patients testing positive for SARS-CoV-2** by province and detection rate by week, pneumonia surveillance public hospitals, 03/01/2022 – 28/08/2022

*Specimens from patients hospitalized with pneumonia at 6 sentinel sites in 5 provinces

**SARS-CoV-2 was detected in 6 of 16 (38%) specimens from patients who met suspected SARS-CoV-2 case definition but did not meet pneumonia (SRI) case definition. These are not included in the epidemiological curve.

Table 11. Number of patients positive for SARS-CoV-2* and total number of samples tested by hospital, pneumonia surveillance public hospitals, 03/01/2022 – 28/08/2022

Hospital (Province)	SARS-CoV-2 positive	Total samples tested
Edendale (KZ)	86	664
Helen Joseph-Rahima Moosa (GP)	48	1016
Klerksdorp-Tshepong (NW)	44	389
Livingstone (EC)	21	256
Mapulaneng-Matikwana (MP)	33	380
Red Cross (WC)	48	516
Mitchell's Plain (WC)	36	924
Tembisa (GP)	11	158
Tintswalo (MP)	20	253
Tygerberg (WC)	4	94
Total:	351	4650

EC: Eastern Cape (Livingstone started enrolling on the 3rd of May 2022); GP: Gauteng (Tembisa started enrolling on the 10th March 2022); KZ: KwaZulu-Natal; NW: North West; MP: Mpumalanga; WC: Western Cape (Tygerberg started enrolling on the 20th April 2022)

*SARS-CoV-2 was detected in 6 of 16 (38%) specimens from patients who met suspected SARS-CoV-2 case definition but did not meet pneumonia (SRI) case definition. These are not included in the table.

Page 16 of 19

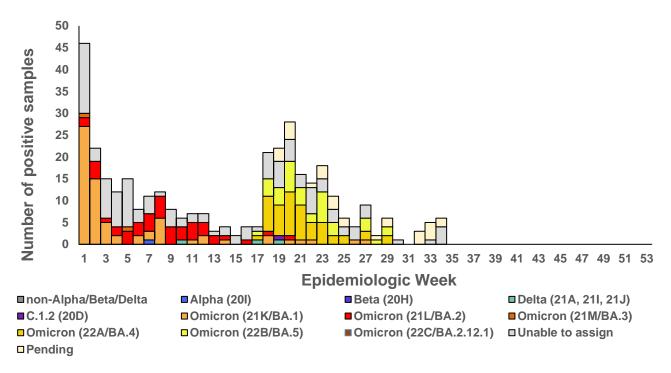


Figure 17. Number and detection rate of laboratory-confirmed SARS-CoV-2 cases* by variant type (variant PCR/sequencing), pneumonia surveillance public hospitals, 03/01/2022 – 28/08/2022

*Specimens are from hospitalized patients at 10 sentinel sites in 6 provinces who met suspected SARS-CoV-2 case definition and met pneumonia (SRI) 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 12. Number of SARS-CoV-2 positive cases* by variant (variant PCR and/or sequencing) identified andtotal number of samples tested by hospital, pneumonia surveillance public hospitals, 03/01/2022 –28/08/2022

Hospital (Province)	Delta (21A, 21I, 21J)	Omicron (21K/BA.1)	Omicron (21L/BA.2)	Omicron (21M/BA.3)	Omicron (22A/BA.4)	Omicron (22B/BA.5)	Omicr on (22C/ BA.2.1 2.1)	Unable to assign	Pending	Total SARS- CoV-2 positive	Total sample s tested
Helen Joseph-Rahima Moosa (GP)	0	7	9	0	6	5	0	16	4	48	1016
Klerksdorp-Tshepong (NW)	0	10	2	1	3	4	0	19	6	45	389
Livingstone (EC)	0	1	1	0	7	4	0	3	5	21	256
Mapulaneng- Matikwana (MP)	0	6	8	0	3	0	0	18	0	35	387
Red Cross (WC)	0	12	1	0	13	3	0	14	5	48	516
Mitchell's Plain (WC)	0	4	6	0	12	3	0	11	0	36	924
Tembisa (GP)	2	1	0	0	2	1	0	4	1	11	158
Tintswalo (MP)	0	4	4	0	1	1	0	9	1	20	253
Tygerberg (WC)	0	1	0	0	1	1	0	0	1	4	94
Total:	3	70	44	2	51	38	0	120	28	358	4666

EC: Eastern Cape (Livingstone started enrolling on the 3rd of May 2022); GP: Gauteng (Tembisa started enrolling on the 10th March 2022); KZ: KwaZulu-Natal; NW: North West; MP: Mpumalanga; WC: Western Cape (Tygerberg started enrolling on the 20th April 2022)

*Specimens are from hospitalized patients at 10 sentinel sites in 6 provinces who met suspected SARS-CoV-2 case definition and met pneumonia (SRI) case definition

One case of Alpha variant from Helen Joseph-Rahima Moosa (GP), no cases of Beta variant and one case of 20D (C.1.2) variant detected from Edendale (KZ). **Unable to assign: no lineage assigned due to poor- sequence quality **OR** low viral load (C_t≥35) **OR** variant PCR could not assign variant and no sequencing result **Pending**: outstanding variant results

Page **17** of **19**

Summary of individuals with laboratory-confirmed SARS-CoV-2

Table13: Characteristics of individuals with laboratory-confirmed SARS-CoV-2, enrolled in influenza-like illness (ILI) and pneumonia surveillance programmes, South Africa, 03 January 2022 - 28 August 2022

Characteristic	Influenza-like illness (ILI), public- sector, n=145 (%)	Pneumonia, public-sector, n=358 (%)			
Age group (years)	50000, 11-145 (76)	(/0)			
0-9	27/145 (19)	90/358 (25)			
10-19	12/145 (8)	8/358 (2)			
20-39	37/145 (26)	93/358 (26)			
40-59	53/145 (37)	85/358 (24)			
60-79	15/145 (10)	71/358 (20)			
≥80	1/145 (1)	11/358 (3)			
Sex-female	93/145 (64)	182/358 (51)			
Province*					
Eastern Cape		21/358 (6)			
Gauteng		59/358 (16)			
KwaZulu-Natal	17/145 (12)	90/358 (25)			
Mpumalanga	30/145 (21)	55/358 (15)			
North West	46/145 (32)	45/358 (13)			
Western Cape	52/145 (36)	88/358 (25)			
Race	01/14F (FC)	262/268 (22)			
Black	81/145 (56)	263/358 (73)			
Coloured Asian (Indian	36/145 (25)	58/358 (16) 1/258 (0)			
Asian/Indian White	0/145 (0) 15 (145 (10)	1/358 (0)			
White	15/145 (10) 12/145 (0)	12/358 (3)			
Other Variant	13/145 (9)	24/358 (7)			
Non-Alpha/Beta/Delta	0/145 (0)	0/358 (0)			
Alpha(20I)	0/145 (0)	1/358 (0)			
Beta(20H)	0/145 (0)	0/358 (1)			
Delta(21A, 21I, 21J)	0/145 (0)	3/358 (1)			
C.1.2(20D)	0/145 (0)	1/358 (0)			
Omicron (21K/BA.1)	11/145 (8)	70/358 (20)			
Omicron (21L/BA.2)	13/145 (9)	44/358 (12)			
Omicron (21M/BA.3)	0/145 (0)	2/358 (1)			
Omicron (22A/BA.4)	22/145 (15)	51/358 (14)			
Omicron (22B/BA.5)	16/145 (11)	38/358 (11)			
Omicron (22C/ BA.2.12.1)	0/145 (0)	0/358 (0)			
Unable to assign**	71/145 (49)	120/358 (34)			
Pending results***	12/145 (8)	28/358 (8)			
Presentation	04/422 (74)	422 (220 (20)			
Fever	94/132 (71)	133/339 (39)			
Cough	131/133 (98)	315/339 (93)			
Shortness of breath	56/132 (42)	222/332 (67)			
Chest pain	57/132 (43)	132/332 (40)			
Diarrhoea	19/132 (14)	34/332 (10)			
Underlying conditions Hypertension	30/132 (23)	50/323 (18)			
Cardiac	30/132 (23) 3/145 (2)	59/333 (18) 13/358 (4)			
Lung disease	3/145 (2) 0/132 (0)	1/333 (0)			
Diabetes		37/333 (0)			
Cancer	8/132 (6) 0/145 (0)	4/358 (1)			
Tuberculosis - Previous	1/145 (1)	4/558 (1) 5/358 (1)			
Tuberculosis - Previous	2/145 (1)	41/358 (11)			
HIV-infection	2/145 (1) 18/145 (12)	41/358 (11) 127/358 (35)			
Other ****	5/131 (4)	32/330 (10)			
SARS-CoV-2 Vaccine	-,(.,				
Pfizer-BioNTech (1 st dose)	20/145 (14)	35/358 (10)			
Pfizer-BioNTech (2 nd dose)	19/145 (13)	28/358 (8)			
Johnson & Johnson (1 st dose)	17/145 (12)	26/358 (7)			
Johnson & Johnson (2 nd dose)	3/145 (2)	2/358 (1)			
Unknown	15/145 (10)	31/145 (21)			
No vaccine	73/145 (50)	258/358 (72)			
Management					
Oxygen therapy	0/131 (0)	183/323 (57)			
ICU admission	0/131 (0)	3/323 (1)			
Ventilation	0/131 (0)	7/323 (2)			
Outcome****					
Died	0/131 (0)	21/312 (7)			

*ILI surveillance not conducted in Gauteng or Eastern Cape province **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 results: outstanding variant results

****Chronic lung, liver and kidney disease, organ transplant, pregnancy, malnutrition, obesity, tracheostomy, prematurity, seizure, stroke, anaemia, asplenia, burns, Systemic lupus erythematosus, seizures *****Outcome includes patients who are still hospitalised, have been discharged or referred, and those who died

Note: Children may be over-represented amongst hospitalised patients due to the inclusion of a large paediatric hospital in Cape Town.

Of the 21 patients who died, six were in the 20-39-year age group, seven were in 40-59 age group and eight were ≥60 years; 13/21 (62%) were female.

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 \geq 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

Allplex^{IM} 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 cleanup 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).

Page 19 of 19