Respiratory Viruses in Luxembourg (ReViLux)

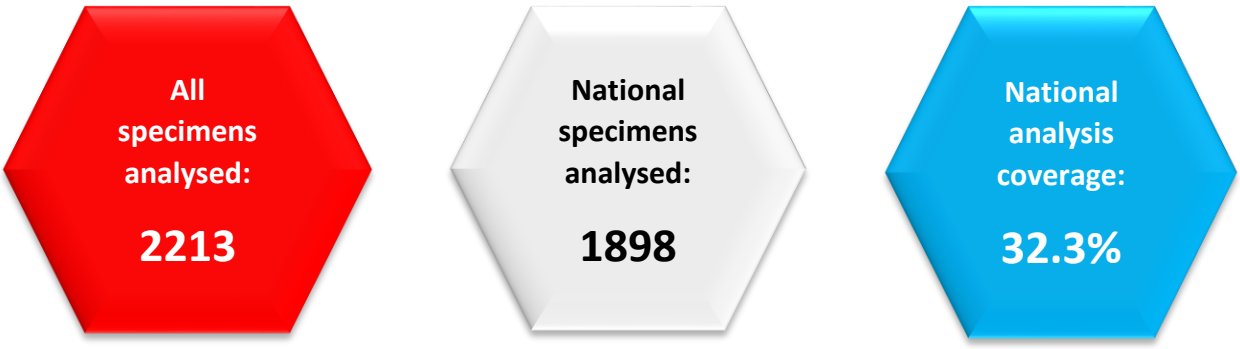
Weekly report (7 – 13 February 2022)

Executive Summary

The Sentinel Surveillance Network reported 2.33% of influenza-like illness, thus staying at baseline transmission according to the European Center for Disease Prevention and Control (ECDC). SARS-CoV-2 remains the most frequently detected virus over the last four weeks (61.8%), followed by influenzavirus type A (17.1%) and human rhinovirus (14.5%).

Regarding SARS-CoV-2 genomic surveillance, the LNS sequenced 675 specimens from residents in Luxembourg in week 6 (from 5871 total cases in the Grand Duchy of Luxembourg; 11.5%). This exceeds the ECDC recommendations to detect emerging variants at 2.5%. Including PCR screening results, 1898 national specimens were analysed globally (32.3%).

The Omicron variant was assigned to 99.4% of national cases collected during week 6, remaining the dominant one. The most frequent lineage was BA.1 (80.3%), followed by BA.2 (19.1%). The analysis of target groups returned no statistically significant differences between BA.1 and BA.2 by collection setting (hospital vs. community specimens) or vaccination status.



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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, 2.33% of consultations were reported as ILI, which falls below the threshold for the epidemic season (2.59%) according to the ECDC.

Table 1. Syndromic surveillance over the last 4 weeks.

Week	ARI		ILI		Total consultations
	N	%	N	%	
2022/3	72	16.78	13	3.03	429
2022/4	65	18.47	15	4.26	352
2022/5	36	17.65	7	3.43	204
2022/6	65	16.80	9	2.33	387

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

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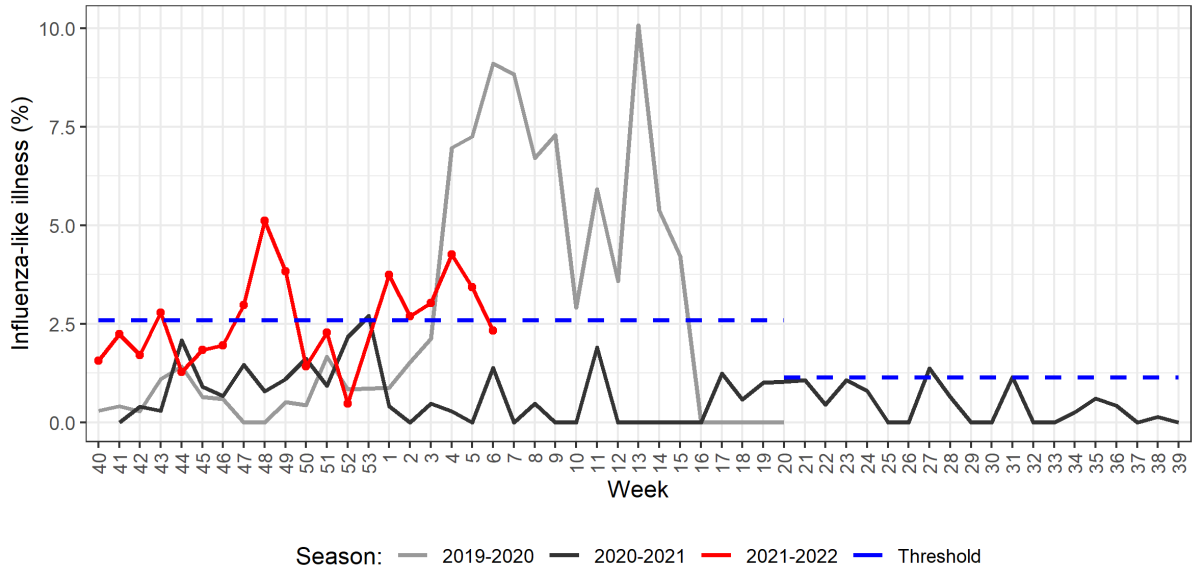

Figure 1. Percentage of patients with influenza-like illness over the last three seasons

Additionally, a selection of cases is further studied in order to monitor different respiratory viruses circulating in the country, as shown in Figure 2. Over the last 4 weeks, influenzavirus type A was detected in 13 specimens from the Sentinel Network, including 1 co-infection with human rhinovirus. A triple co-infection was also detected for adenovirus, metapneumovirus and respiratory syncytial virus. These results are displayed more in detail in Table 2.

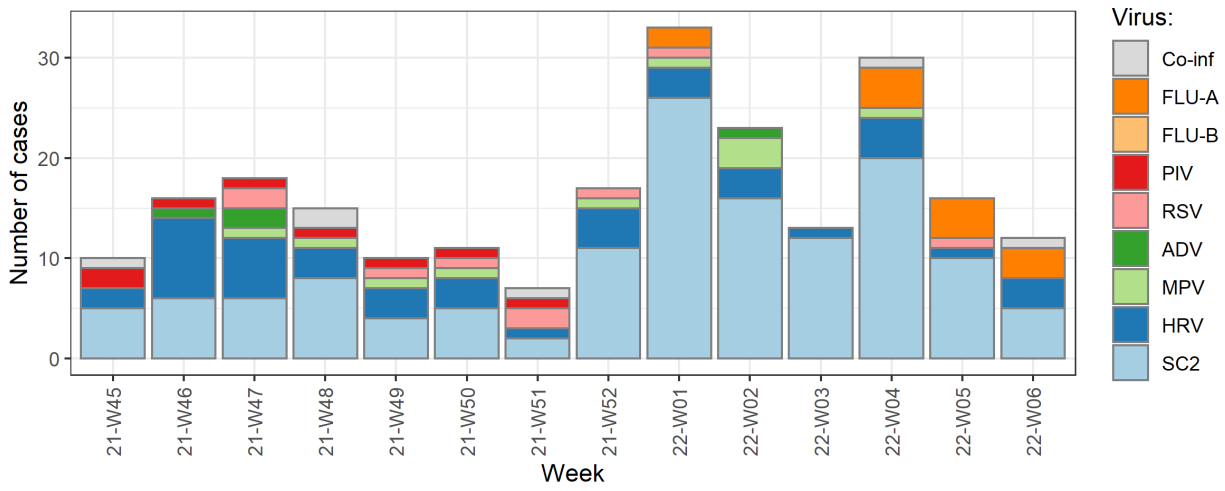

Figure 2. Distribution of respiratory viruses detected within the Sentinel Network, by week. Results from last two weeks are not yet consolidated.

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.

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	47	61.8	225	59.1
Human rhinovirus	11	14.5	95	24.9
Influenzavirus type A	13	17.1	15	3.9
Parainfluenzavirus	0	0.0	14	3.7
Respiratory syncytial virus	2	2.6	14	3.7
Metapneumovirus	2	2.6	13	3.4
Adenovirus	1	1.3	5	1.3
Influenzavirus type B	0	0	0	0
Total	**81**	**100.0**	**363**	**100.0**

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

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 all specimens from hospital cases.
2) Sequencing all specimens from post-vaccination cases.
3) Sequencing specimens from clusters with high transmission.
4) Sequencing a representative sample of community cases.

The representative sample of community cases 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.

Due to the emergence of the new Omicron variant of concern, as well as the high incidence rates in the European context, targeted PCR tests are carried systematically in order to detect potential Omicron cases within 24h from reception of the specimen. The PCR kits used target the following spike mutations: 69/70del, K417N, N501Y. The potential cases identified this way are then prioritised for confirmation by sequencing.

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 2022-02-02). 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).

Screening and sequenced specimens

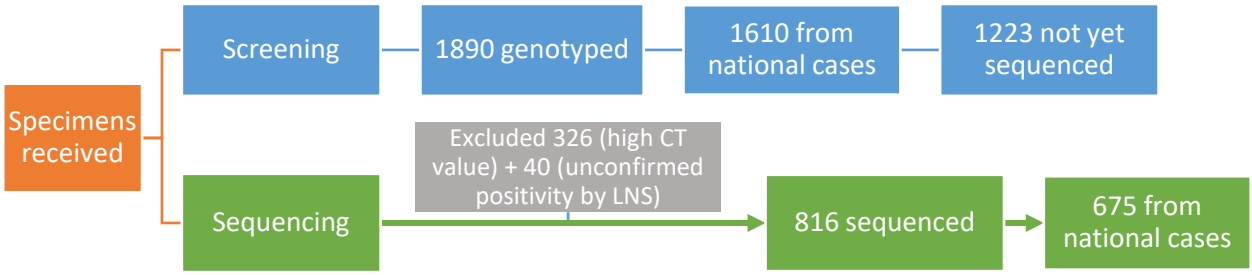

Figure 3. Flowchart of specimens collected during week 6/2022

In week 5, 5871 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 544 specimens (9.3%).

As shown in Figure 3, of all specimens received from the week of study, 1725 were screened by targeted PCR for the Omicron variant (including 1475 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 816 specimens, including 675 national ones. The weekly sequencing coverage remains at 11.5% (675 out of 5871 cases registered in Luxembourg; see coverage trend in Figure 4), which exceeds the recommended sample size. Overall, 1898 national specimens were analysed either by sequencing or screening (32.3%).

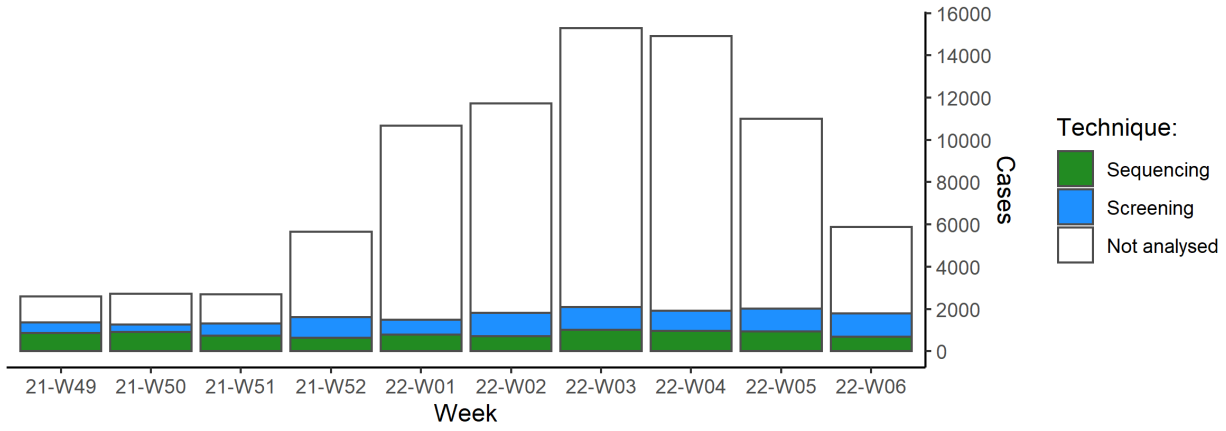

Figure 4. National coverage based on weekly number of positive cases in Luxembourg. The coverage from the latest weeks might not be consolidated yet.

Omicron screening results

As shown in Figure 5, of the 1890 specimens from week 6 screened by targeted PCR, 1860 were identified as potential Omicron cases (98.9%).

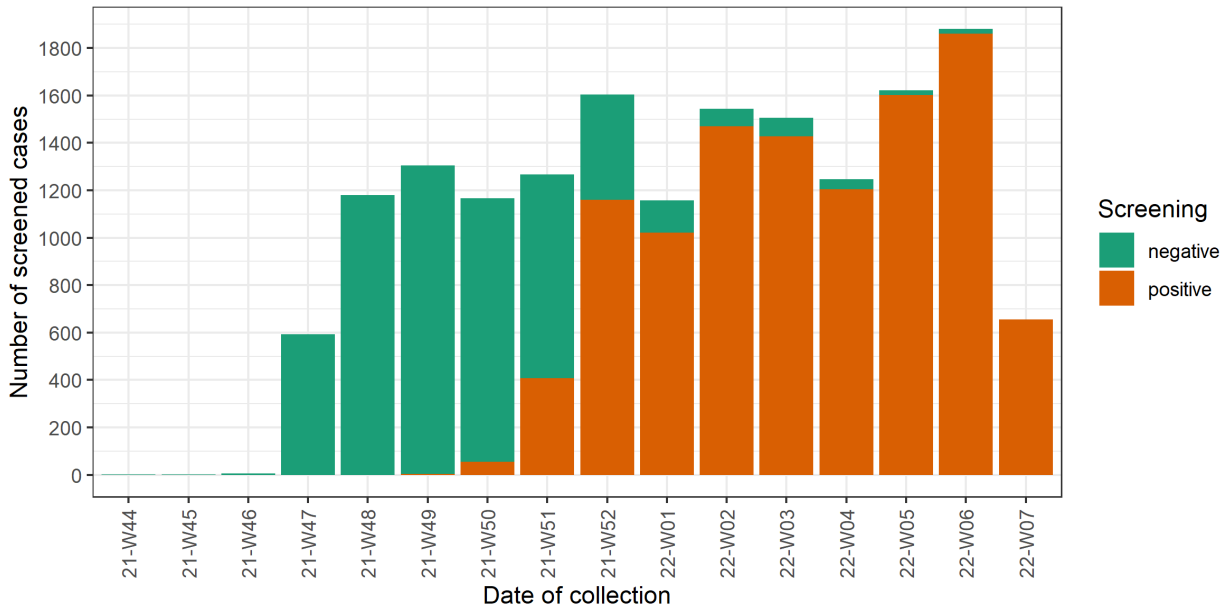

Figure 5. Number of specimens included in the screening for the Omicron variant by date of collection. Results more recent than the week of study are not yet consolidated.

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 (99.8%) within the representative sample. The most frequent lineage is BA.1, followed by BA.2, which continues to increase. Concerning the Delta variant, 1 specimen was assigned to the lineage AY.43.

Table 4. Distribution of SARS-CoV-2 lineages detected within the representative sample in weeks 4-5 (previously reported cases might be updated by retrospective analysis).

Variant	Week 5			Week 6		
	N	%	CI %	N	%	CI %
Omicron	568	99.8	99.5 – 100.0	541	99.4	98.8 – 100.0
BA.1	530	93.1	91.1 – 95.2	437	80.3	77.0 – 83.7
BA.2	36	6.3	4.3 – 8.3	104	19.1	15.8 – 22.4
BA.1.1	2	0.4	0.0 – 0.8	0	-	-
Delta	1	0.2	0.0 – 0.5	3	0.6	0.0 – 1.2
Beta	0	-	-	0	-	-
Gamma	0	-	-	0	-	-
Others	0	-	-	0	-	-
Total	**569**	**100.0**	**-**	**544**	**100.0**	**-**

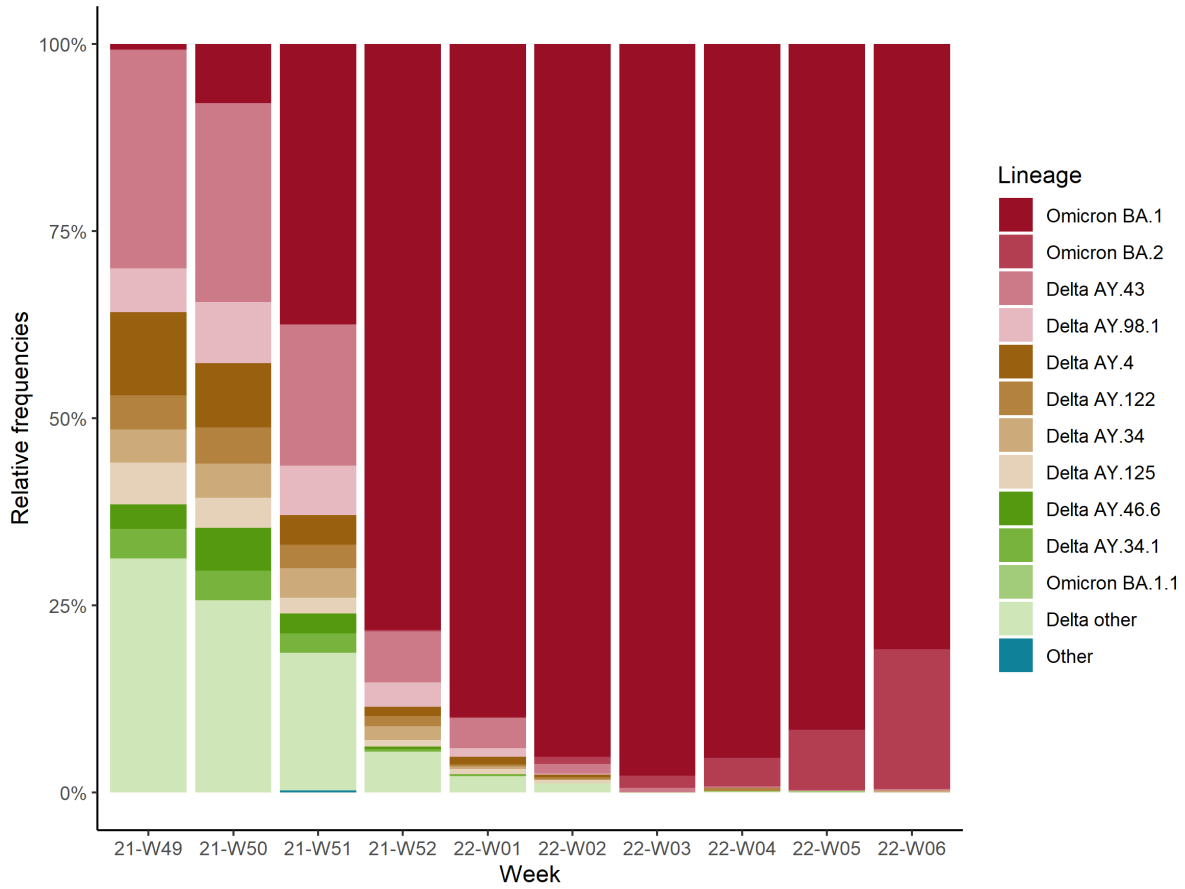

Figure 6. Distribution of lineages within the national selection during the last 10 weeks.

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), but no statistically significant difference was found ($p > 0.1$). Only specimens from the latest 3 weeks were included in order to avoid bias by differing epidemiological situations.

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

	Community	**Hospital**
Omicron BA.1	89.3%	90.0%
Omicron BA.2	10.7%	10.0%
Total	100.0%	100.0%

Number of cases: General = 2169, Hospital = 659. P value > 0.1 (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 no statistically significant difference was found either ($p > 0.1$).

Table 7. Comparison of VOC distribution by vaccination status.

	Not vaccinated	**Fully vaccinated**
Omicron BA.1	89.0%	84.9%
Omicron BA.2	11.0%	15.1%
Total	100.0%	100.0%

Number of cases: Not vaccinated = 326, Fully vaccinated = 456. P value > 0.1 (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 LNS found the following mutations (minimum 1% prevalence):

- BA.1 specimens:
  - A701V (in at least 7.4%), frequently associated to the Beta variant.
- BA.2 specimens:
  - T95I and L981F (each in at least 1.3%), frequently associated to Omicron BA.1.
  - A67V, del69-70 and N856K (each in at least 1.0%), frequently associated to Omicron BA.1.

Nevertheless, all aforementioned mutations were found in lower or similar proportions to the European region, according to GISAID.

References

Centers for Disease Control and Prevention. SARS-CoV-2 Variant Classifications and Definitions. Retrieved 22 February 2022, from https://www.cdc.gov/coronavirus/2019-ncov/variants/variant-info.html

COVID-19 Data Portal - accelerating scientific research through data. (2021). Retrieved 22 February 2022, from https://www.covid19dataportal.org/sequences

Direção-Geral da Saúde. Relatório de Situação. Retrieved 22 February 2022, from https://covid19.min-saude.pt/relatorio-de-situacao/

European Centre for Disease Prevention and Control. Guidance for representative and targeted genomic SARS-CoV-2 monitoring – 3 May 2021. ECDC : Stockholm ; 2021

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European Centre for Disease Prevention and Control. SARS-CoV-2 variants of concern. Retrieved 22 February 2022, from https://www.ecdc.europa.eu/en/covid-19/variants-concern

Genomic sequencing of SARS-CoV-2: a guide to implementation for maximum impact on public health. Geneva: World Health Organization; 2021.

GISAID. EpiCoV – Pandemic coronavirus causing COVID-19. Retrieved 22 février 2022, from https://www.gisaid.org

GitHub - cov-lineages/pangolin: Software package for assigning SARS-CoV-2 genome sequences to global lineages. (2021). Retrieved 22 February 2022, from https://github.com/cov-lineages/pangolin

Hadfield J., Megill C., Bell S., Huddleston J., Potter B., Callender C. et al. (2018). Nextstrain: real-time tracking of pathogen evolution. Bioinformatics, 34(23), 4121-4123. doi: 10.1093/bioinformatics/bty407

Instituto Nacional de Saúde Doutor Ricardo Jorge. Diversidade genética do novo coronavírus SARS-CoV-2 (COVID-19) em Portugal. Retrieved 22 February 2022, from https://insaflu.insa.pt/covid19/

Pango Network. New AY lineages. Retrieved 22 February 2022, from: https://www.pango.network/new-ay-lineages-and-an-update-to-ay-4-ay-12/

Rambaut A., Holmes E., O'Toole Á., Hill V., McCrone J., Ruis C. et al. (2020). A dynamic nomenclature proposal for SARS-CoV-2 lineages to assist genomic epidemiology. Nature Microbiology, 5(11), 1403-1407. doi: 10.1038/s41564-020-0770-5

Robert Koch Institut. Aktueller Lage-/Situationsbericht des RKI zu COVID-19. Retrieved 22 February 2022, from https://www.rki.de/DE/Content/InfAZ/N/Neuartiges_Coronavirus/Situationsberichte/Gesamt.html;jsessionid=69BC18053F9591C56EB148E463103DB7.internet101

Santé publique France. Coronavirus : chiffres clés et évolution de la COVID-19 en France et dans le Monde. Retrieved 22 February 2022, from https://www.santepubliquefrance.fr/dossiers/coronavirus-covid-19/coronavirus-chiffres-cles-et-evolution-de-la-covid-19-en-france-et-dans-le-monde

Santé publique France. Enquêtes Flash : évaluation de la circulation des variants du SARS-CoV-2 en France. Retrieved 22 February 2022, from https://www.santepubliquefrance.fr/etudes-et-enquetes/enquetes-flash-evaluation-de-la-circulation-des-variants-du-sars-cov-2-en-france

Sciensano. COVID-19 – Bulletin épidémiologique hebdomadaire. Retrieved 22 February 2022, from https://covid-19.sciensano.be/fr/covid-19-situation-epidemiologique

The Luxembourgish data platform. COVID-19: Rapports hebdomadaires. Retrieved 22 February 2022, from https://data.public.lu/en/datasets/covid-19-rapports-hebdomadaires/

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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.*

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