Respiratory Viruses in Luxembourg (ReViLux)

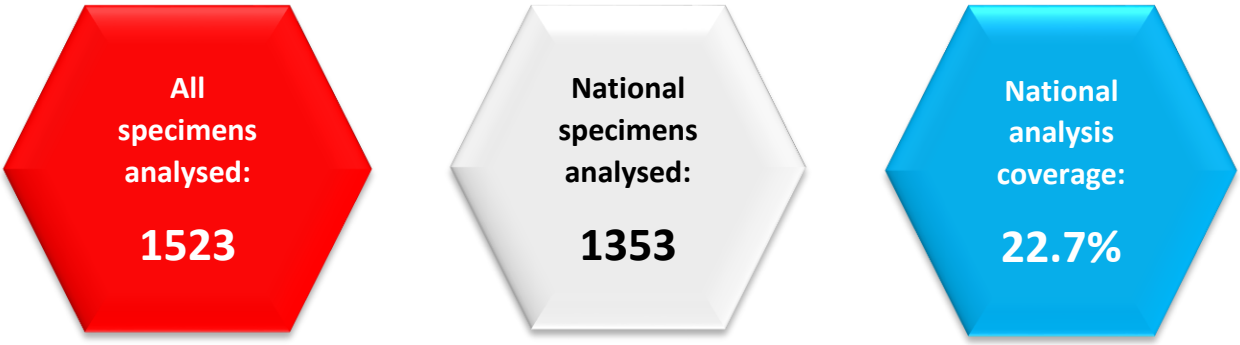
Weekly report (7 – 13 March 2022)

Executive Summary

The Sentinel Network reported 5.33% of consultations for influenza-like illness, thus exceeding the baseline circulation threshold, according to the European Center for Disease Prevention and Control (ECDC). Within the specimens collected by the Sentinel Network, the influenzavirus A is the most frequently detected virus over the last four weeks (47.0%), followed by SARS-CoV-2 (30.1%) and human rhinovirus (12.0%).

Regarding the SARS-CoV-2 genomic surveillance, the LNS sequenced 564 specimens from residents in Luxembourg in week 10 (of 5968 total cases in the Grand Duchy of Luxembourg; 9.5%). This meets the ECDC recommendations to detect emerging variants. Including PCR screening results, 1353 national specimens were analysed globally (22.7%).

The Omicron variant was the dominant one in the representative sample. The BA.2 lineage remains the most frequent one (78.2%), followed by BA.1 (21.8%). The analysis of target groups showed a statistically significant difference in the distribution of BA.1 and BA.2 according to collection setting and vaccination status, suggesting a potential lower severity and immune escape rate for BA.2.



Introduction

The Laboratoire national de santé, as **National Reference Laboratory for Acute Respiratory Infections in Luxembourg**, performs close surveillance on respiratory viruses, with a special focus on SARS-CoV-2. There are currently two active projects:

- **The Sentinel Surveillance Network**. It provides a broad picture of respiratory diseases affecting the Luxembourgish population, based on its double monitoring system (syndromic and virological).
- **The National SARS-COV-2 Genomic Surveillance Program**. It enables detailed observation of SARS-CoV-2 mutations and variants through time and space, and also monitoring specific groups of interest.

The objective of the ReViLux is to inform public health actions in Luxembourg.

Sentinel Surveillance Network

The **Sentinel Surveillance Network** aims at monitoring the circulating respiratory viruses, including SARS-CoV-2, and hence underpin public health actions. Following the World Health Organization (WHO) and European Centre for Disease Prevention and Control (ECDC) guidance, it focuses on cases of acute respiratory infection (ARI) and influenza-like illness (ILI).

The current influenza season started in week 40/2021, and the history of ILI consultations is displayed in Figure 1. A detailed summary of the number of ARI and ILI cases during the last four weeks is included in Table 1. In the week of study, 5.33% of consultations were reported as ILI, thus exceeding the threshold for baseline circulation, according to the ECDC (2.59%).

Table 1. Syndromic surveillance over the last 4 weeks.

Week	ARI		ILI		Total consultations
	N	%	N	%	
2022/7	26	15.03	1	0.58	173
2022/8	55	18.33	7	2.33	300
2022/9	64	14.55	11	2.50	440
2022/10	81	18.00	24	5.33	450

ARI: Acute Respiratory Infections; ILI: Influenza-Like Illness.

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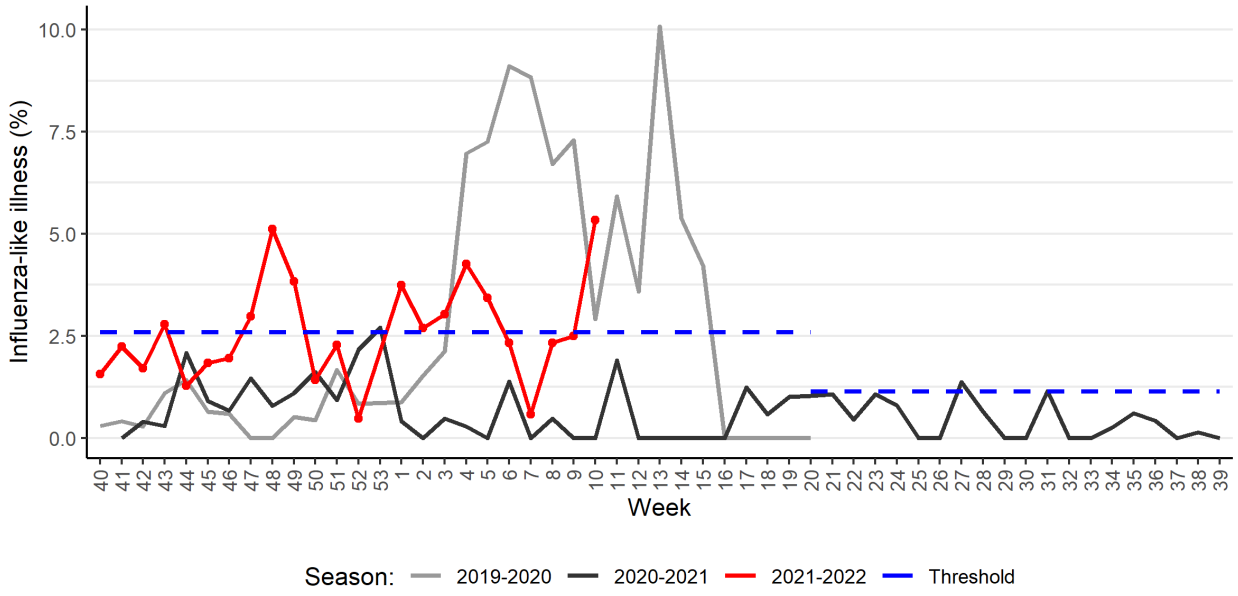

Figure 1. Percentage of patients with influenza-like illness over the last three seasons

Additionally, a selection of sentinel cases is further studied in order to monitor the circulation of respiratory viruses in the country, as shown in Figure 2. Over the last 4 weeks, influenzavirus A was detected in 47.0% of positive specimens (including 3 co-infection cases), and influenzavirus B in 4.8% (including 1 co-infection). These results are displayed more in detail in Table 2.

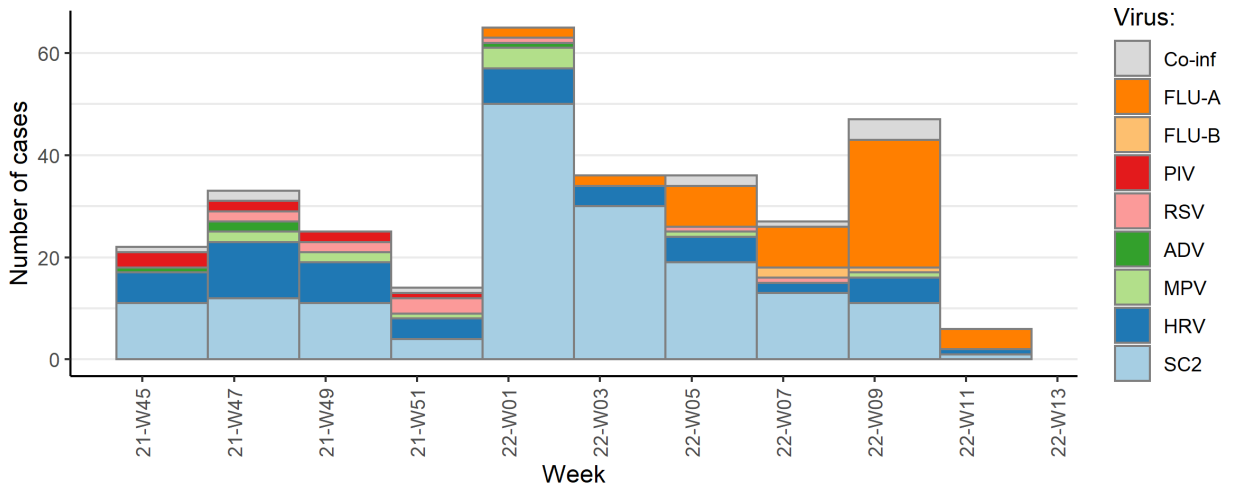

Figure 2. Distribution of respiratory viruses detected within the Sentinel Network, by two weeks. Results from last weeks are not yet consolidated.

Co-inf: co-infection; FLU-A: influenzavirus A; FLU-B: influenzavirus B; PIV: parapneumovirus; RSV: respiratory syncytial virus; ADV: adenovirus; MPV: metapneumovirus; HRV: human rhinovirus; SC2: SARS-CoV-2.

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Table 2. Distribution of respiratory viruses detected within the Sentinel Network over the last 4 weeks and during the current season.

Virus	Last 4 weeks		Current season	
	N*	%	N*	%
SARS-CoV-2	25	30.1	252	54.3
Human rhinovirus	10	12.0	104	22.4
Influenzavirus A	39	47.0	53	11.4
Metapneumovirus	3	3.6	16	3.4
Respiratory syncytial virus	1	1.2	15	3.2
Parainfluenzavirus	0	0.0	14	3.0
Adenovirus	1	1.2	6	1.3
Influenzavirus B	4	4.8	4	0.9
Total	**83**	**100.0**	**464**	**100.0**

**Co-infection cases counted once for each virus detected.*

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SARS-CoV-2 Genomic Surveillance

The current sequencing strategy

The National Reference Laboratory for Acute Respiratory Infections at LNS receives SARS-CoV-2 positive samples (nasopharyngeal or oropharyngeal swabs analysed by RT-PCR) from the national network of laboratories and proceeds as follows:

1) Sequencing a representative sample of specimens
2) Sequencing specimens from target groups (i.e. hospital cases and post-vaccination cases)
3) Sequencing specimens from clusters with high transmission.

The representative sample of specimens is a systematic selection from all SARS-CoV-2 positive cases registered in Luxembourg to detect emerging variants and early increases in their incidence and transmission within the community in Luxembourg. This sample is selected according to the ECDC guidelines.

Since the emergence of the Omicron variant of concern, and given the high incidence rates in the European context, targeted PCR tests are carried systematically in order to get an earlier interim evaluation of the variants in circulation. The PCR kits currently used target the following spike mutations: 69/70del, K417N, N501Y.

The LNS shares its sequencing results with GISAID EpiCov database periodically. SARS-CoV-2 lineages have been assigned based on Rambaut et al. using the Phylogenetic Assignment of Named Global Outbreak LINeages (pangolin) software (v3.1.20, pangoLEARN 2022-02-28). The Pango nomenclature is used in addition to the WHO nomenclature to enable easier visualization of links between any evolving variants and their ancestor (See nomenclature equivalences in Appendix 1).

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Screening and sequenced specimens

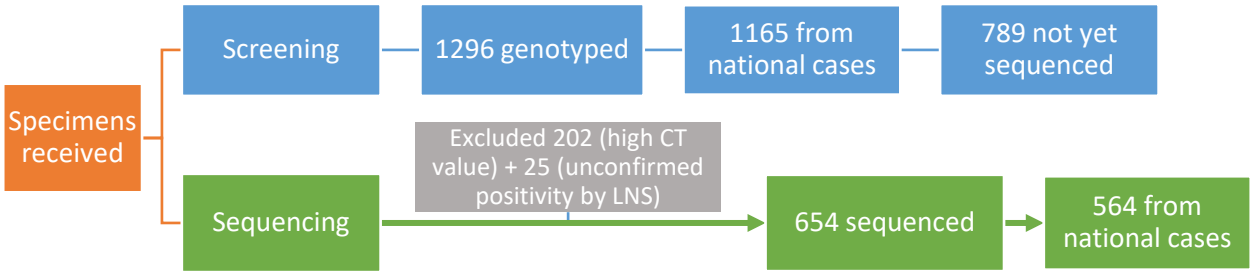

Figure 3. Flowchart of specimens collected during week 10/2022

In week 10, 5968 new cases were registered in Luxembourg; hence, the minimum sample size required to detect emerging variants at a 2.5% incidence is estimated to be 545 specimens (9.1%).

As shown in Figure 3, of all specimens received from the week of study, 1296 were screened by targeted PCR for the Omicron variant (including 1165 national specimens), in order to enable an earlier detection of potential Omicron cases (see results in the following section). In parallel, the microbial genomics unit at the LNS sequenced 654 specimens, including 564 national ones. The weekly sequencing coverage remains at 9.5% (564 out of 5968 cases registered in Luxembourg; see coverage trend in Figure 4), which exceeds the recommended sample size. Overall, 1353 national specimens were analysed either by sequencing or screening (22.7%).

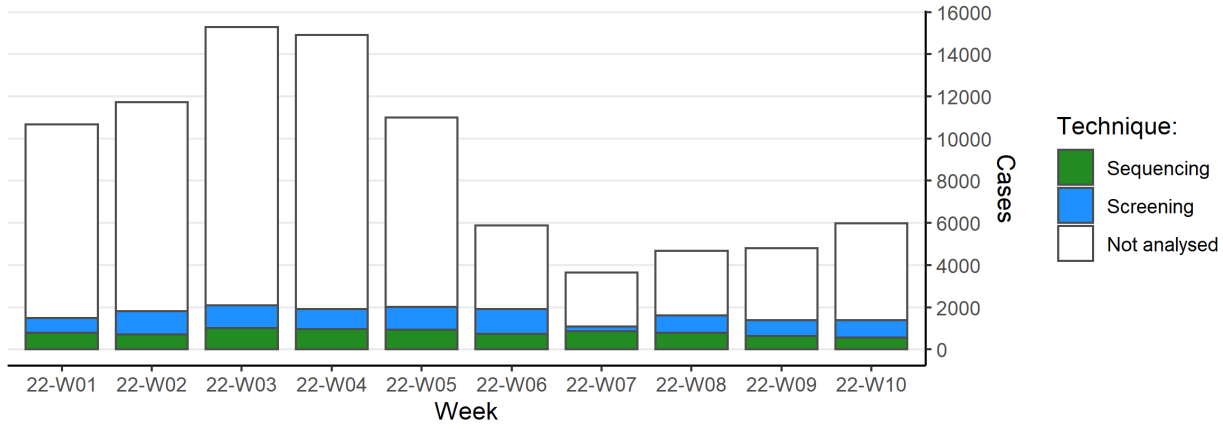

Figure 4. National coverage based on weekly number of positive cases in Luxembourg. The coverage from the latest weeks might not be consolidated yet.

Omicron screening results

As shown in Figure 5, of the 1296 specimens from week 10 which were screened by targeted PCR, all were identified as potential Omicron cases: 74.1% BA.2 and 25.9% BA.1.

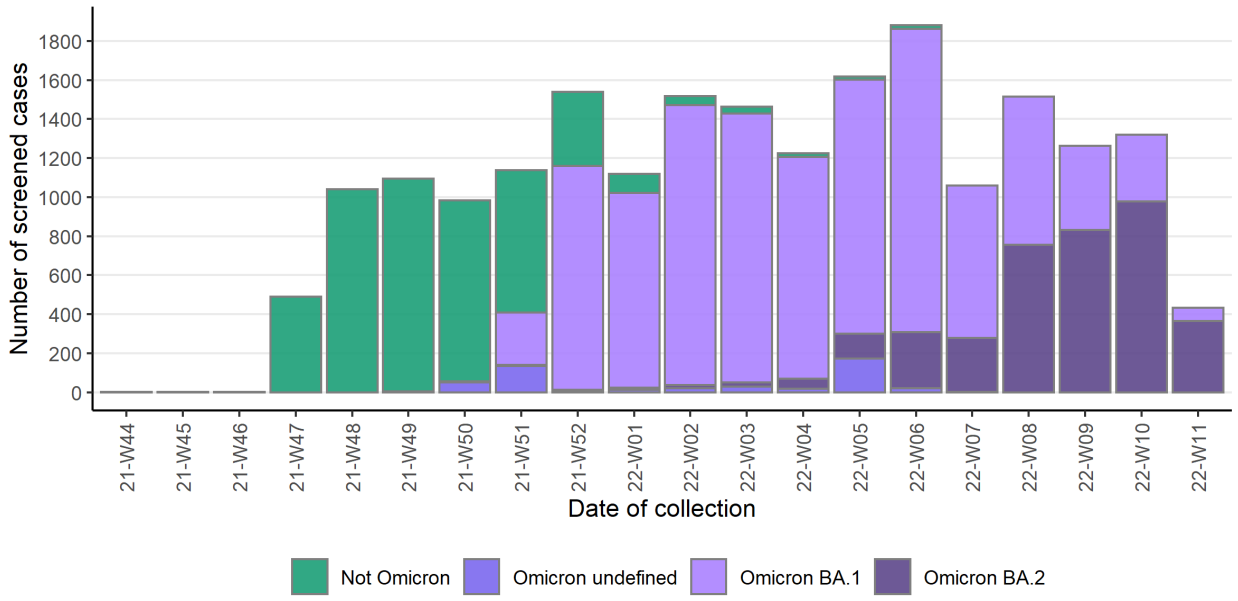

Figure 5. Number of specimens included in the screening for the Omicron variant by date of collection. Results more recent than the week of study are not yet consolidated.

Circulating lineage detection

The distribution of successfully assigned lineages within the national selection is shown in Figure 6. Regarding Delta AY sublineages, only a selection is displayed, based on their prevalence during the last 10 weeks (min. 1%). This distribution is further detailed for the last 2 weeks in Table 4.

The Omicron variant remains the dominant one within the representative sample. The most frequent lineage is now BA.2 (78.2%), followed by BA.1 (21.8%).

A summary of the VOCs assigned among all specimens sequenced (including non-residents) during the last two weeks and since the beginning of the sequencing activity is shown in Table 5.

Table 4. Distribution of SARS-CoV-2 lineages detected within the representative sample during the last two weeks (previously reported cases might be updated by retrospective analysis).

Variant	Previous week			Current week		
	N	%	CI %	N	%	CI %
Omicron	532	99.8	99.4 – 100.0	545	100.0	-
BA.1	175	32.8	28.8 – 36.8	119	21.8	18.4 – 25.3
BA.2	357	67.0	63.0 – 71.0	426	78.2	74.7 – 81.6
BA.1.1	0	-	-	0	-	-
Delta	1	0.2	0.0 – 0.6	0	-	-
Beta	0	-	-	0	-	-
Gamma	0	-	-	0	-	-
Others	0	-	-	0	-	-
Total	**532**	**100.0**	**-**	**545**	**100.0**	**-**

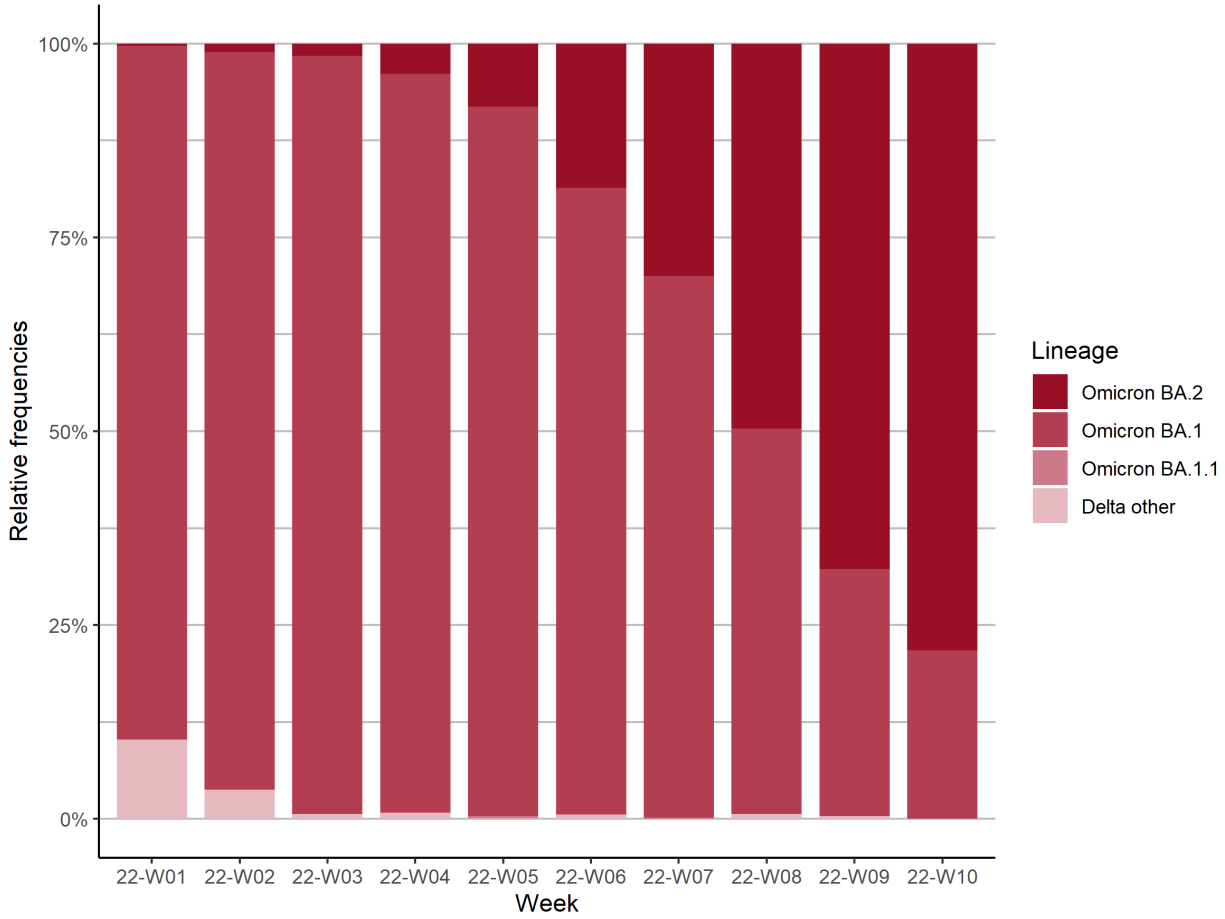

Figure 6. Distribution of lineages within the representative sample during the last 10 weeks.

Table 5. Distribution of VOCs within all samples sequenced (including non-residents)

Variant	**Previous week**		**Current week**		**Cumulative count**
	N	**%**	**N**	**%**	**N**
Delta	2	0.3	0	-	12 502
Omicron	652	69.7	612	100.0	9273
BA.1	201	30.7	130	21.2	7313
BA.2	451	69.0	482	78.8	1956
BA.1.1	0	-	0	-	4
Gamma	0	-	0	-	1305
Beta	0	-	0	-	1255
Other	0	-	0	-	12 170
Total	**654**	**100.0**	**612**	**100.0**	**36 505**

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Target groups

The LNS prioritises the sequencing of specimens collected at hospitals. These may belong to either outpatients or inpatients, and patients hospitalised for reasons other than COVID-19 might also be included. Therefore, not all hospital specimens can be attributed to severe COVID-19 cases. The distribution of Omicron lineages (BA.1 and BA.2) was studied by origin of the specimen (hospital or community) (Table 6), and a statistically significant difference was found ($p < 0.1$). This means that the proportion of BA.2 is lower among hospital specimens, suggesting a potential lower risk of hospitalisation that should be further studied. Only specimens from the latest 3 weeks were included in order to avoid bias by differing epidemiological contexts.

Table 6. Comparison of Omicron lineages distribution by sampling setting.

	Community	**Hospital**
Omicron BA.1	37.0%	31.5%
Omicron BA.2	63.0%	68.5%
Total	100.0%	100.0%

Number of cases: General = 1510, Hospital = 572. P value = 0.022 (χ^2).

Similarly, the distribution of VOC cases was studied by vaccination status (fully vaccinated with 2 or more doses vs non-vaccinated) (Table 7), and a statistically significant difference was found ($p < 0.05$). This suggests that BA.2 may have a lower vaccine escape rate, which should be further studied.

Table 7. Comparison of VOC distribution by vaccination status.

	Not vaccinated	**Fully vaccinated**
Omicron BA.1	31.2%	37.8%
Omicron BA.2	68.8%	62.2%
Total	100.0%	100.0%

Number of cases: Not vaccinated = 413, Fully vaccinated = 760. P value = 0.03 (χ^2).

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Mutation surveillance

In addition to the surveillance of SARS-CoV-2 variants, the LNS monitors the occurrence of SARS-CoV-2 mutations reported to have a clinical and epidemiological relevance. This complementary surveillance enables us to detect unexpected mutations among the specimens sequenced. Newly acquired mutations may occur and their early detection might be key to expect changes in the epidemic evolution. Following ECDC guidance, the LNS is currently monitoring 42 mutations to the spike protein frequently associated to VOCs. As each VOC is characterised by a set of defining mutations, which are expected to be highly present, it is interesting to analyse the non-defining ones. Among the specimens collected over the last four weeks, the following mutations were detected (minimum 1% prevalence):

- BA.1 specimens:
    - A701V (in at least 3.8%), frequently associated to the Beta variant.
- BA.2 specimens:
    - del69-70, L981F (each in at least 1.04%), frequently associated to Omicron BA.1.

Concerning BA.1 specimens, the A701V mutation continues to show a decreasing trend and remains below the rate in the European region. Concerning BA.2 specimens, all aforementioned mutations also showed decreasing trends, but still higher than in the European region (<1%, according to GISAID), so they will remain under monitoring.

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Appendices

Appendix 1: SARS-CoV-2 variants of concern

According to the ECDC

Table A1-a. Nomenclature for variants of concern by the European Centre for Disease Prevention and Control (ECDC)

WHO label	Pango lineage*	Spike mutations of interest	First detection	Evidence for impact on: transmission	immunity	severity
Beta	B.1.351	K417N, E484K, N501Y, D614G, A701V	South Africa, Sept 2020	Increased (v)	Increased (v)	Increased (v)
Gamma	P.1	K417T, E484K, N501Y, D614G, H655Y	Brazil, Dec 2020	Increased (v)	Increased (v)	Increased (v)
Delta	B.1.617.2	L452R, T478K, D614G, P681R	India, Dec 2020	Increased (v)	Increased (v)	Increased (v)
Omicron	B.1.1.529	**	South Africa, Botswana, Nov 2021	Increased (v)	Increased (v)	Reduced (v)

WHO: World Health Organization. (v): evidence derived from the variant itself; (m): evidence derived from mutations associated with the variant.

**All sub-lineages included.*

***A67V, Δ69-70, T95I, G142D, Δ143-145, N211I, Δ212, ins215EPE, G339D, S371L, S373P, S375F, K417N, N440K, G446S, S477N, T478K, E484A, Q493R, G496S, Q498R, N501Y, Y505H, T547K, D614G, H655Y, N679K, P681H, N764K, D796Y, N856K, Q954H, N969K, L981F.*

Adapted from ECDC – SARS-CoV-2 variants of concern (https://www.ecdc.europa.eu/en/covid-19/variants-concern).

According to the WHO

Table A1-b. Nomenclature for variants of concern by the World Health Organization (WHO)

WHO label	Pango lineage*	GISAID clade/lineage	Nextstrain clade	Additional amino acid changes monitored	Earliest documented samples	Date of designation
Alpha	B.1.1.7[#]	GRY (formerly GR/501Y.V1)	20I (V1)	+S:484K +S:452R	United Kingdom, Sep-2020	18-Dec-2020
Beta	B.1.351	GH/501Y.V2	20H (V2)	+S:L18F	South Africa, May-2020	18-Dec-2020
Gamma	P.1	GR/501Y.V3	20J (V3)	+S:681H	Brazil, Nov-2020	11-Jan-2021
Delta	B.1.617.2[§]	GK	21A, 21I, 21J	+S:417N +S:484K	India, Oct-2020	VOI: 4-Apr-2021 VOC: 11-May-2021
Omicron	B.1.1.529°	GRA	21K, 21L, 21M	+S:R346K	Multiple countries, Nov-2021	VUM: 24-Nov-2021 VOC: 26-Nov-2021

**All sublineages included. [#] includes all Q sublineages. [§] includes all AY sublineages. ° includes all BA sublingeages*

Adapted from WHO - Tracking SARS-CoV-2 variants (https://www.who.int/en/activities/tracking-SARS-CoV-2-variants/)