VIROLOGICAL SURVEILLANCE REPORT OF THE NRC INFLUENZA FOR SEASON 2021-2022

Activity Report

CYRIL BARBEZANGE • SARAH DENAYER • FRANÇOIS DUFRASNE • ASSIA HAMOUDA • REINOUT VAN EYCKEN • ILHAM FDILLATE • BERT MONSIEURS • STEVEN VAN GUCHT

WHO WE ARE

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As our name suggests, science and health are central to our mission. Sciensano's strength and uniqueness lie within the holistic and multidisciplinary approach to health. More particularly we focus on the close and indissoluble interconnection between human and animal health and their environment (the "One health" concept). By combining different research perspectives within this framework, Sciensano contributes in a unique way to everybody's health.
For this, Sciensano builds on the more than 100 years of scientific expertise.

Sciensano

Infectious diseases in humans - Viral diseases

Respiratory Viruses

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Cyril Barbezange

•

Sarah Denayer

•

François Dufrasne

•

Assia Hamouda

•

Reinout Van Eycken

•

Ilham Fdillate

•

Bert Monsieurs

•

Steven Van Gucht

•

In collaboration with

Nathalie Bossuyt, Epidemiology of infectious diseases
Koen Blot, Epidemiology of infectious diseases
Floriane Rouvez, Health services research
Robrecht De Schreye, Health services research
Milena Callies, Health-associated infections and antimicrobial resistances
Boudewijn Catry, Health-associated infections and antimicrobial resistances

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INAMI-RIZIV

Santé publique Sécurité de la Chaîne alimentaire Environnement

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ABSTRACT

Based on the sentinel surveillance networks for influenza-like illness (ILI) and severe acute respiratory infections (SARI), the 2021-2022 season was characterized by the return of seasonal influenza viruses, cause an epidemic wave in Spring, so later than pre-COVID season. Seasonal influenza A viruses of the H3N2 subtype clearly dominated over the H1N1 subtype and the influenza B viruses. SARS-CoV-2 virus continued to intensively circulate during the whole period with several epidemic waves occurring throughout the season. Respiratory syncytial virus was not responsible for a true epidemic wave this season. Other respiratory viruses such as metapneumoviruses, parainfluenza viruses, seasonal coronaviruses, rhino- and enteroviruses, and adenoviruses were also regularly detected, without clear epidemic wave.

BACKGROUND

Influenza viruses are a major cause of human morbidity and mortality worldwide. Yearly epidemics during the winter months have also a huge impact on the health care systems and the economic.
Surveillance of influenza viruses is coordinated at the global level by WHO (World Health Organisation) through the GISRS network implemented in 1952. The network is organised with reference national laboratories in each country (National Influenza Centre), regional supranational organisations (such as ECDC, European Centre for Disease Control and prevention, in the European Union) and WHO Collaborating Centres, all working together to exchange information and viruses. The main objectives of the surveillance are to monitor the influenza activity (start, intensity, duration) over the whole year, to determine the type and subtype/lineage of influenza viruses circulating, to characterise at the viruses at the antigenic and genetic level, to contribute to the decision process on the yearly influenza vaccine content, to assess the overall vaccine effectiveness, to monitor the susceptibility to antivirals of the circulating viruses, and to detect the appearance of new (non-seasonal) influenza viruses in the human population.
Since the beginning of the COVID-19 pandemic in 2020, it has become clear that an integrated surveillance of several respiratory viruses is needed and such a recommendation has been made by WHO [a] and ECDC [b].

Traditionally, an “influenza” season was defined by the period running from week 40 of one year to week 20 of the following year in the Northern Hemisphere. The rest of the year was defined as the inter-seasonal period.
Since the COVID-19 pandemic, the surveillance in Belgium is officially running all year round and a season is defined from week 40 of the year to week 39 of the following year. The season 2021-2022 thus started on week 40-2021 and ended at the end of week 39-2022.

The surveillance relies on different systems. ‘Sentinel’ surveillance involves dedicated networks of general practitioners, hospitals, nursing homes, or other settings, who recruit cases based and a precise clinical case definitions. In Belgium, sentinel surveillance included networks of general practitioners (ILI: influenza-like illness), nursing homes (NH-ILI) and hospitals (SARI: severe acute respiratory infection). All other types of surveillance are designated as ‘non sentinel’ and cover the collection of data from different partners.

For the ILI surveillance (mild cases), several case definitions are available.

- WHO-ILI: sudden onset of symptoms, with fever and cough or dyspnoea.
- ECDC-ARI: sudden onset of symptoms with at least one of the following: cough, sore throat, shortness of breath, coryza.
- ECDC-ILI: sudden onset of symptoms with at least one general symptom among fever, history of fever, malaise, headache or myalgia, and at least one respiratory symptom among cough, sore throat or shortness of breath.

The WHO ILI case definition strictly includes fever, when the ECDC ILI case definition is broader and is not restricted to fever as general symptoms. For an integrated surveillance of influenza, SARS-CoV-2 and RSV, the WHO case definition might have to evolve.

For the SARI surveillance (hospitalised cases), the WHO-SARI case definition is used:

[a] https://www.who.int/initiatives/mosaic-respiratory-surveillance-framework
[b] https://www.ecdc.europa.eu/en/seasonal-influenza/surveillance-and-disease-data/facts

- Onset of symptoms within 10 days of hospitalisation/sampling
- Fever or history of fever
- Cough or dyspnoea

Since season 2020-2021, the hospitals had the opportunity to enrol cases matching the BE-COVID case definition:

- At least one of the following: fever, history of fever, cough, shortness of breath, fatigue, anosmia, ageusia, diarrhoea, loss of appetite.

The BE-COVID case definition is slightly broader than the ECDC-COVID:

- At least one of the following: fever, history of fever, cough, shortness of breath, anosmia, ageusia.

All samples collected through the sentinel surveillance networks are sent to the NRC (National Reference Centre) influenza for testing. All samples are tested to detect the presence of influenza viruses and determine the type and subtype/lineage for seasonal influenza viruses. Since season 2015-2016, all samples are also tested for other respiratory viruses. This testing algorithm was implemented because only about 30-40% of samples were positive for influenza viruses in the SARI surveillance compared to about 70% in the ILI surveillance in the preceding seasons. In January 2020, testing for SARS-CoV-2 was also included.
For season 2021-2022, samples were tested by multiplex PCRs for: influenza A virus, influenza B virus, SARS-CoV-2, respiratory syncytial virus (RSV), human metapneumovirus, parainfluenza virus (types 1, 2, 3 and 4), seasonal coronavirus (229E, OC43, NL43), rhinovirus, enterovirus, specifically for enterovirus D68, parechovirus, adenovirus, bocavirus. Influenza positive samples were further tested by PCR to determine the subtype of influenza A viruses (H1N1pdm09 or H3N2) and the lineage of influenza B viruses (Victoria or Yamagata). All these PCR tests are following standard operating procedures (SOP) and are accredited according to the ISO 15189 norm.
Other PCR tests are available at the NRC influenza to determine the subtype of non-seasonal influenza A viruses that have already been responsible for severe cases of human infection (H5, H7 and H9) and for MERS coronavirus.

Samples positive for influenza viruses by PCR and with a good viral load are selected to attempt viral isolation and further characterisation by sequencing and phenotypic tests to evaluate the susceptibility to neuraminidase inhibitor antivirals. Representative samples are sent to the WHO Collaborating Center at the Crick Institute (United-Kingdom) for detailed characterisation, according to the terms of reference of the WHO-recognised National Influenza Centres.
Samples positive for SARS-CoV-2 by PCR and with a good viral load are selected for whole genome sequencing using the ARTIC protocol for Oxford Nanopore MinION technology. There is currently no official mechanism in place to exchange SARS-CoV-2 viruses at the global level.

SENTINEL SURVEILLANCE

1. Influenza-like illness (ILI)

1.1. NETWORK OF GENERAL PRACTITIONERS

The surveillance of influenza-like illness (ILI) is organised through a network of general practitioners spread all over Belgium. The network is involved in the surveillance of many diseases, reporting weekly information to the Department of Infectious Diseases at Sciensano. More information on the network can be found on Sciensano's website [c].

Regarding the surveillance of respiratory infections, the general practitioners are requested to weekly report the total number of consultations they had during the previous week, and the specific number of consultations for influenza-like illness (ILI) and for acute respiratory infection (ARI). These numbers are used to calculate incidence rates that allow to follow the epidemic situation throughout the year and that are presented in the weekly bulletin for acute respiratory infections [d].

A subset of the general practitioners are also taking part in an active virological surveillance for influenza viruses and other respiratory viruses. They are requested to take a nasopharyngeal swab from the first 3 ILI and first 2 ARI cases of the week belonging to different households. The NRC influenza provides the sampling kits (nasopharyngeal swab and UTM universal transport medium) and the packaging for sending the samples (prepaid envelops). All the samples are sent to the NRC influenza for testing.

1.2. SAMPLE INFORMATION

During the 2021-2022 season, 38 general practitioners took part in the virological surveillance and collected samples for the NRC influenza. This represents a drop by about 45% compared to the last pre-COVID19 season 2019-2020 (Table 1).

Table 1 • Number of ILI/ARI samples and contributing general practitioners per season

Season	n	Nb of GP
2021-2022	394	38
2020-2021	*29*	*8*
2019-2020	*698*	*69*
2018-2019	*512*	*59*
2017-2018	*677*	*65*
2016-2017	*651*	*72*
2015-2016	*752*	*78*

n: number of samples; GP: general practitioner

A total of 394 nasopharyngeal swabs were collected during the 2021-2022 season and were sent to the NRC influenza for testing. Regarding the administrative region of origin, the participating GP in Flanders and the Brussels region collected on average more than 10 samples each, when participating GP in Wallonia only collected on average 7 samples each (Table 2).

[c] https://www.sciensano.be/en/network-general-practitioners
[d] https://www.sciensano.be/en/health-topics/acute-respiratory-tract-infection/numbers

Table 2 • Number of ILI/ARI samples and contributing general practitioners per province, season 2021-2022

Region	n	Nb of GP
Brussels	75	6
Flanders	269	25
Wallonia	50	7

n: number of samples; GP: general practitioner

Out of the 394 enrolled cases, 86.8% and 92.6% matched the ECDC case definitions for ILI and ARI, respectively, which is similar to the percentages obtained during the last pre-COVID19 season 2019-2020 (Table 3). On the contrary, only 50.8% of the enrolled cases matched the very narrow WHO case definition for ILI, which is much lower than during the last pre-COVID19 season 2019-2020.

Table 3 • Number of ILI/ARI samples responding to the different clinical case definitions (with percentages)

Season	n	ECDC-ILI	ECDC-ARI	WHO-ILI	p_ECDC-ILI	p_ECDC-ARI	p_WHO-ILI
2021-2022	394	342	365	200	86.8	92.6	50.8
2020-2021	*29*	*21*	*26*	*10*	*72.4*	*89.7*	*34.5*
2019-2020	*698*	*663*	*668*	*555*	*95.0*	*95.7*	*79.5*
2018-2019	*512*	*381*	*413*	*381*	*74.4*	*80.7*	*74.4*
2017-2018	*677*	*528*	*575*	*528*	*78.0*	*84.9*	*78.0*
2016-2017	*651*	*473*	*534*	*473*	*72.7*	*82.0*	*72.7*
2015-2016	*752*	*415*	*599*	*415*	*55.2*	*79.6*	*55.2*

n: number of samples; p: percentage

The median time between sampling date and reception date was 5 days, but it varied by region (4 days for Flanders and Wallonia, and 6 days for Brussels).
The median time between reception date and reporting date (i.e. turnaround time, TAT) was 7 days, well below the target of 15 days.

The age distribution showed a predominance of adult (between 15 and 65 years old) patients within the ILI/ARI surveillance (Figure 1). For cases with known age, 81.1% (270/333) of the patients were adults (Table 4). Children (below the age of 15) and older adults (above the age of 65) are structurally not well covered by the network of general practitioners. No samples were collected after week 28-2023.

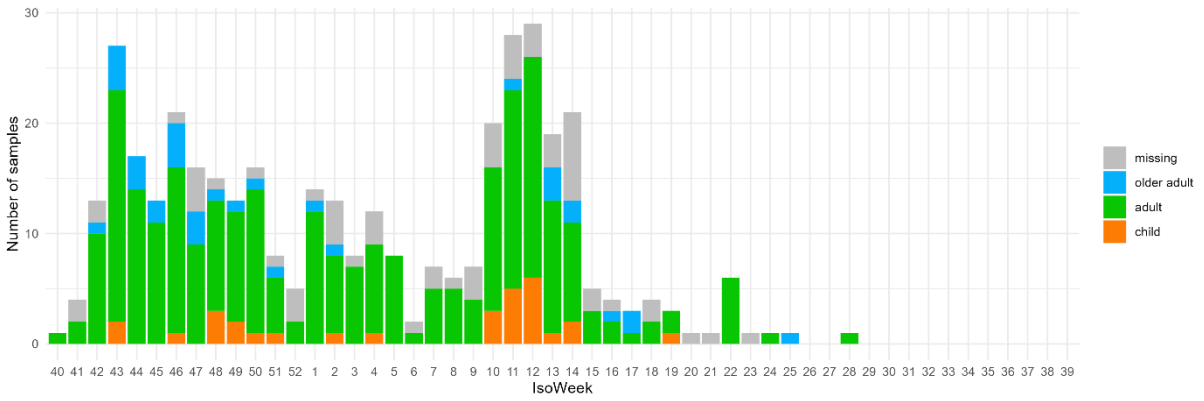


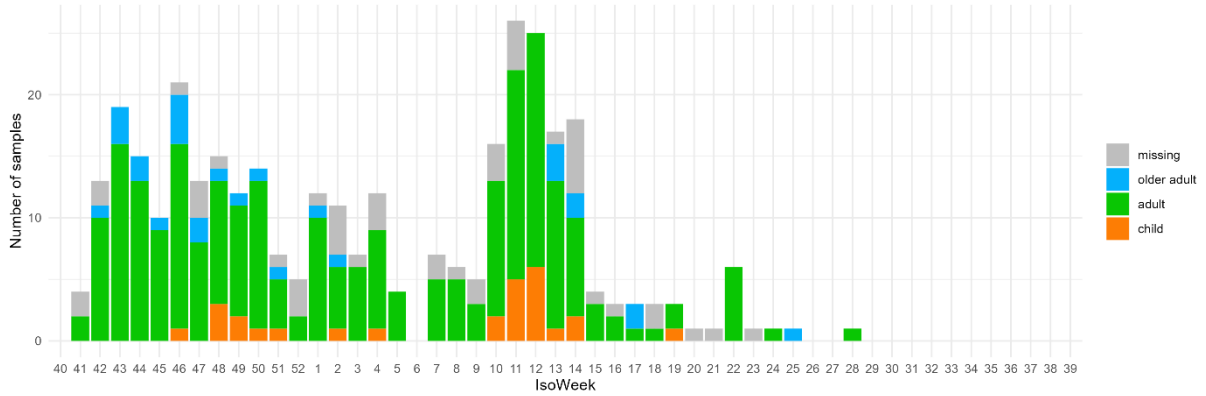
Figure 1 • Age distribution of ILI/ARI samples per week of collection, season 2021-2022

Table 4 • Number of ILI/ARI samples per age group and region, season 2021-2022

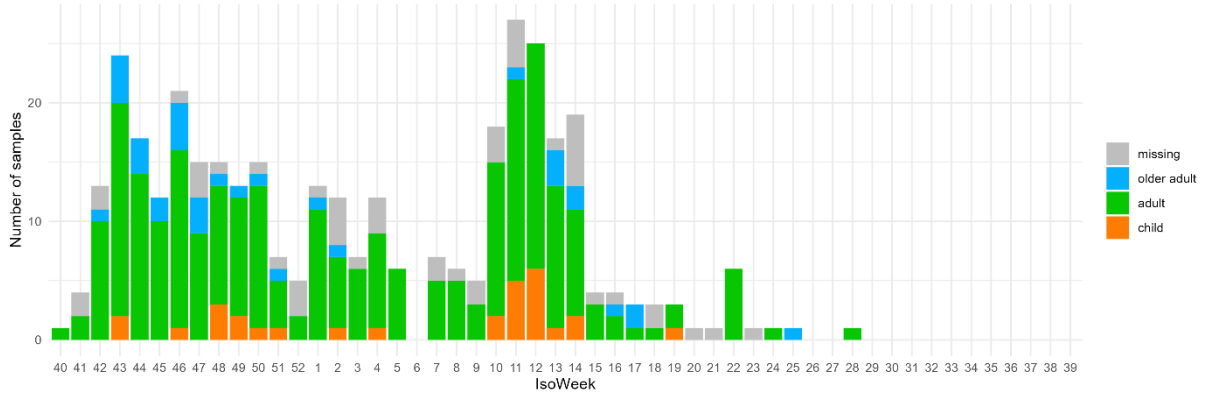
Age group	Brussels	Flanders	Wallonia	*Total*
child	5	20	5	*30*
adult	27	214	29	*270*
older adult	4	27	2	*33*
missing	39	8	14	*61*
Total	*75*	*269*	*50*	*394*

When looking by case definition (ECDC-ILI, ECDC-ARI, WHO-ARI), similar weekly age group distributions were observed (Figure 2).

ECDC-ILI



ECDC-ARI



WHO-ILI

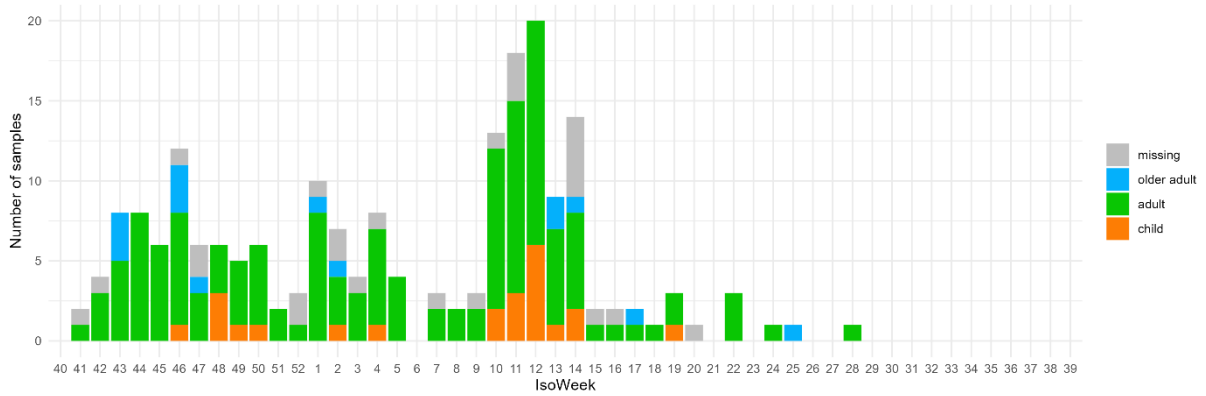


Figure 2 • Weekly age distribution of ILI cases matching the different case definitions, season 2021-2022

1.3. INFLUENZA VIRUS

The result for the influenza virus typing PCR was not available for one sample of bad quality, which was reported as 'undetermined' and was not tested for the other respiratory viruses.
Out of the 393 samples with a result for the influenza virus typing PCR, the first positive sample was detected in week 47-2021 and the last in week 22-2022 (Figure 3). The active period of circulation of influenza viruses truly started in week 07-2022. The highest proportion of positive samples was reached in week 12-2022.

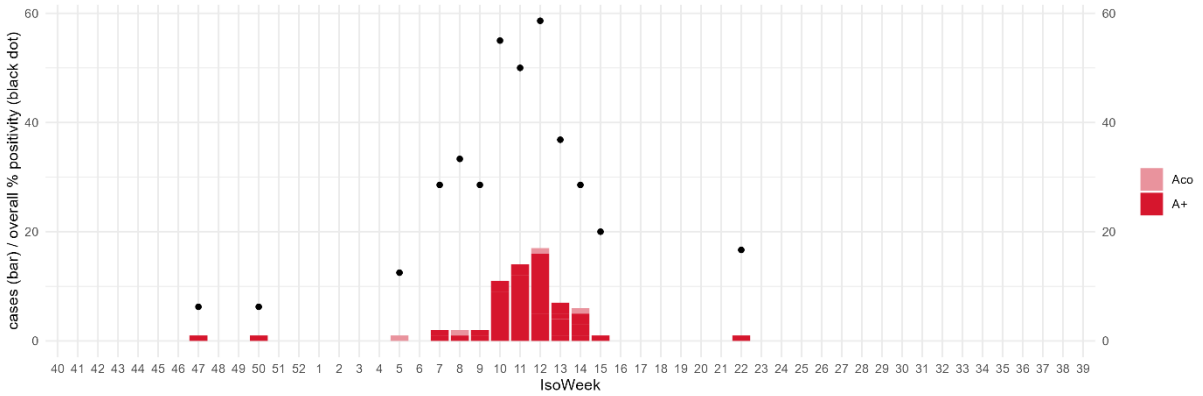


Figure 3 • Weekly number of ILI samples positive for influenza viruses A or B and percentage of influenza positivity, season 2022-2023

A+: influenza A virus detected alone; Aco: co-detection of influenza A virus and another respiratory virus; B+: influenza B virus detected alone; Bco: co-detection of influenza B virus and another respiratory virus

Overall the percentage of positivity for the season 2021-2022 was 16.8% (Table 5), with only influenza type A viruses being detected. Since adults represent the majority of cases, the percentage of positivity and the distribution between types A and B among adults were similar to the overall values. On the other hand, the percentage of positivity was higher among children (below 15 years old; 40%; 12/30) and lower among older adults (above 65 years old; 3%; 1/33).

Table 5 • Age distribution of the influenza positive ILI cases, season 2021-2022

Influenza virus typing PCR result	child	adult	older adult	missing	*Total*
Influenza A virus detected alone	11	40	1	10	*62*
Influenza A + another respiratory virus	1	2	0	1	*4*
Influenza B virus detected alone	0	0	0	0	*0*
Influenza B + another respiratory virus	0	0	0	0	*0*
Influenza virus not detected	18	227	32	50	*327*
Total	*30*	*269*	*33*	*61*	*393*

Although only 50.1% (200/393) of the cases matched the WHO ILI case definition, compared to 86.8% (341/393) or 92.4% (364/393) matching the ECDC ILI or ARI case definition, respectively, the percentage of positivity was higher. Out of the 200 cases matching the WHO ILI case definition, 53 (26.5%) were positive for influenza A. Out of the 341 cases matching the ECDC ILI case definition, 61 (17.9%) were positive for influenza A (Table 6).

Table 6 • Influenza typing PCR results for each ILI/ARI case definition, season 2021-2022

Case definition	number	negative for influenza	positive for type A	positive for type B
WHO-ILI	200	147	53	0
ECDC-ILI	341	280	61	0
ECDC-ARI	364	303	61	0

The subtype could be determined for all 66 influenza A positive samples. H3N2 and H1N1dpm09 subtypes were identified in 59 and 6 samples, respectively (Figure 4).

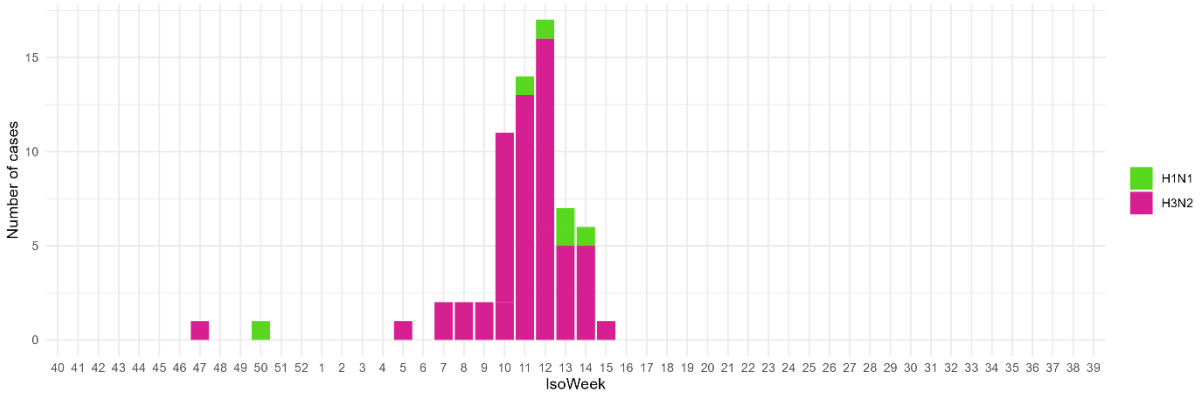


Figure 4 • Weekly distribution of influenza A viruses per subtype among ILI samples, season 2021-2022

NT: no subtype available; H1N1: subtype H1N1pdm09; H3N2: subtype H3N2

1.4. SARS-COV-2 VIRUS

Positive samples were detected during almost the entire surveillance period when samples were collected (Figure 5). The highest proportion for positive samples were found before the period of intense circulation of influenza viruses (weeks 07-2022 to 14-2023).

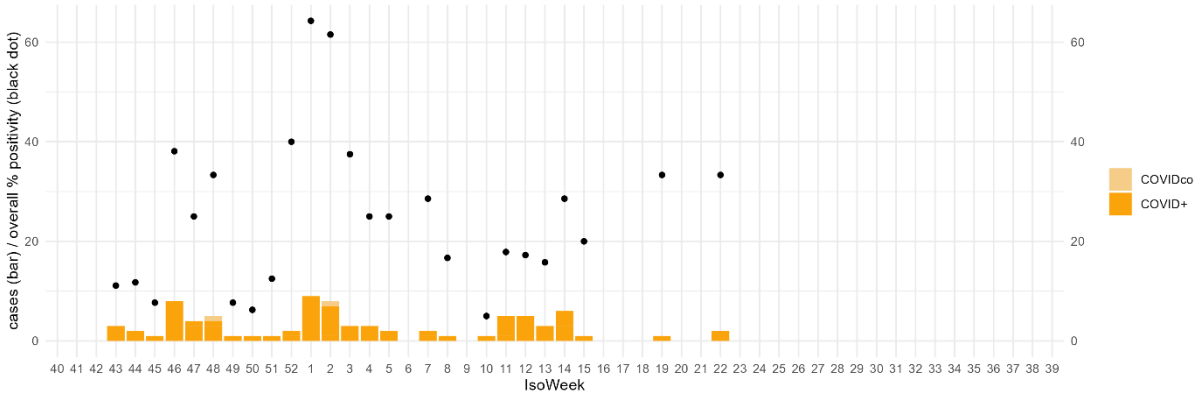


Figure 5 • Weekly number of ILI samples positive for SARS-CoV-2 coronavirus and percentage of positivity, season 2021-2022

COVID+: SARS-CoV-2 detected alone; COVIDco: co-detection of SARS-CoV-2 and another respiratory virus

Overall the percentage of positivity for the season 2021-2022 was 20.4% (Table 7), but reaching 33.3% (11/33) among older adults (above 65 years old).

Table 7 • Age distribution of the SARS-CoV-2 positive ILI cases, season 2021-2022

SARS-CoV-2 PCR result	child	adult	older adult	missing	*Total*
SARS-CoV-2 detected alone	3	55	11	9	*78*
SARS-CoV-2 + another respiratory virus	1	0	0	1	*2*
SARS-CoV-2 virus not detected	26	214	22	51	*313*
Total	*30*	*269*	*33*	*61*	*393*

The differences in percentage of positivity were less important between case definitions than for influenza viruses. Out of the 200 cases matching the WHO ILI case definition, 44 (22%) were positive SARS-CoV-2. Out of the 341 and 264 cases matching the ECDC ILI and ARI case definitions, 70 (20.5%) and 75 (20.6%) were positive for SARS-CoV-2, respectively (Table 8). The WHO ILI case definition was slightly better at capturing the circulation of SARS-CoV-2 in the general population.

Table 8 • SARS-CoV-2 PCR results for each ILI/ARI case definition, season 2021-2022

Case definition	number	negative for SARS-CoV-2	positive for SARS-CoV-2
WHO-ILI	200	156	44
ECDC-ILI	341	271	70
ECDC-ARI	364	289	75

1.5. RESPIRATORY SYNCYTIAL VIRUS

RSV was only sporadically detected during the 2021-2022 season (Table 9).

Table 9 • Age distribution of the RSV positive ILI cases, season 2021-2022

RSV PCR result	child	adult	older adult	missing	*Total*
RSV type A detected alone	2	0	0	2	*4*
RSV-A + another respiratory virus	1	1	0	0	*2*
RSV type B detected alone	0	2	0	4	*6*
RSV-B + another respiratory virus	0	0	0	0	*0*
RSV not detected	27	266	33	55	*381*
Total	*30*	*269*	*33*	*61*	*393*

1.6. OTHER RESPIRATORY VIRUSES

Rhinoviruses and enteroviruses were detected in 13.9% of the samples, followed by seasonal coronaviruses in 4.3%, parainfluenzaviruses in 3.6%, human metapneumoviruses in 3.1% and adenoviruses in 0.5%.

2. Nursing home - ILI

The surveillance of influenza-like illness (ILI) in nursing homes started in January 2022, but sampling only started in March 2022. The network comprised 10 nursing homes in the country, that weekly report to the Department of Infectious Diseases at Sciensano the total number of influenza-like infection identified in the nursing home [e].
In addition, they were requested to take a nasopharyngeal swab from the first 2 ILI cases of the week. The NRC influenza provided the sampling kits (nasopharyngeal swab and UTM transport medium) and the packaging for sending the samples (prepaid envelops). All the samples were sent to the NRC influenza for testing.

[e] https://www.sciensano.be/fr/biblio/influenza-illness-including-covid-19-sentinel-surveillance-belgian-nursing-homes-amended-protocol-v2

In total, 5 nursing homes submitted 57 samples between weeks 08-2022 and 20-2022. The nursing homes participating to the virological surveillance were predominantly from the Flemish region (Table 10).

Table 10 • Number of samples and contributing nursing homes per province, season 2021-2022

Region	n	Nb of nursing homes
Brussels	4	1
Flanders	51	3
Wallonia	2	1

n: number of samples; Nb: number

Twenty-two samples (38.6%) were positive for respiratory viruses. SARS-CoV-2 was detected in 10 samples, followed by RSV in 7 and rhino-/enteroviruses in 4. Influenza virus was detected in only 1 samples.

3. Severe Acute Respiratory Infections (SARI)

3.1. NETWORK OF SENTINEL HOSPITALS

The surveillance of severe acute respiratory infections (SARI) is organised through a network of 6 hospitals in Belgium, 2 in each region (Flanders, Wallonia, Brussels). The network was implemented in 2012, following the recommendations of WHO after the 2009 H1N1 pandemic to reinforce the surveillance of severe cases. More information on the network can be found on Sciensano's website [f]. The hospitals are requested to recruit all cases matching the case definition and to take a nasopharyngeal swab. The NRC influenza can provide the sampling kits (nasopharyngeal swab and UTM transport medium) and the packaging for sending the samples (prepaid envelops), but following the COVID-19 pandemic, less hospitals require the sampling kits. All the samples are sent to the NRC influenza for testing, even if they already have been tested in the hospital microbiological laboratory.

3.2. SAMPLE INFORMATION

A total of 2621 nasopharyngeal swabs were collected during the 2021-2022 season and were sent to the NRC influenza for testing, with the hospitals of Flanders and the Brussels region slowly coming back to exhaustive recruitment after the COVID-19 pandemic disruption (Table 11).

Table 11 • Number of SARI samples per province, season 2021-2022

Region	n
Brussels	1094
Flanders	1307
Wallonia	220

n: number of samples

Out of the 2621 enrolled cases, 68.9% matched the case definition defined in the protocol and following the WHO SARI case definition (Table 12). When considering broader case definitions, 90.4% and 91.1%

[f] https://www.sciensano.be/en/projects/severe-acute-respiratory-infection-surveillance-a-sentinel-network-hospitals

of the enrolled cases matched the ECDC-COVID and BE-COVID case definitions, respectively. These proportions remained relatively stable over the seasons within the SARS-CoV-2 pandemic area.

Table 12 • Number of SARI samples responding to the different clinical case definitions (with percentages)

Season	n	WHO-SARI	ECDC-COVID	BE-COVID	p_WHO-SARI	p_ECDC-COVID	p_BE-COVID
2021-2022	**2621**	**1806**	**2370**	**2387**	**68.9**	**90.4**	**91.1**
2020-2021	*1331*	*982*	*1231*	*1253*	*73.8*	*87.1*	*94.1*

n: number of samples; p: percentage

The median time between sampling date and reception date was 34 days, but it varied by region (142 days for Flanders, 8 days for Wallonia, and 22 days for Brussels). For convenience, samples collected for the SARI surveillance were often sent by batch covering a few weeks of collection.

Age was missing or not yet communicated for 7.1% of the samples. The age distribution showed a slight predominance of older adult (above the age of 65) patients within the SARI surveillance (Figure 6). Overall, when age was known, 41.2% (1003/2434) of the patients were older adults (Table 13). Children (below the age of 15) and adults (between 15 and 65 years old) were nonetheless well covered too, representing 35.6% (884/2434) and 23.2% (565/2434), respectively. As requested in the protocol for this season, sample collection took place each week during the season.

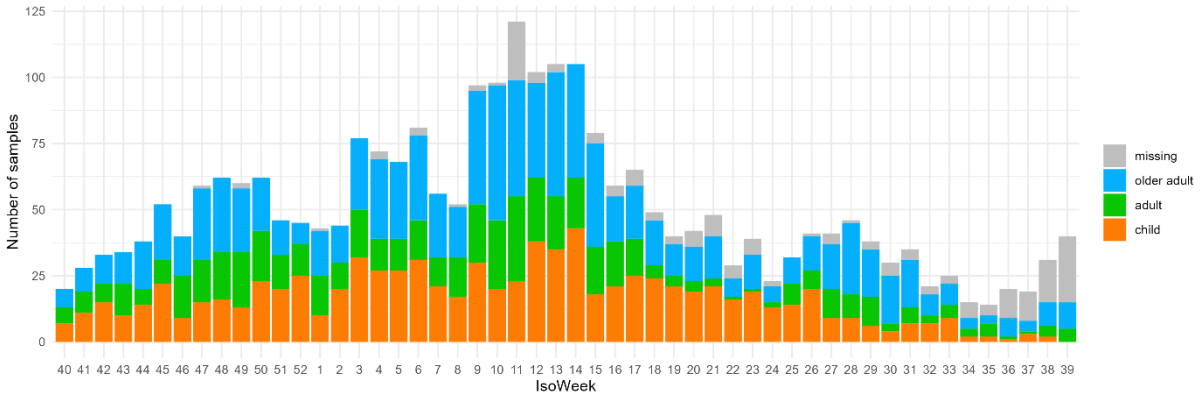


Figure 6 • Age distribution of SARI samples per week of collection, season 2021-2022

Table 13 • Number of SARI samples per age group and region, season 2021-2022

Age group	Brussels	Flanders	Wallonia	*Total*
child	390	457	19	*866*
adult	198	276	91	*565*
older adult	319	574	110	*1003*
missing	187	0	0	*187*
Total	*1094*	*1307*	*220*	*2621*

When looking by case definition, more than 80% of child cases matched the WHO-SARI case definition, and around 70% for adult and older adult cases (Table 14). Nearly all cases matched the broader ECDC-COVID and BE-COVID case definitions.

Table 14 • Number of SARI samples per age group and case definition, season 2021-2022

Case definition	Child	Adult	Older adult	Missing
WHO-SARI	708	382	714	2
ECDC-COVID	857	546	963	4
BE-COVID	857	553	1003	4
Total	*866*	*565*	*1003*	*187*

3.3. INFLUENZA VIRUS

The result for the influenza virus typing PCR was not available for 16 samples of bad quality, which were reported as 'undetermined' and were not tested for the other respiratory viruses.
Out of the 2605 samples with a result for the influenza virus typing PCR, the first positive sample was detected in week 49-2021 and the last in week 20-2022 (Figure 7). Sporadic positive samples were detected afterwards. The highest proportion of positive samples was reached in week 11-2022.

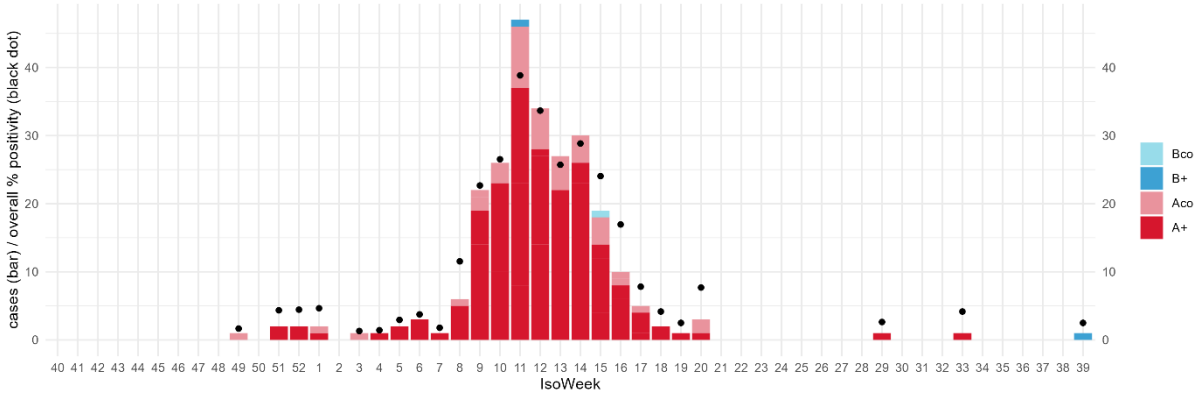


Figure 7 • Weekly number of SARI samples positive for influenza viruses A or B and percentage of influenza positivity, season 2021-2022

A+: influenza A virus detected alone; Aco: co-detection of influenza A virus and another respiratory virus; B+: influenza B virus detected alone; Bco: co-detection of influenza B virus and another respiratory virus

Overall, 250 samples were positive for an influenza virus and the percentage of positivity for the season 2021-2022 was 9.5% (Table 15), with 98.8% (247/250) positive for influenza type A. The percentage of positivity decreased with age, ranging from 13.0% (111/856) for children (below 15 years old), to 8.5% (48/563) for adults (between 15 and 65 years old) and 6.2% (62/1001) for older adults (above 65 years old).

Table 15 • Age distribution of the influenza positive SARI cases, season 2021-2022

Influenza virus typing PCR result	child	adult	older adult	missing	*Total*
Influenza A virus detected alone	86	41	56	21	*204*
Influenza A + another respiratory virus	23	7	6	7	*43*
Influenza B virus detected alone	1	0	0	1	*2*
Influenza B + another respiratory virus	1	0	0	0	*1*
Influenza virus not detected	745	515	939	156	*2355*
Total	*856*	*563*	*1001*	*185*	*2605*

Among the 247 influenza A positive samples, the subtypes could be determined for 233 samples. The viral load of the remaining 14 samples was too low to allow subtype determination. H3N2 and H1N1pdm09 subtypes were identified in 153 and 80 samples, respectively, without a clear pattern in time (Figure 8).

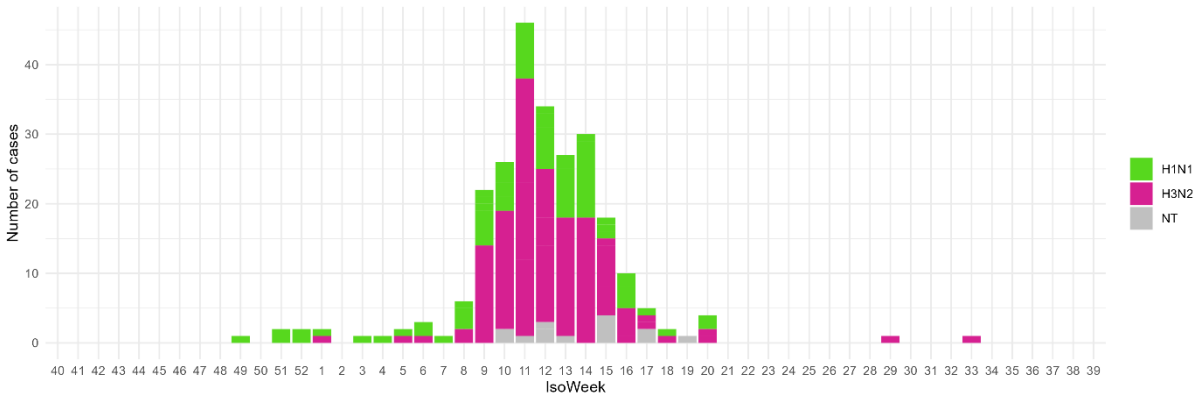


Figure 8 • Weekly distribution of influenza A viruses per subtype among SARI samples, season 2021-2022

NT: no subtype available; H1N1: subtype H1N1pdm09; H3N2: subtype H3N2

All 3 influenza B positive samples were of the Victoria lineage.

3.4. SARS-COV-2 VIRUS

Positive samples were detected during the entire surveillance period when samples were collected (Figure 9). Several waves occurred before and during the influenza virus circulation period.

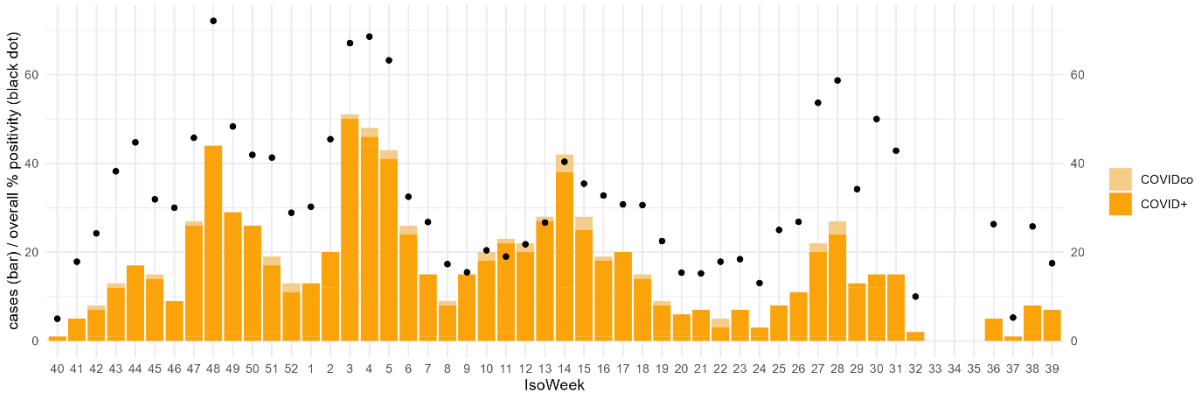


Figure 9 • Weekly number of SARI samples positive for SARS-CoV-2 coronavirus and percentage of positivity, season 2021-2022

COVID+: SARS-CoV-2 detected alone; COVIDco: co-detection of SARS-CoV-2 and another respiratory virus

Overall, 854 samples were positive for SARS-CoV-2 and the percentage of positivity for the season 2021-2022 was 33.0% (Table 16), reaching 41.2% (230/558) among adults (between 15 and 65 years old) and 50.2% (497/991) among older adults (above 65 years old), but only 12.1% (104/856) among children (below 15 years old).

Table 16 • Age distribution of the SARS-CoV-2 positive SARI cases, season 2021-2022

SARS-CoV-2 PCR result	child	adult	older adult	missing	*Total*
SARS-CoV-2 detected alone	87	225	481	22	*815*
SARS-CoV-2 + another respiratory virus	17	5	16	1	*39*
SARS-CoV-2 virus not detected	752	328	494	160	*1734*
Total	*856*	*558*	*991*	*183*	*2588*

3.5. RESPIRATORY SYNCYTIAL VIRUS

Positive samples were detected during the entire surveillance period when samples were collected (Figure 10). The highest proportions for positive samples were found after the period of intense circulation of influenza viruses.

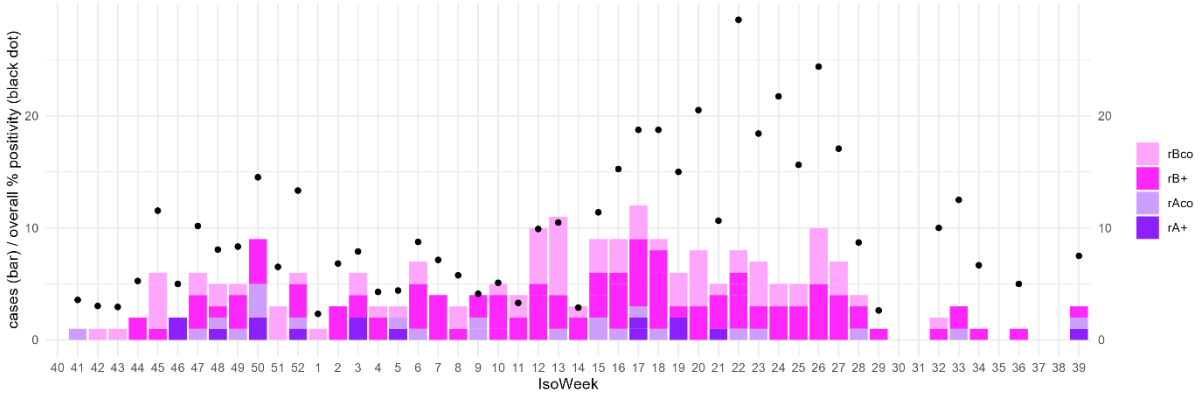


Figure 10 • Weekly number of SARI samples positive for respiratory syncytial viruses A or B and percentage of RSV positivity, season 2021-2022

rA+: RSV type A virus detected alone; rAco: co-detection of RSV type A virus and another respiratory virus; rB+: RSV B virus detected alone; rBco: co-detection of RSV B virus and another respiratory virus

Overall, 229 samples were positive for a respiratory syncytial virus and the percentage of positivity for the season 2021-2022 was 8.8% (Table 17), with RSV type B (83.4%, 191/229) dominating type A viruses (16.6%, 38/229). Eighty-two percent (188/229) of the positive samples were from children (below 15 years old).

Table 17 • Age distribution of the RSV positive SARI cases, season 2021-2022

RSV PCR result	child	adult	older adult	missing	*Total*
RSV type A detected alone	12	1	1	1	*15*
RSV-A + another respiratory virus	19	1	1	2	*23*
RSV type B detected alone	92	9	5	5	*111*
RSV-B + another respiratory virus	65	4	6	5	*80*
RSV not detected	667	548	988	173	*2376*
Total	*855*	*563*	*1001*	*186*	*2605*

3.6. OTHER RESPIRATORY VIRUSES

3.6.1. Picornavirus (rhinovirus, enterovirus, parechovirus)

Positive samples were detected at relatively high proportion during the entire surveillance period when samples were collected (Figure 11). Viruses were mostly entero- and rhinoviruses. Very few parechoviruses were detected. The specific enterovirus D68 was detected as a little epidemic wave at the beginning of the season (weeks 40-2021 to 45-2021). Overall, picornaviruses were frequently co-detected with another respiratory viruses (Table 18).

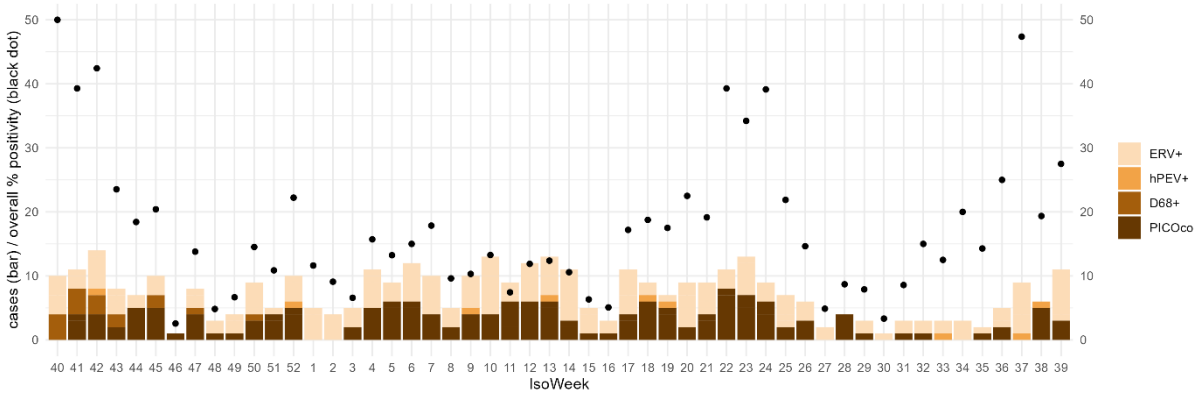


Figure 11 • Weekly number of SARI samples positive for rhino-, entero- and parechoviruses and percentage of positivity, season 2021-2022

ERV+: entero- or rhinovirus detected alone; hPEV+: parechovirus detected alone; D68+: enterovirus D68 detected alone; PICOco: co-detection of entero-, rhino- or parechovirus and another respiratory virus

Table 18 • Age distribution of the picornavirus positive SARI cases, season 2021-2022

PCR result	child	adult	older adult	missing	*Total*
Rhino- / enterovirus detected alone	103	29	41	24	*197*
Enterovirus D68 detected alone	17	0	0	0	*17*
Parechovirus detected alone	5	1	0	3	*9*
Picornavirus + another respiratory virus	128	2	7	23	*160*
Picornavirus not detected	600	530	952	136	*2218*
Total	*853*	*562*	*1000*	*186*	*2601*

3.6.2. Human metapneumovirus

Positive samples were detected a little bit throughout the season, but without clear epidemic wave (Figure 12). All age groups were concerned, but co-detection with another respiratory virus was more frequent among children (Table 19).

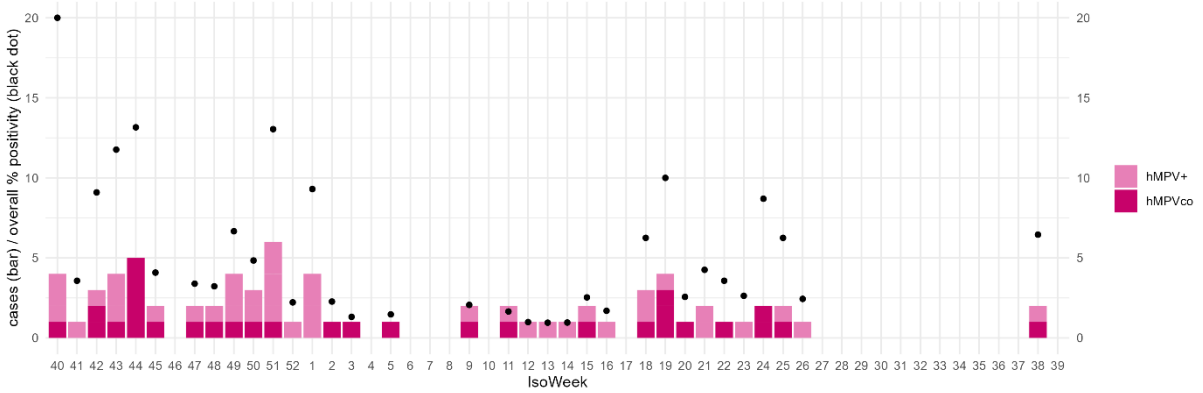


Figure 12 • Weekly number of SARI samples positive for human metapneumoviruses and percentage of positivity, season 2021-2022

hMPV+: human metapneumovirus detected alone; hMPVco: co-detection of human metapneumovirus and another respiratory virus

Table 19 • Age distribution of the metapneumovirus positive SARI cases, season 2021-2022

PCR result	child	adult	older adult	missing	*Total*
Metapneumovirus detected alone	16	12	11	3	*42*
Metapneumovirus + another resp. virus	22	2	3	4	*31*
Metapneumovirus not detected	815	548	984	179	*2526*
Total	*853*	*562*	*998*	*186*	*2599*

3.6.3. Parainfluenzavirus

Positive samples were detected throughout the entire surveillance period when samples were collected, without a clear epidemic wave (Figure 13). Parainfluenzavirus type 3 dominated with all age groups being concerned (Table 20). Co-detection with another respiratory virus was more frequent among children.

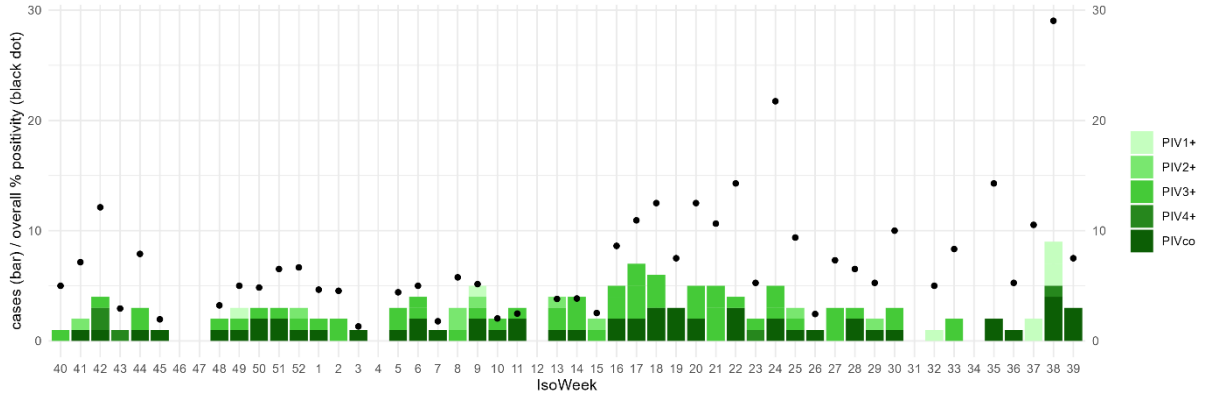


Figure 13 • Weekly number of SARI samples positive for human parainfluenzaviruses and percentage of positivity, season 2021-2022

PIV1+: parainfluenzavirus type 1 detected alone; PIV2+: parainfluenzavirus type 2 detected alone; PIV3+: parainfluenzavirus type 3 detected alone; PIV4+: parainfluenzavirus type 4 detected alone; PIVco: co-detection of parainfluenzavirus and another respiratory virus

Table 20 • Age distribution of the parainfluenzavirus positive SARI cases, season 2021-2022

PCR result	child	adult	older adult	missing	*Total*
Parainfluenzavirus type 1 detected alone	3	0	1	5	*9*
Parainfluenzavirus type 2 detected alone	6	1	2	0	*9*
Parainfluenzavirus type 3 detected alone	32	11	15	1	*59*
Parainfluenzavirus type 4 detected alone	0	0	4	1	*5*
PIV + another respiratory virus	34	3	3	17	*57*
Parainfluenzavirus not detected	781	548	974	160	*2463*
Total	*856*	*563*	*999*	*184*	*2602*

PIV: parainfluenzavirus

3.6.4. Seasonal coronavirus

Positive samples were detected at relatively low proportion throughout the surveillance period (Figure 14). Human coronavirus 229E and OC43 were the most frequently detected, with all age groups being concerned (Table 21). Co-detection with another respiratory virus was more frequent among children.

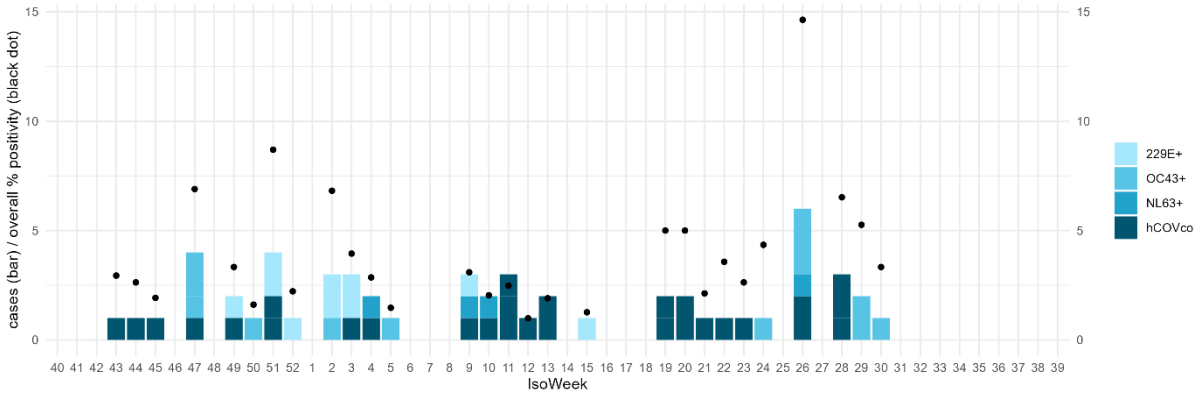


Figure 14 • Weekly number of SARI samples positive for seasonal coronaviruses and percentage of positivity, season 2021-2022

229E+: coronavirus 229E detected alone; OC43+: coronavirus OC43 detected alone; NL63+: coronavirus NL63 detected alone; hCOVco: co-detection of seasonal coronavirus and another respiratory virus

Table 21 • Age distribution of the seasonal coronavirus positive SARI cases, season 2021-2022

PCR result	child	adult	older adult	missing	*Total*
Coronavirus 229E detected alone	2	3	5	0	*10*
Coronavirus NL63 detected alone	2	2	0	0	*4*
Coronavirus OC43 detected alone	7	2	4	0	*13*
hCOV + another respiratory virus	21	1	5	2	*29*
Seasonal coronavirus not detected	824	555	986	184	*2549*
Total	*856*	*563*	*1000*	*186*	*2605*

3.6.5. Adenovirus

Positive samples were detected at relatively low proportion during the entire surveillance period when samples were collected (Figure 15). Adenoviruses were frequently co-detected with another respiratory virus, and almost exclusively among children (Table 22).

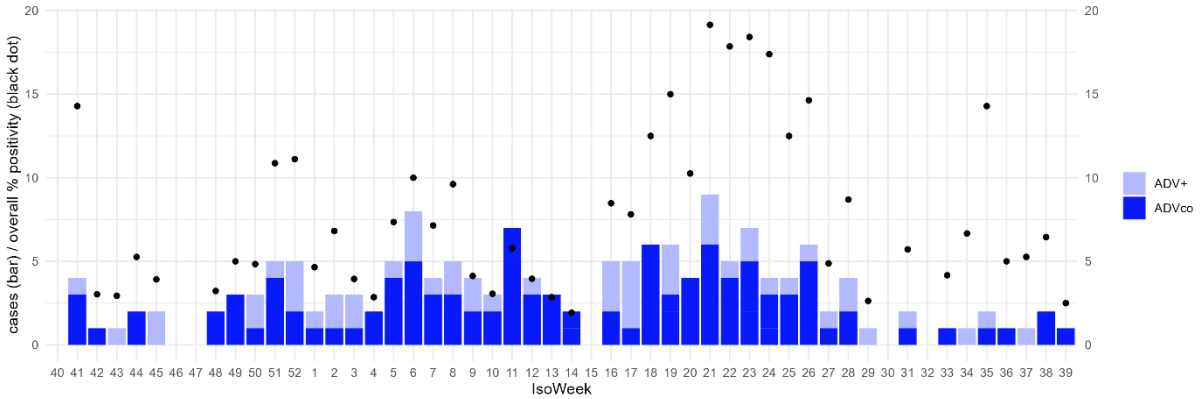


Figure 15 • Weekly number of SARI samples positive for adenoviruses and percentage of positivity, season 2021-2022

ADV+: adenovirus detected alone; ADVco: co-detection of adenovirus and another respiratory virus

Table 22 • Age distribution of the adenovirus positive SARI cases, season 2021-2022

PCR result	child	adult	older adult	missing	*Total*
Adenovirus detected alone	44	2	2	5	*53*
Adenovirus + another resp. virus	90	0	2	17	*109*
Adenovirus not detected	718	561	997	164	*2440*
Total	*852*	*563*	*1001*	*186*	*2602*

3.6.6. Bocavirus

Positive samples were detected at very low proportion throughout the entire surveillance period when samples were collected (Figure 16). Bocaviruses were almost exclusively detected among children and in co-detection with another respiratory virus (Table 23).

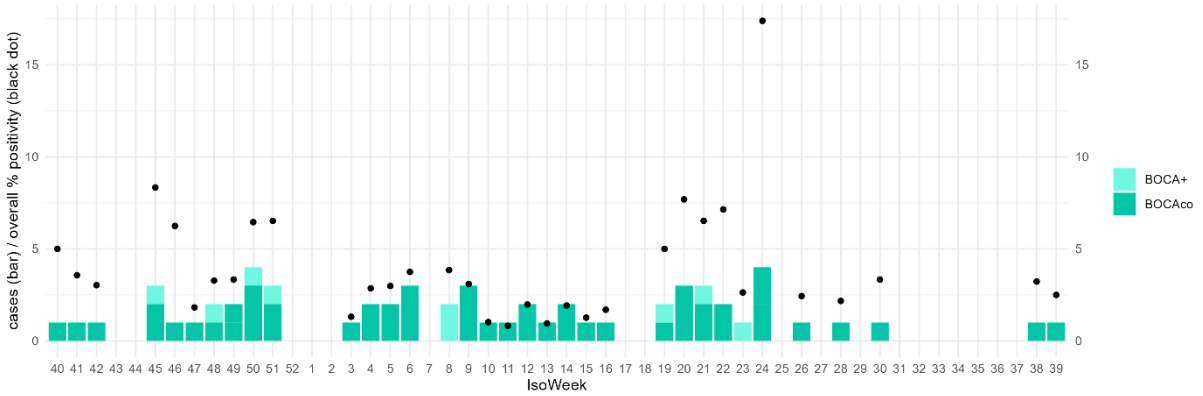


Figure 16 • Weekly number of SARI samples positive for bocaviruses and percentage of positivity, season 2021-2022

BOCA+: bocavirus detected alone; BOCAco: co-detection of bocavirus and another respiratory virus

Table 23 • Age distribution of the bocavirus positive SARI cases, season 2021-2022

PCR result	child	adult	older adult	missing	*Total*
Bocavirus detected alone	8	0	1	0	*9*
Bocavirus + another resp. virus	45	0	0	7	*52*
Bocavirus not detected	789	550	979	179	*2497*
Total	*842*	*550*	*980*	*186*	*2558*

NON-SENTINEL SURVEILLANCE

1. Hospital laboratories (HOSPI)

The laboratories from all the hospitals in Belgium can send samples to the NRC influenza for confirmation of influenza positive cases or for the determination of the subtype (influenza A) or the lineage (influenza B) or for a differential diagnostic with other respiratory viruses in particular MERS-CoV (very specific cases).

In January 2022, following the absence of influenza epidemic in 2020-2021, a communication was sent to the hospitals to encourage them to send as many samples positive for influenza virus as possible. At that time, influenza viruses have not yet been detected in the sentinel networks and the NRC influenza was unable to provide strains to the WHO Collaborating Centre at the Crick Institute in London (United-Kingdom).
During the season 2021-2022, 31 hospitals submitted 567 samples to the NRC influenza. The hospitals sending samples for characterisation were predominantly from Flanders and Wallonia (Table 24).

Table 24 • Number of HOSPI samples and contributing hospitals per province, season 2021-2022

Region	n	Nb of hospitals
Brussels	8	1
Flanders	381	17
Wallonia	178	13

n: number of samples; Nb: number

Only 5 samples were confirmed as positive for influenza type B virus: all belonged to the Victoria lineage. Among the 494 samples confirmed positive for influenza type A virus, 472 were positive only for influenza A viruses. For the remaining 22 samples, another respiratory virus was also detected, including SARS-CoV-2 (in 5 samples). Subtype determination when viral load was sufficient gave 160 H1N1pdm09 and 304 H3N2. The remaining samples were negative for influenza viruses: they were either not confirmed by the NRC influenza or sent for another purpose (tested for other respiratory viruses).

2. Zoonotic influenza

The NRC influenza did not receive any samples to test from a true suspicion of a human case of infection with a non-seasonal influenza virus (infection with an influenza virus of animal origin).

CHARACTERISATION

1. Virus isolation

Virus isolation is routinely performed only for influenza viruses at the NRC influenza. SARS-CoV-2 virus isolation is not currently performed routinely.

In total, 59 ILI and 143 SARI samples positive for influenza viruses were used to attempt influenza virus isolation (Table 25 and Table 26). H3N2 virus positive samples were inoculated in MDCK-SIAT cells, and H1N1 and Victoria virus positive samples were inoculated in MDCK cells. After inoculation, cells were observed daily over a period of 72 hours post infection to check for the appearance of a characteristic cytopathic effect.

Table 25 • Virus isolation from ILI samples, season 2021-2022

Subtype or lineage	isolated	negative	contaminated	*Total*
A/H3N2	47	1	5	*53*
A/H1N1pdm09	5	0	1	*6*
B/VIC	0	0	0	*0*
Total	*52*	*1*	*6*	*59*

Table 26 • Virus isolation from SARI samples, season 2021-2022

Subtype or lineage	isolated	negative	contaminated	*Total*
A/H3N2	74	8	9	*91*
A/H1N1pdm09	37	5	8	*50*
B/VIC	2	0	0	*2*
Total	*113*	*13*	*17*	*143*

A representative selection of these isolated influenza viruses were sent to the WHO Collaborating Centre at the Crick Institute in London (United-Kingdom), as part of the WHO-recognised National Influenza Centre's terms of reference, to be further characterised and compared with isolates from other countries in preparation of the Vaccine Composition Meetings to select the vaccine strains.
There is currently no similar system of systematic exchange in place for SARS-CoV-2 viruses.

2. Sequencing

Whole genome sequencing using NGS Oxford Nanopore MinION technology is performed at the NRC influenza for SARS-CoV-2 and influenza viruses.

2.1. SARS-COV-2 CORONAVIRUS

Sequencing was attempted on all SARI samples positive for SARS-CoV-2 with a sufficient viral load, as part of a specifically-funded project.

A total of 730 samples positive for SARS-CoV-2 coronavirus were processed for sequencing. Two systems are used for further subdivision to classify SARS-CoV-2 based on genomic analysis: Nextclade (Table 27) and Pangolin lineage (Table 28). Figure 17 presents a summary of the evolution of SARS-

CoV-2 viruses with a correspondence between the Nextclade system and the main representant in the Pangolin lineage (taken from [g]).
During the 2021-2022 season, viruses belonging to the Delta and Omicron groups were detected. Clades 21J, for Delta, and 21K (BA.1), 21L (BA.2) and 22B (BA.5), for Omicron, (XBB) were the most prominent groups.

Table 27 • Clade distribution of the SARS-CoV-2 positive samples based on Nextclade system, season 2021-2022

Nextclade	ILI	SARI	*Total*
20B		1	*1*
21A (Delta)		1	*1*
21I (Delta)	1	11	12
21J (Delta)	23	143	166
21K (Omicron)	27	192	219
21L (Omicron)	12	198	210
22A (Omicron)	1	4	5
22B (Omicron)		106	106
22C (Omicron)		5	5
22E (Omicron)		1	1
recombinant		4	4

Table 28 • Clade distribution of the SARS-CoV-2 positive samples based on Pangolin lineage system, season 2021-2022

Pangolin	ILI	SARI	*Total*
AY.122	1	5	*6*
AY.123	1	11	*12*
AY.123.1		3	*3*
AY.125		8	*8*
AY.126	1		*1*
AY.127	1	1	*2*
AY.25.1		4	*4*
AY.33	1	3	*4*
AY.34		1	*1*
AY.36		1	*1*
AY.39		4	*4*
AY.4	1	9	*10*
AY.4.9	1		*1*
AY.4.11		3	*3*
AY.4.2.3		2	*2*
AY.4.6		4	*4*
AY.4.8		2	*2*
AY.4.9		1	*1*
AY.42		1	*1*
AY.43	11	45	*56*
AY.46		1	*1*
AY.46.6	1	2	*3*
AY.47		1	*1*
AY.5		1	*1*
AY.5.2		1	*1*
AY.73	1		*1*
AY.87		1	*1*
AY.9		2	*2*
AY.9.2		10	*10*

[g] https://clades.nextstrain.org/

AY.98.1	1	2	*3*
B.1.617.2	3		*3*
BA.1	11	47	*58*
BA.1.1	12	82	*94*
BA.1.1.1	2	14	*16*
BA.1.1.10		1	*1*
BA.1.1.14		1	*1*
BA.1.1.15		1	*1*
BA.1.1.18		1	*1*
BA.1.14	2	9	*11*
BA.1.15		5	*5*
BA.1.16		1	*1*
BA.1.17		8	*8*
BA.1.17.2		5	*5*
BA.1.18	1	16	*17*
BA.1.20		1	*1*
BA.2	12	147	*159*
BA.2.1		2	*2*
BA.2.12.1		3	*3*
BA.2.13		1	*1*
BA.2.22		3	*3*
BA.2.3		4	*4*
BA.2.3.12		1	*1*
BA.2.36		4	*4*
BA.2.37		1	*1*
BA.2.44		1	*1*
BA.2.9		34	*34*
BA.4.1	1	3	*4*
BA.4.2		1	*1*
BA.5		7	*7*
BA.5.1		28	*28*
BA.5.1.17		2	*2*
BA.5.1.22		5	*5*
BA.5.1.23		1	*1*
BA.5.1.3		1	*1*
BA.5.1.30		1	*1*
BA.5.2		8	*8*
BA.5.2.1		15	*15*
BA.5.2.20		3	*3*
BA.5.2.30		1	*1*
BA.5.2.44		1	*1*
BA.5.2.9		1	*1*
BA.5.3.3		1	*1*
BA.5.6		2	*2*
BE.1.1		9	*9*
BE.1.3		1	*1*
BF.11.2		1	*1*
BF.27		1	*1*
BF.36		1	*1*
BF.39		1	*1*
BF.5		2	*2*
BF.7		10	*10*
BF.7.4		2	*2*
BF.8		1	*1*
BG.2		2	*2*
BQ.1		1	*1*
XAB		1	*1*
XAN		1	*1*
XAZ		2	*2*

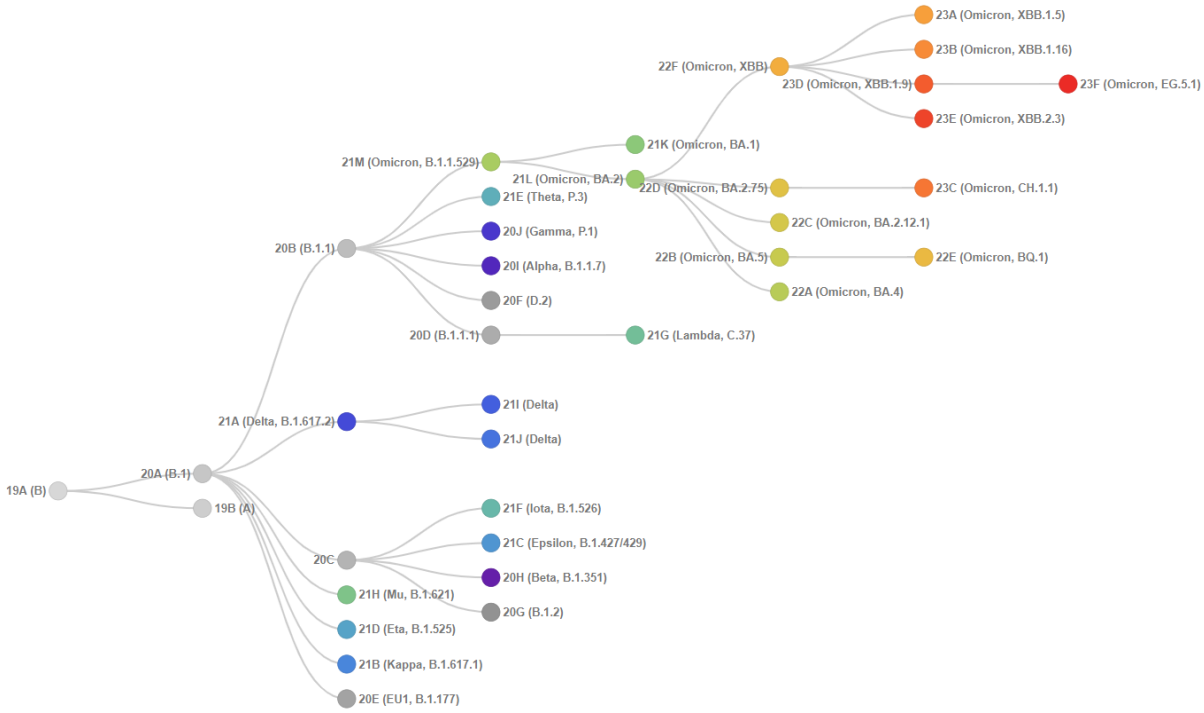


Figure 17 • Simplified evolution tree of SARS-CoV-2 viruses indicating the correspondence between the different classification system.

2.2. INFLUENZA VIRUSES

Because of the ongoing pandemic of COVID-19, no sequencing of influenza viruses was performed directly at the NRC influenza during the season 2021-2022. Samples were sent to the WHO Collaborating Centre at the Crick Institute in London (United-Kingdom).

QUALITY MANAGEMENT

1. Quality System

The NRC influenza is accredited by BELAC (Belgian Accreditation Body) according to the ISO 15189 norm. In 2022, there was no external audit conducted by BELAC of all the NRCs hosted within the Viral Diseases service, including the NRC influenza.

2. External Quality Assessment

As part of its requirements to maintain ISO 15189 accreditation and its recognition as National Influenza Centre by WHO, the NRC influenza successfully took part in several Proficiency Tests / External Quality Assessments (EQA) during the 2021-2023 season.

For influenza viruses:
The NRC influenza took part in the annual EQA organised by WHO during the summer to control the capacities of National Influenza Centres to detect influenza viruses and to correctly identify seasonal influenza viruses (influenza A virus subtype H1N1pdm09, seasonal influenza A virus subtype H3N2, seasonal influenza B virus), and non-seasonal influenza A viruses (subtype H5, H7 and H9). The EQA also includes some samples for sequencing characterisation to detect potential mutations associated with increased resistance to antivirals.

The NRC influenza also registered to commercial EQAs organised by QCMD: INFRNA21S_QAV54134 (only seasonal influenza viruses), INFTP21S_QAV064138 (also including non-seasonal influenza viruses).

For SARS-CoV-2 coronavirus:
WHO organised a parallel EQA to test the capacity of the laboratories to detect SARS-CoV-2.

The NRC influenza also registered to commercial EQAs organised by QCMD: SCV2_22C1B_QAV204215.

For other respiratory viruses:
The NRC influenza registered to several commercial EQAs organised by QCMD covering the different viruses that are targeted by the PCRs routinely used for the surveillance:

- Respiratory syncytial viruses: RSV21S_QAV054142
- Human metapneumoviruses: MPV22S_QAV054135
- Parainfluenza viruses: PINFRNA22S_QAV064136
- Seasonal coronaviruses: CVRNA22S_QAV064137
- MERS coronavirus: MERS22S_QAV154181
- Picornaviruses: RVRNA22S_QAV064143, EVRNA22S_QAV984104, PeVRNA22S_QAV114145
- Adenoviruses: end of 2022

The NRC influenza also took part in a dedicated EQA for RSV detection organised by WHO in November 2021.

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- The coordinators in the hospitals of the SARI network:
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    - Hasselt: Koen Magerman, Door Jour, Marieke Bleyven
    - UZB, Brussels: Lucie Seyler, Thomas Demuyser, Arne Witdouck, Els Van Nedervelde
    - Hôpital Universitaire St-Pierre: Nicolas Dauby, Marc Hainaut
    - Grand Hôpital de Charleroi: Xavier Holemans, Bénédicte Lissoir
    - Mont-Godinne: Marc Bourgeois, Bénédicte Delaere

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- The federal public service Public Health
- The regional health agencies of the federated entities:
    - AZG
    - AVIQ
    - COCOM

PUBLICATIONS

Scientific articles published during the 2021-2022 season:

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CONTACT: respirvir@sciensano.be

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Visit our website
>www.sciensano.be or
contact us at
>info@sciensano.be

Sciensano • Rue Juliette Wytsmanstraat 14 • Brussels • Belgium • T + 32 2 642 51 11 • T press + 32 2 642 54 20 • info@sciensano.be • www.sciensano.be

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