### MONTHLY RESPIRATORY PATHOGENS SURVEILLANCE REPORT

SOUTH AFRICA WEEK 52 2021

COMMUNICABLE DISEASES

NATIONAL INSTITUTE FOR

# CONTENTS

Surveillance programme description	2
Comments	3
Systematic Influenza-like illness (ILI) surveillance Influenza Respiratory syncytial virus <i>Bordetella pertussis</i> SARS-CoV-2	4-9
Influenza-like illness (ILI) Viral Watch Influenza Respiratory syncytial virus SARS-CoV-2	10-13
National syndromic surveilance for pneumonia Influenza Respiratory syncytial virus <i>Bordetella pertussis</i> SARS-CoV-2	14-19
Summary of laboratory confirmed SARS-CoV-2 cases	20-21
SARS-CoV-2 Testing Methods	22

# **HIGHLIGHTS: WEEK 52**

In 2021, although there was sustained detections of influenza from week 48 to week 52 in all surveillance programmes, transmission remained below seasonal threshold. In week 52, transmission and impact were below threshold. One new case of influenza from Western Cape surveillance site was detected in week 52. To date, 440 influenza cases have been detected from Gauteng, Western Cape, North West, Eastern Cape, Mpumalanga and KwaZulu-Natal sentinel surveillance sites. From 1 January 2021 to date, influenza A(H1N1)pdm09 was the most commonly detected subtype in both influenza-like illness (ILI) surveillance (n=94/171, 55%) and pneumonia surveillance (n=103/207, 50%).

· RSV activity remains below seasonal threshold in both ILI and pneumonia surveillance programmes. From 1 January 2021 to date, RSV subgroup A was the most commonly detected subgroup in both ILI surveillance (n=43/75, 57%) and pneumonia surveillance (n=217/418, 52%).

From 2 March 2020 to 2 Jan 2022, a total of 3 211 COVID-19 cases were detected from all surveillance programmes. In week52, a decrease in detection rate of COVID-19 cases has been noted in ILI surveillance programme whereas pneumonia surveillance had a slight increase. Of the 2 245 hospitalised COVID-19 cases reported with available data on outcome, 369 (17%) died.

From 1 Jan 2021 to 2 Jan 2022, of the 1607/1881 (85%) SARS-CoV-2 positive cases with variant type results, Delta was the most detected variant in both pneumonia surveillance (631/1175, 54%) and in ILI (216/432, 50%), respectively. Delta variant predominated in both programmes (from week 22, week starting 31st May 2021 until week47). In 2021, Omicron has been detected from week46 in pneumonia surveillance (90/1175, 8%) and from week47 in ILI (34/432, 8%).

# INFLUENZA, RESPIRATORY SYNCYTIAL VIRUS AND SARS-CoV-2 SURVEILLANCE REPORT

WEEK **52** 2021

# **PROGRAMME DESCRIPTIONS**

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

### www.nicd.ac.za TOLL-FREE NUMBER 0800 029 999

PAGE 2

### WEEK **52** 2021

### **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 (Viral Watch Programme) 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.

\* EC: Eastern Cape; FS: Free State; GP: Gauteng; KZ: KwaZulu-Natal; LP: Limpopo; MP: Mpumalanga: NC: Northern Cape; NW: North West; WC: Western Cape \*\*Started in 2019

\*\*\*Started in November 2020

\*\*\*\*INF: influenza virus, RSV: respiratory syncytial virus, BP: Bordetella pertussis; SARS-CoV-2: severe acute respiratory syndrome coronavirus 2

#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).

# COMMENTS

### Influenza

In 2021, although there was sustained detection of influenza from week 48 to week 52, transmission remained below seasonal threshold. Since the first influenza positive case of 2021 was detected in pneumonia surveillance in week 9 of 2021 (week ending on the 07 March 2021), sporadic cases have been reported from week 16 to date. Of the 440 influenza cases detected in surveillance sites in 2021, the majority (n=220, 50%) were influenza A(H1N1) pdm09. In week 52, transmission and impact were below threshold.

**ILI programme:** In 2021 to date, specimens from 1 941 patients meeting ILI case definition were received from 4 ILI sites. Influenza was detected in 171 (9%) patients, of which 94 (55%) were influenza A(H1N1) pdm09, 26 (15%) influenza A(H3N2), five (3%) influenza A(inconclusive), 42 (25%) influenza B(Victoria) and four (2%) influenza B(lineage inconclusive). (Fig1, Table1).

**Viral Watch programme:** From 1 Jan 2021 to 2 Jan 2022, specimens were received from 253 patients from Viral Watch sites in 6 of the 8 provinces participating in surveillance. Influenza was detected in 35 (14%) patients, of which 17 (49%) were influenza A(H1N1)pdm09, six (17%) influenza A(H3N2), one (3%) influenza A(pending results), eight (23%) influenza B(Victoria) and three (9%) influenza B(lineage inconclusive). (Fig7, Table6)

**Pneumonia surveillance:** From 1 Jan 2021 to 2 Jan 2022, specimens from 6 183 patients with severe respiratory illness (SRI) were received from the 6 sentinel sites. Influenza was detected in 207 (3%) patients, of which 103 (50%) were influenza A(H1N1)pdm09, 34 (16%) influenza A(H3N2), 11 (5%) influenza A(subtype inconclusive), 52 (25%) influenza B(Victoria) and seven (3%) were influenza B(lineage inconclusive). Table10)

In addition, influenza was detected in 27 (3%) of 883 specimens, of which six (22%) were influenza A(H1N1)pdm09, one (4%) influenza A(H3N2), two (7%) influenza A(subtype pending results), 17 (63%) influenza B(Victoria) and one (4%) was influenza B(lineage inconclusive) from patients who met suspected SARS-CoV-2 case definition but did not meet pneumonia/ILI surveillance case definition.

### **Respiratory syncytial virus**

In 2021 to date, RSV detection has been reported from all surveillance programmes, activity remains below seasonal threshold. Of the 520 RSV cases detected in 2021, the majority (n=270, 52%) were RSV subgroup A

**ILI programme:** From 1 Jan 2021 to 2 Jan 2022, 1 941 specimens from patients meeting the ILI case definition were tested and RSV was detected in 75 (4%) patients. Of which, 43 (57%) were RSV subgroup A, 31 (41%) RSV subgroup B and one (1%) was RSV (subgroup inconclusive). (Fig3, Table2)

**Viral Watch programme:** From 1 Jan 2021 to 2 Jan 2022, 253 specimens from viral watch patients were tested and RSV was detected in specimens of six (2%) patients. Of which, three (50%) were RSV subgroup A, two (33%) RSV subgroup B and one (17%) was RSV (subgroup inconclusive). (Fig8, Table7)

**Pneumonia surveillance:** From 1 Jan 2021 to 2 Jan 2022, 6 183 specimens were tested and RSV was detected in specimens of 418 (7%) patients. Of which, 217 (52%) were RSV subgroup A, 196 (47%) RSV subgroup B and five (1%) RSV (subgroup inconclusive). (Fig13, Table11)

In addition, RSV was detected in 21 of 883 (2%) specimens, of which seven (33%) were RSV subgroup A, 13 (62%) RSV subgroup B and one (5%) was RSV (subgroup inconclusive) from patients who met suspected SARS-CoV-2 case definition but did not meet pneumonia/ILI surveillance case definition.

### Bordetella pertussis

**ILI programme:** From 1 Jan 2021 to 2 Jan 2022, combined nasopharyngeal and oropharyngeal specimens were tested from 1 921 patients and *B. pertussis* was not detected. (Fig4, Table3)

**Pneumonia surveillance:** During the same period, combined nasopharyngeal and oropharyngeal specimens were tested from 6 180 patients and *B. pertussis* was detected in one (0.02%). (Fig14, Table12). The case was a two month old female, identified in week 33 from Rahima Moosa Hospital, Gauteng Province.

In addition, *B. pertussis* was not detected in 883 specimens from patients who met suspected SARS-CoV-2 case definition but did not meet the pneumonia/ILI surveillance case definition.

# SARS-CoV-2 (Severe acute respiratory syndrome coronavirus 2)

**ILI programme:** From March 2020 to 2 Jan 2022, 3 256 patients were tested and SARS-CoV-2 was detected in 628 (19%) patients. From 1 Jan 2021 to 2 Jan 2022, of the 432/487 (89%) with data on variant type, majority (216/432, 50%) were Delta variant which predominated from week25 to week40, followed by Beta (157/432, 36%) variant which predominated from week1 to week 24. Omicron has been detected from week47 (34/432, 8%). (Fig5, Table4)

**Viral Watch programme:** From March 2020 to 2 Jan 2022, 532 patients presenting with ILI were tested and SARS-CoV-2 was detected in 98 (18%) patients. From 1 Jan 2021 to 2 Jan 2022, of the 40/61 (66%) with data on variant type, majority were (24/40, 60%) were Delta variant which dominated from week 25 to week 30. Omicron has been detected from week47 (11/40, 28%). (Fig10, Table9)

**Pneumonia surveillance:** From March 2020 to 2 Jan 2022, 10 198 patients with severe respiratory illness (SRI) were tested and SARS-CoV-2 was detected in 2 245 (22%) patients. From 1 Jan 2021 to 2 Jan 2022, of the 1175/1394 (84%) with data on variant type, majority were (631/1175, 54%) were Delta variant which dominated from week 22 to week 47 followed by Beta (402/1175, 34%) variant which dominated from week 1 to 25. Omicron has been detected from week46 (90/1175, 8%). (Fig16, Table14)

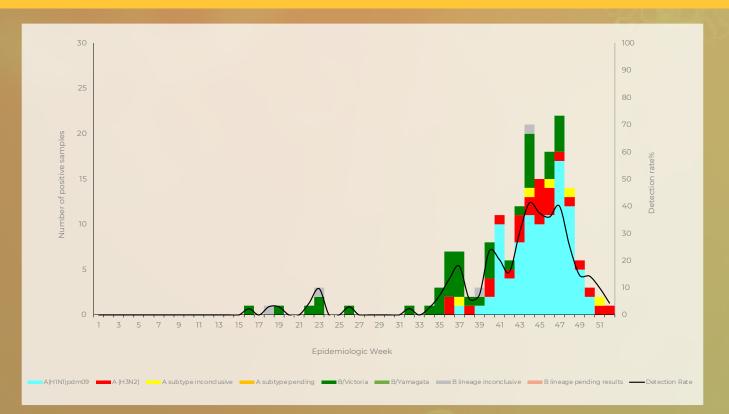
In addition, SARS-CoV-2 was detected in 240 of 1085 (22%) specimens from patients who met suspected SARS-CoV-2 case definition but did not meet the pneumonia/ILI surveillance case definitions.

### www.nicd.ac.za TOLL-FREE NUMBER 0800 029 999

PAGE 3

### WEEK **52** 2021

### INFLUENZA-LIKE ILLNESS (ILI) SURVEILLANCE PRIMARY HEALTH CARE CLINICS



**Figure 1.** Number of influenza positive cases\* by influenza subtype and lineage\*\* and detection rate\*\*\* by week, Influenza-like illness (ILI) surveillance primary health care clinics, 04/01/2021 – 02/01/2022

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

\*\*Influenza was detected in 20 (3%) of 654 specimens, of which three (15%) were influenza A(H1N1)pdm09, one (5%) influenza A(H3N2), two (10%) influenza A(subtype inconclusive), 13 (65%) influenza B(Victoria) and one (5%) was influenza B(inconclusive) 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

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 primary health care clinics, 04/01/2021 – 02/01/2022

Clinic (Province)	A(H1N1) pdm09	A(H3N2)	A subtype inconclusive	A subtype pending results <sup>®</sup>	B/ Victoria	B/ Yamagata	B lineage inconclusive	B lineage pending results <sup>ଃ</sup>	Total samples
Agincourt (MP)		3		0	0	0	0	0	246
Eastridge (WC)	9	11	0	0		Ο	0	0	262
Edendale Gateway (KZ)	14			0	16	0		0	304
Jouberton (NW)	46	5		О	13	0	О	0	798
Mitchell's Plain (WC)	24	6	2	0	6	0	1	0	331
Total:	94	26	5	0	42	0	4	0	1 941

KZ: KwaZulu-Natal; NW: North West; WC: Western Cape; MP: Mpumalanga Inconclusive: insufficient viral load in sample and unable to characterise further

\*\*Influenza was detected in 20 (3%) of 654 specimens, of which three (15%) were influenza A(H1N1)pdm09, one (5%) influenza A(H3N2), two (10%) influenza A(subtype inconclusive), 13 (65%) influenza B(Victoria) and one (5%) was influenza B(inconclusive) 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.

www.nicd.ac.za TOLL-FREE NUMBER 0800 029 999

PAGE 4

### WEEK **52** 2021

### **INFLUENZA-LIKE ILLNESS (ILI) SURVEILLANCE PRIMARY HEALTH CARE CLINICS**

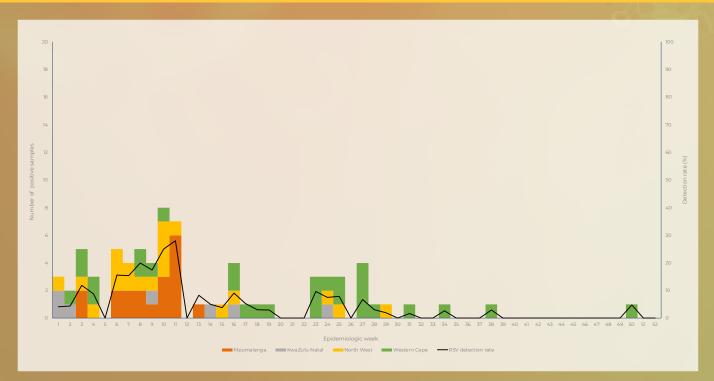


Figure 2. Number of patients testing positive for respiratory syncytial virus\* by province and detection rate\*\* by week, Influenzalike illness (ILI) surveillance primary health care clinics, 04/01/2021 – 02/01/2022

\*\*RSV was detected from 15 of 654 (2%) 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.

### www.nicd.ac.za TOLL-FREE NUMBER 0800 029 999

PAGE 5

### WEEK 52 2021

### **INFLUENZA-LIKE ILLNESS (ILI) SURVEILLANCE PRIMARY HEALTH CARE CLINICS**



Figure 3. Number of patients testing positive for respiratory syncytial virus\*\* by subgroup and detection rate by week,

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 primary health care clinics, 04/01/2021 - 02/01/2022

Clinic (Province)	RSVA	RSVB	RSVAB	RSV subgroup inconclusive	RSV subgroup pending*	Total samples
Agincourt (MP)	12	7	0	0	0	246
Eastridge (WC)	25		0	О	Ο	262
Edendale Gateway (KZ)		6	0	О	Ο	304
Jouberton (NW)	2	17	0		О	798
Mitchell's Plain (WC)	3	0	0	0	О	331
Total	43	31	0	1	0	1 941

Inconclusive: insufficient viral load in sample and unable to characterise further RSV AB: Both RSV A and B subgroup identified \*RSV results for subgroups are pending

\*\*RSV was detected from 15 of 654 (2%) 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.

#### www.nicd.ac.za TOLL-FREE NUMBER 0800 029 999

PAGE 6

### WEEK **52** 2021

# 

### **INFLUENZA-LIKE ILLNESS (ILI) SURVEILLANCE PRIMARY HEALTH CARE CLINICS**

**Figure 4.** Number of samples testing positive for *B. pertussis* and detection rate by month, Influenza-like illness (ILI) surveillance primary health care clinics, 04/01/2021 – 02/01/2022

\*\*No *B. pertussis* was detected in 654 specimens of patients who met the suspected *B. pertussis* case definition but did not meet Influenza-like illness case definition. These are not included in the epidemiological curve

Table 3. Cumulative number of B. pertussis identified and total number of samples\*\* tested by province, Influenza-like illness(ILI) surveillance primary health care clinics, 04/01/2021 – 02/01/2022

Clinic (Province)	B. pertussis Positive**	Total samples tested		
Agincourt (MP)	0	245		
Eastridge (WC)	0	262		
Edendale Gateway (KZ)	0	304		
Jouberton (NW)	0	794		
Mitchell's Plain (WC)	0	316		
Total:	0	1 921		

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

\*\*No B. pertussis was detected in 654 specimens of patients who met the suspected B. pertussis case definition but did not meet Influenza-like illness case definition. These are not included in the table.

### www.nicd.ac.za TOLL-FREE NUMBER 0800 029 999

PAGE 7

### WEEK **52** 2021

### INFLUENZA-LIKE ILLNESS (ILI) SURVEILLANCE PRIMARY HEALTH CARE CLINICS

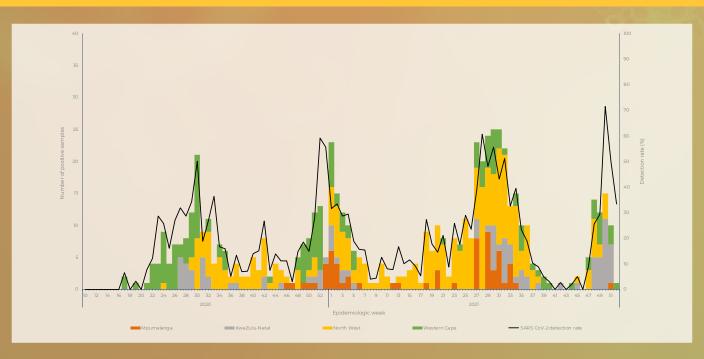


Figure 5. Number of patients testing positive for SARS-CoV-2\* by province and detection rate by week, Influenza-like illness (ILI) surveillance primary health care clinics, 02/03/2020 – 02/01/2022

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

\*\*SARS-CoV-2 was detected in 161 of 785 (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.

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, 02/03/2020 – 02/01/2022

Clinic (Province)	SARS-CoV-2 positive	Total samples tested
Agincourt (MP)	63	277
Eastridge (WC)	62	751
Edendale Gateway (KZ)	97	447
Jouberton (NW)	304	1121
Mitchell's Plain (WC)	102	660
Total:	628	3 256

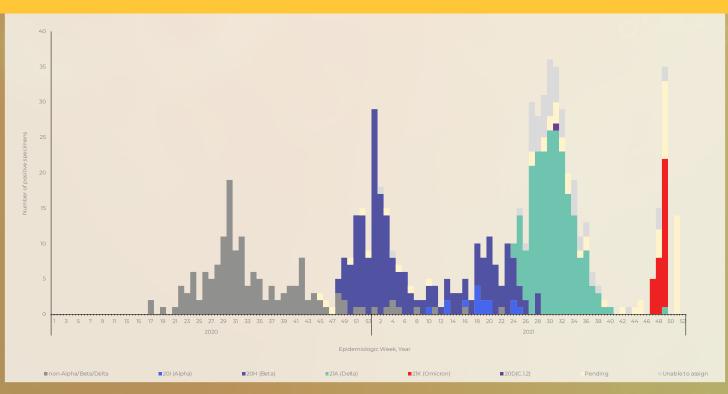
KZ: KwaZulu-Natal; NW: North West; WCP: Western Cape; MP: Mpumalanga (started enrolling on the 10th November 2020)

\*\*SARS-CoV-2 was detected in 161 of 785 (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.

### www.nicd.ac.za TOLL-FREE NUMBER 0800 029 999

PAGE 8

### WEEK 52 2021



### **INFLUENZA-LIKE ILLNESS (ILI) SURVEILLANCE PRIMARY HEALTH CARE CLINICS**

Figure 6. Number and detection rate of laboratory confirmed SARS-CoV-2\* cases by variant type (variant PCR/sequencing) and

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 5. Number of SARS-CoV-2\* positive cases 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, 02/03/2020 - 02/01/2022

Clinic (Province)	Non-Alpha/ Beta/Delta	20I (Alpha)	20H (Beta)	21A (Delta)	20D (C.1.2)	21K (Omicron)	Pending	Unable to assign	Total SARS-CoV-2 positive
Agincourt (MP)	4	0	29	34	0	0	11	6	84
Eastridge (WC)	20	Ο	19	9	Ο	0	5	2	55
Edendale Gateway (KZ)	27	0	25	48	0	14	23	9	146
Jouberton (NW)	67	15	105	110		14	15	32	359
Mitchell's Plain (WC)	35	О	28	15	0	6	7	8	99
Total:	153	15	206	216	1	34	61	57	743

\*Specimens are from patients with influenza-like illness at 5 sentinel sites in 4 provinces who met suspected SARS-CoV-2 case definition and met pneumonia (SRI) case definition as wel did not meet the ILL case definition.

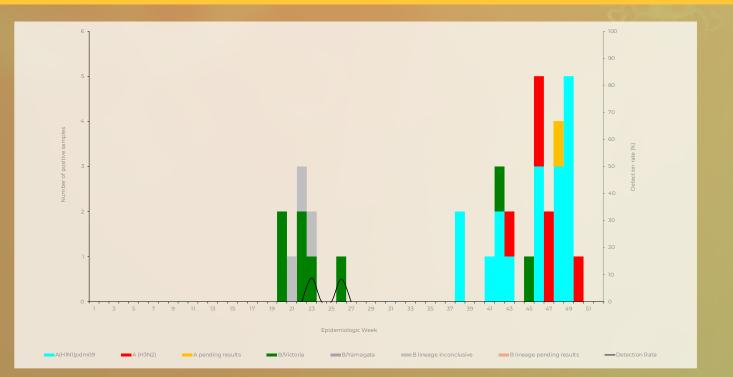
Unable to assign: no lineage assigned due to poor- sequence quality OR low viral load (ct=>35) OR variant PCR could not assign variant and no sequencing result Pending: outstanding variant results

#### TOLL-FREE NUMBER 0800 029 999 www.nicd.ac.za

PAGE 9

### WEEK **52** 2021

### **INFLUENZA-LIKE ILLNESS (ILI) SURVEILLANCE VIRAL WATCH**



**Figure 7.** Number of positive patients\* by influenza subtype and lineage and detection rate\*\* by week, ILI surveillance - Viral Watch, 04/01/2021 – 02/01/2022

\*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 6. Number of laboratory confirmed influenza cases by influenza subtype and lineage and total number of samples tested by province, ILI surveillance - Viral Watch, 04/01/2021 – 02/01/2022

Province	A(H1N1) pdm09	A(H3N2)	A subtype inconclusive	A subtype pending results*	B/ Victoria	B/ Yamagata	B lineage inconclusive	B lineage pending results*	Total samples
Eastern Cape	0	0	0	0	2	0	0	0	4
Free State		0	0	0	Ο	О	О	0	4
Gauteng	6	2	0	0	5	О	3	0	180
Limpopo	0	0	0	0	0	О	О	0	О
Mpumalanga	0	0	0	0	0	О	О	0	3
North West	0	0	0	0	0	О	О	0	2
Northern Cape	0	0	0	0	0	О	О	0	О
Western Cape	10	4	О	1		0	0	0	60
Total:	17	6	0	1 1	8	0	3	0	253

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

From 04 January 2021 to date, no patients were tested for influenza at the time of entry into South Africa following travel abroad.

Patients known to have acquired influenza abroad are not included in the table or epidemiological curve.

### www.nicd.ac.za TOLL-FREE NUMBER 0800 029 999

PAGE **10** 

### WEEK **52** 2021

### **INFLUENZA-LIKE ILLNESS (ILI) SURVEILLANCE VIRAL WATCH**

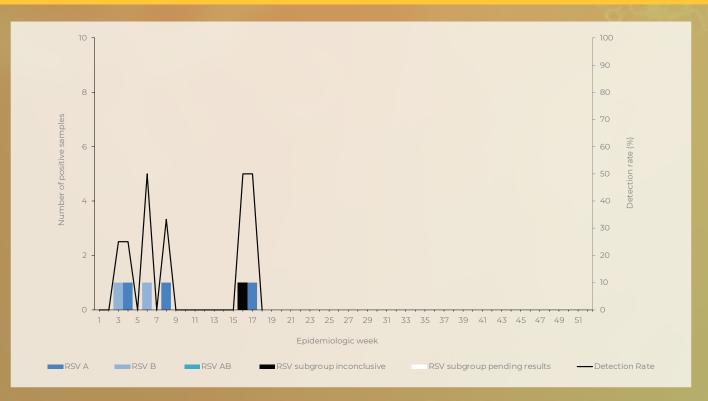


Figure 8. Number of RSV positive cases testing positive for respiratory syncytial virus (RSV)\* by subgroup and detection rate by week, ILI surveillance - Viral Watch, 04/01/2021 – 02/01/2022

\*Specimens from patients with Influenza-like illnesses at 92 sentinel sites in 8 provinces \*RSV results for subgroups are pending

 Table 7. Number of RSV positive cases identified and total number of samples tested by province, ILI surveillance - Viral Watch, 04/01/2021 – 02/01/2022

Province	RSV A	RSV B	RSV AB	RSV subgroup inconclusive	RSV subgroup pending results*	Total samples tested
Eastern Cape	0	0	0	0	0	4
Free State	О	О	0	0	0	4
Gauteng	2	2	О	0	0	180
Limpopo	О	О	О	0	0	0
Mpumalanga	О	0	0	0	0	3
North West	0	0	О	0	0	2
Northern Cape	0	Ο	О	0	0	О
Western Cape	<b>nesi</b> ()	<b>o</b>	0	1	0	60
Total:	3	2	0	1	0	253

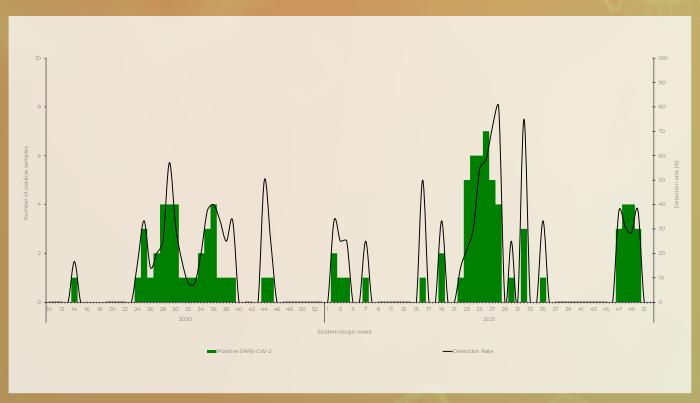
\*RSV results for subgroups are pending

www.nicd.ac.za TOLL-FREE NUMBER 0800 029 999

PAGE 11

### WEEK **52** 2021

### **INFLUENZA-LIKE ILLNESS (ILI) SURVEILLANCE VIRAL WATCH**



**Figure 9.** Number of patients testing positive for SARS-CoV-2\*, by site and detection rate by week, ILI surveillance - Viral Watch, 02/03/2020 – 02/01/2022

\*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,02/03/2020 - 02/01/2022

Province	SARS-CoV-2 positive	Total samples tested
Eastern Cape	1	8
Free State		18
Gauteng	71	340
Limpopo	0	2
Mpumalanga		8
North West	0	2
Northern Cape	0	2
Western Cape	24	152
Total:	98	532

www.nicd.ac.za TOLL-FREE NUMBER 0800 029 999

PAGE **12** 

WEEK **52** 2021

### INFLUENZA-LIKE ILLNESS (ILI) SURVEILLANCE VIRAL WATCH



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, 02/03/2020 – 02/01/2022

\*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 (ct=>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, 02/03/2020 – 02/01/2022

Clinic (Province)	Non-Alpha/ Beta/Delta	201 (Alpha)	20H (Beta)	21A (Delta)	20D (C.1.2)	21K (Omicron)	Pending	Unable to assign	Total SARS-CoV-2 positive
Eastern Cape	0	0	0	0	0	0	1	0	1.1
Free State	0	0	О	0	О	0		0	
Gauteng	0	3	О	23	2	5	35	3	71
Limpopo	0	0	О	Ο	Ο	0	0	Ο	0
Mpumalanga	0	Ο	О	О	Ο	0		Ο	
North West	0	0	О	0	0	0	0	Ο	0
Northern Cape	0	0	0	0	О	0	0	Ο	0
Western Cape	0	0	О	1755	О	6	17	О	24
Total:	0//	3	0	24	2	n	55	3	98

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

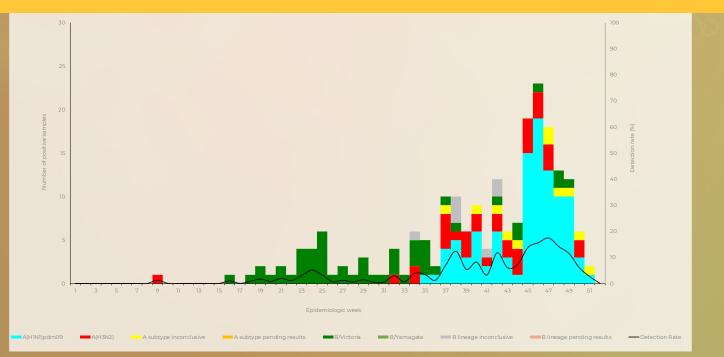
Unable to assign: no lineage assigned due to poor- sequence quality OR low viral load (ct=>35) OR variant PCR could not assign variant and no sequencing result Pending: outstanding variant results

### www.nicd.ac.za TOLL-FREE NUMBER 0800 029 999

PAGE **13** 

### WEEK **52** 2021

### NATIONAL SYNDROMIC SURVEILLANCE FOR PNEUMONIA



**Figure 11.** Number of positive influenza positive cases\* by influenza subtype and lineage\*\* and detection rate\*\*\* by week, pneumonia surveillance public hospitals, 04/01/2021 – 02/01/2022

\*Specimens from patients hospitalized with pneumonia at 7 sentinel sites in 5 provinces

\*\*Influenza was detected in seven (3%) of 229 specimens, of which three (43%) was influenza A(HINI)pdm09 and four (57%) were influenza B(Victoria) 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.

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

**Table 10.** Number of laboratory confirmed influenza cases by subtype and lineage\*\* and total number of samples tested by hospital, pneumonia surveillance public hospitals, 04/01/2021 – 02/01/2022

Hospital (Province)	A(H1N1) pdm09	A(H3N2)	A subtype inconclusive	A subtype pending results***	B/ Victoria	B/ Yamagata	B lineage inconclusive	B lineage pending results***	Total samples
Edendale (KZ)	8	3	0	0	13	Ο	0	0	985
Helen Joseph- Rahima Moosa (GP)	24	14	3	0	17	0	5	Ο	1641
Klerksdorp- Tshepong (NW)	18		3	0	4	0	2	0	955
Mapulaneng- Matikwana (MP)	8		0	0	2	0	Ο	0	578
Red Cross (WC)	25	11		0	13	О	О	0	815
Mitchell's Plain (WC)	16	3	2	0	2	0	Ο	0	948
Tintswalo (MP)	4	<b>MAN</b>	2	0	1	0	0	0	261
Total:	103	34	11	0	52	0	7	0	6 183

GP: Gauteng; KZ: KwaZulu-Natal; NW: North West; MP: Mpumalanga (Tintswalo started enrolling on the 10th Feb 2021); WC: Western Cape Inconclusive: insufficient viral load in sample and unable to characterise further

\*\*Influenza was detected in seven (3%) of 229 specimens, of which three (43%) was influenza A(H1N1)pdm09 and four (57%) were influenza B(Victoria) 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.

www.nicd.ac.za TOLL-FREE NUMBER 0800 029 999

PAGE **14** 

### WEEK **52** 2021

### NATIONAL SYNDROMIC SURVEILLANCE FOR PNEUMONIA

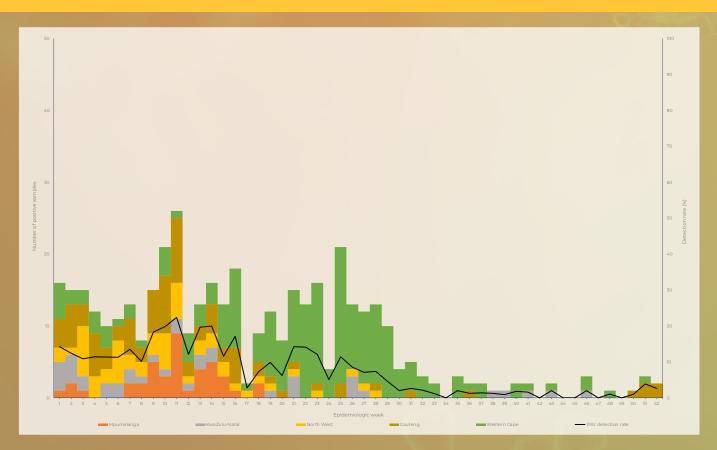


Figure 12. Number of patients testing positive for respiratory syncytial virus\* by province and detection rate by week, pneumonia surveillance public hospitals, 04/01/2021 – 02/01/2022

\*RSV was detected in six of 229 (3%) 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.

### www.nicd.ac.za TOLL-FREE NUMBER 0800 029 999

PAGE **15** 

### WEEK 52 2021

### NATIONAL SYNDROMIC SURVEILLANCE FOR PNEUMONIA

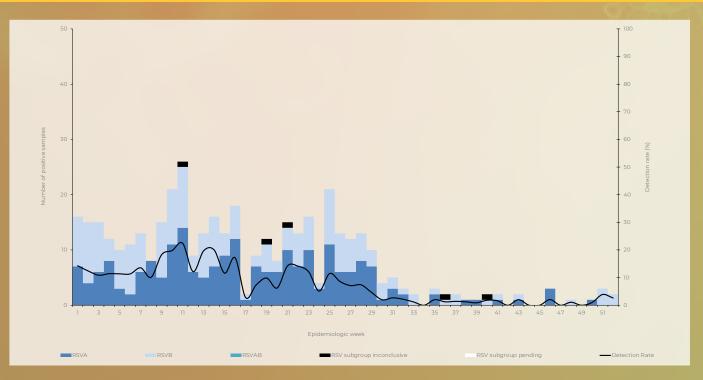


Figure 13. Number of patients testing positive for respiratory syncytial virus\* by subgroup and detection rate by week,

 Table 11: Number of patients positive for respiratory syncytial virus subgroups\*\* by subgroups identified and total number of

 samples tested by hospital, pneumonia surveillance public hospitals, 04/01/2021 – 02/01/2022

Hospital (Province)	RSVA	RSVB	RSVAB	RSV subgroup inconclusive	RSV subgroup pending*	Total samples
Edendale (KZ)	10	29	0	0	Ο	985
Helen Joseph-Rahima Moosa (GP)	38	46	0		Ο	1641
Klerksdorp-Tshepong (NW)	8	45	О		Ο	955
Mapulaneng-Matikwana (MP)	21	7	0	Ο	О	578
Red Cross (WC)	88	53	0	2	Ο	815
Mitchell's Plain (WC)	42	13	О		Ο	948
Tintswalo (MP)	10	3	0	О	Ο	261
Total:	212	189	0	5	0	5187

GP: Gauteng; KZ: KwaZulu-Natal; NW: North West; MP: Mpumalanga (Tintswalo started enrolling on the 10th Feb 2021); WC: Western Cape Inconclusive: insufficient viral load in sample and unable to characterise further

RSV AB: Both RSV A and B subgroup identified \*RSV results for subgroups are pending

\*\*RSV was detected in six of 229 (3%) 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

#### www.nicd.ac.za TOLL-FREE NUMBER 0800 029 999

PAGE **16** 

### WEEK **52** 2021

### NATIONAL SYNDROMIC SURVEILLANCE FOR PNEUMONIA

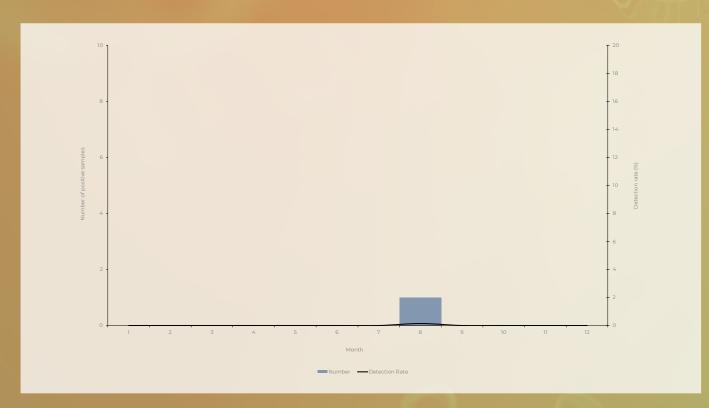


Figure 14. Number of patients testing positive for B. pertussis and detection rate by month, pneumonia surveillance public hospitals, 04/01/2021 – 02/01/2022

\*\*No *B. pertussis* was detected in 229 specimens of patients who met the suspected B. pertussis case definition but did not meet Pneumonia Surveillance case definition These are not included in the epidemiologic curve.

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, 04/01/2021 – 02/01/2022

Hospital (Province)	B. pertussis Positive**	Total samples tested		
Edendale (KZ)	Ο	983		
Helen Joseph-Rahima Moosa (GP)		1633		
Klerksdorp-Tshepong (NW)	0	948		
Mapulaneng-Matikwana (MP)	0	587		
Red Cross (WC)	Ο	813		
Mitchell's Plain (WC)	Ο	951		
Tintswalo (MP)	0	265		
Total:		6 180		

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

\*\*No B. pertussis was detected in 229 specimens of patients who met the suspected B. pertussis case definition but did not meet Pneumonia Surveillance case definition. These are not included in the table

### www.nicd.ac.za TOLL-FREE NUMBER 0800 029 999

PAGE **17** 

### WEEK **52** 2021

### NATIONAL SYNDROMIC SURVEILLANCE FOR PNEUMONIA

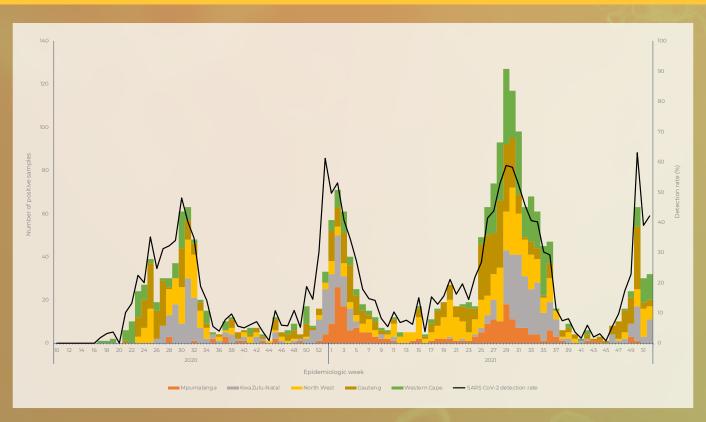


Figure 15. Number of samples testing positive for SARS-CoV-2<sup>\*8</sup> province and detection rate by week, pneumonia surveillance public hospitals, 02/03/2020 – 02/01/2022

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

\*<sup>\$</sup>SARS-CoV-2 was detected in 79 of 300 (26%) 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. These are not included in the epidemiological curve.

Table 13. Number of patients positive for SARS-CoV-2\*\* and total number of samples tested by hospital, pneumonia surveillance public hospitals, 02/03/2020 – 02/01/2022

Hospital (Province)	SARS-CoV-2 positive	Total samples tested		
Edendale (KZ)	503	1832		
Helen Joseph-Rahima Moosa (GP)	563	2 487		
Klerksdorp-Tshepong (NW)	507	1548		
Mapulaneng-Matikwana (MP)	168	880		
Red Cross (WC)	72	1 773		
Mitchell's Plain (WC)	377	1 418		
Tintswalo (MP)	55	260		
Total:	2 245	10 198		

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

\*\*SARS-CoV-2 was detected in 79 of 300 (26%) 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.

### www.nicd.ac.za TOLL-FREE NUMBER 0800 029 999

PAGE **18** 

### WEEK **52** 2021

### NATIONAL SYNDROMIC SURVEILLANCE FOR PNEUMONIA

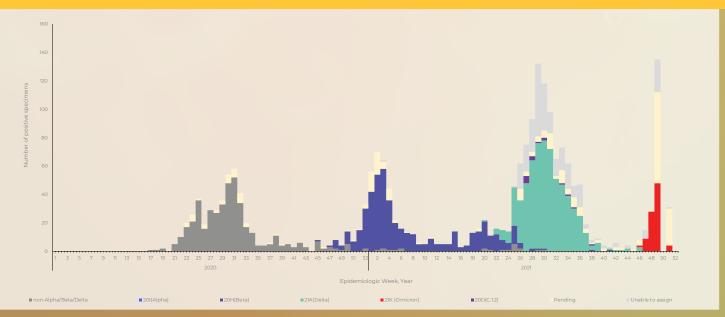


Figure 16. Number and detection rate of laboratory confirmed SARS-CoV-2 cases\* by variant type (variant PCR/sequencing), pneumonia surveillance public hospitals, 02/03/2020 – 02/01/2022

\*Specimens are from hospitalized patients at 7 sentinel sites in 5 provinces who met suspected SARS-CoV-2 case definition and met pneumonia (SRI) case definition as well as those that did not meet the 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 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, 02/03/2020 – 02/01/2022

Hospital (Province)	Non-Alpha/ Beta/Delta	20I (Alpha)	20H (Beta)	21A (Delta)	20D (C.1.2)	21K (Omicron)	Pending	Unable to assign	SARS-CoV-2 positive
Edendale (KZ)	101	1	79	151	2	12	105	56	507
Helen Joseph-Rahima Moosa (GP)	135	5	122	118	7	54	45	80	566
Klerksdorp-Tshepong (NW)	130	9	113	128	3	14	29	51	477
Mapulaneng- Matikwana (MP)	16	0	90	48	Ο	0	18	23	195
Red Cross (WC)	17	Ο	6	11	0	6	13	2	55
Mitchell's Plain (WC)	51	0	50	154	2	4	45	59	365
Tintswalo (MP)	О	1	12	21	1	0	13	5	53
Total:	450	16	472	631	15	90	268	276	2 218

CP: Gauteng; KZ: KwaZulu-Natal; NW: North West; MP: Mpumalanga (Tintswalo started enrolling on the 10th Feb 2021); WC: Western Cape \*Specimens are from hospitalized patients at 7 sentinel sites in 5 provinces who met suspected SARS-CoV-2 case definition and met pneumonia (SRI) case definition as well as those that did not meet the 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

#### www.nicd.ac.za TOLL-FREE NUMBER 0800 029 999

PAGE **19** 

WEEK **52** 2021

### SUMMARY OF LABORATORY CONFIRMED SARS-COV-2 CASES

**Table 15.** Characteristics of individuals with laboratory-confirmed SARS-CoV-2, enrolled in influenza-like illness (ILI) and pneumonia surveillance programmes, South Africa, 2 March 2020 - 02 January 2022

Characteristic	Influenza–like illness (ILI), public-sector, n=789 (%)	Pneumonia, n=2 324 (%)		
Age group				
0-9	58/789 (7)	177/2324 (8)		
10-19	56/789 (7)	19/2324 (1)		
20-39	373/789 (47)	471/2324 (20)		
40-59	242/789 (31)	833/2324 (36)		
60-79	58/789 (7)	742/2324 (32)		
≥80	2/789 (<1)	82/2324 (4)		
Sex-female	478/789 (61)	1415/2324 (61)		
Province*				
Gauteng	N/A	589/2324 (25)		
KwaZulu-Natal	150/789 (19)	520/2324 (22)		
Mpumalanga**	89/789 (11)	258/2324 (11)		
North West	382/789 (48)	507/2324 (22)		
Western Cape	168/789 (21)	450/2324 (19)		
Race				
Black	595/783 (76)	1856/2319 (80)		
Coloured	154/783 (20)	355/2319 (15)		
Asian/Indian	4/783 (1)	52/2319 (2)		
White	24/783 (3)	46/2319 (2)		
Other	6/783 (1)	10/2319 (<1)		
Variant <sup>ss</sup>				
Non-Alpha/Beta/Delta	153/686 (22)	450/1942 (23)		
20I(Alpha)	15/686 (2)	16/1942 (1)		
20H(Beta)	206/686 (30)	472/1942 (24)		
21A(Delta)	216/686 (31)	631/1942 (32)		
20D(C.1.2)	1/686 (0.1)	15/1942 (1)		
21K(Omicron)	34/686 (5)	90/1942 (5)		
Pending results	61/686 (9)	268/1942 (14)		

### www.nicd.ac.za TOLL-FREE NUMBER 0800 029 999

PAGE **20** 

WEEK **52** 2021

Characteristic	Influenza–like illness (ILI), public-sector, n=789 (%)	Pneumonia, n=2 324 (%)		
Presentation				
Fever	600/783 (77)	1061/2319 (46)		
Cough	764/783 (98)	2178/2319 (94)		
Shortness of breath	270/783 (34)	1747/2319 (75)		
Chest pain	339/783 (43)	913/2319 (39)		
Diarrhoea	61/783 (8)	146/2319 (6)		
Underlying conditions				
Hypertension <sup>\$</sup>	69/525 (13)	476/1480 (32)		
Cardiac	3/783 (<1)	51/2319 (2)		
Lung disease	0/783 (0)	4/2319 (<1)		
Diabetes	18/783 (2)	456/2319 (20)		
Cancer	2/783 (<1)	14/2319 (1)		
Tuberculosis	11/783 (1)	61/2319 (3)		
HIV-infection	143/783 (18)	529/2319 (23)		
Other ***	13/783 (2)	94/2319 (4)		
SARS-CoV-2 Vaccine				
Pfizer-BioNTech (1st dose)	20/490 (4)	121/1421 (9)		
Pfizer-BioNTech (2nd dose)	8/490 (2)	35/1421 (2)		
Johnson & Johnson	27/490 (6)	20/1421 (1)		
Management				
Oxygen therapy	9/783 (1)	1738/2319 (75)		
ICU admission	N/A	51/2319 (2)		
Ventilation	N/A	56/2319 (2)		
Outcome***				
Died	0/780 (0)	369/2245 (16)		

\*Mpumalanga (ILI site started enrolling on the 10th November 2020 and an additional SARI site started enrolling on the 10th February 2

\*\*Chronic lung, liver and kidney disease, organ transplant, pregnancy, malnutrition, obesity, tracheostomy, prematurity, seizure, stroke, anaemia, asplenia, burns, Systemic lupus erythematosus, s

<sup>5</sup>Data on hypertension was not collected on all cases.

<sup>55</sup> These individuals with unassigned sequence are not included, ILI (n=181) and SRI (n=594)

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

Of the 369 patients who died, four were in <20 age group, 29 in the 20-39 year age group, 127 in the 40-59 year age group, and 210 were  $\geq$ 60 years; 209/369 (57%) were temale

### www.nicd.ac.za TOLL-FREE NUMBER 0800 029 999

PAGE **21** 

### WEEK **52** 2021

## 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 (Ct) <40 interpreted as positive for SARS-CoV-2. From April 2021 to date the laboratory changed to the Allplex<sup>™</sup> SARS-CoV-2/FluA/FluB/ RSV kit (Seegene Inc., Seoul, South Korea), with positivity assigned if the PCR cycle threshold (Ct) 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

Allplex<sup>™</sup> 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.

Allplex<sup>™</sup> 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  $\mu$ l per sample, in order to increase yields. 300  $\mu$ l of each sample was used for automated magnetic bead-based extraction using the Chemagic 360. RNA was eluted in 60  $\mu$ l 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, (https://sarscov-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).

#### www.nicd.ac.za TOLL-FREE NUMBER 0800 029 999

PAGE 22