LNS LUXEMBOURG

LE GOUVERNEMENT
DU GRAND-DUCHÉ DE LUXEMBOURG
Ministère de la Santé

Direction de la santé

Respiratory Viruses in Luxembourg (ReViLux)

Weekly report (Period 01-07/03/2021)

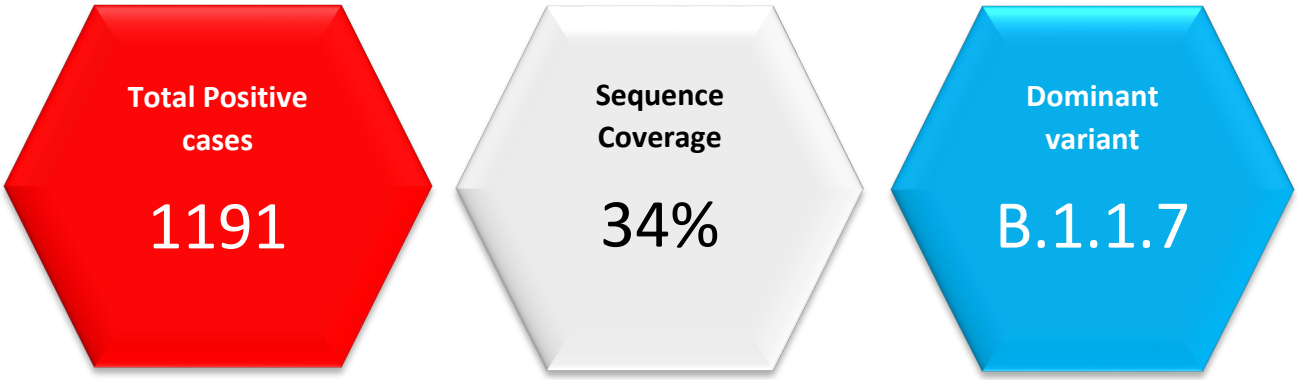
Executive Summary

The aim of the "Sentinel" national surveillance program is to monitor the circulating respiratory viruses, including SARS-CoV-2 variants, and hence underpin public health actions.

In week 9/2021, the overall frequency of the SARS-CoV-2 B.1.1.7 variant in all specimens sequenced remained stable at 62,8% (CI 58,1% - 67,5%, p<0,05). For the SARS-CoV-2 B.1.351 variant, we found an overall frequency of 18,5% (CI 14,7% - 22,2%, p<0,05) within the sequenced specimens.

The representative sample was estimated, based on the number of positive cases in Luxembourg for week 9 (1191). The minimum sample size required to detect prevalence of B.1.1.7 (63%) reported in week 8 with an error margin of 5% was estimated to be **276** specimens. This number corresponds to a coverage of 23.1% which exceeds the minimum coverage recommended by ECDC (10%). Hence our sequencing results this week are representative of the circulating variants in Luxembourg.

The total number of sequences performed this week was 475, with 411 specimens collected in the time frame of week 9/2021. The sequencing coverage this week was 34% of all positive cases in Luxembourg.



Scope

At present, the scope of the ReViLux report is to provide (i) surveillance and descriptive epidemiology data, including data on molecular phylogenies (identification of importation events, changes in outbreak size over time), and (ii) phylogenetic interpretation of regional virus spread and circulation in Luxembourg, and cluster investigation. The scope of the ReViLux report is not to attempt phylogeographical reconstructions in Luxembourg, as a whole.

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Clinical Surveillance

The "Sentinel" surveillance network reported 294 consultations in week 9 (01/MAR/2021 - 07/MAR/2021). There was no case of ILI[1], as shown in Figure 1. The percentage of consultations for ARI[2] was 7,8%.

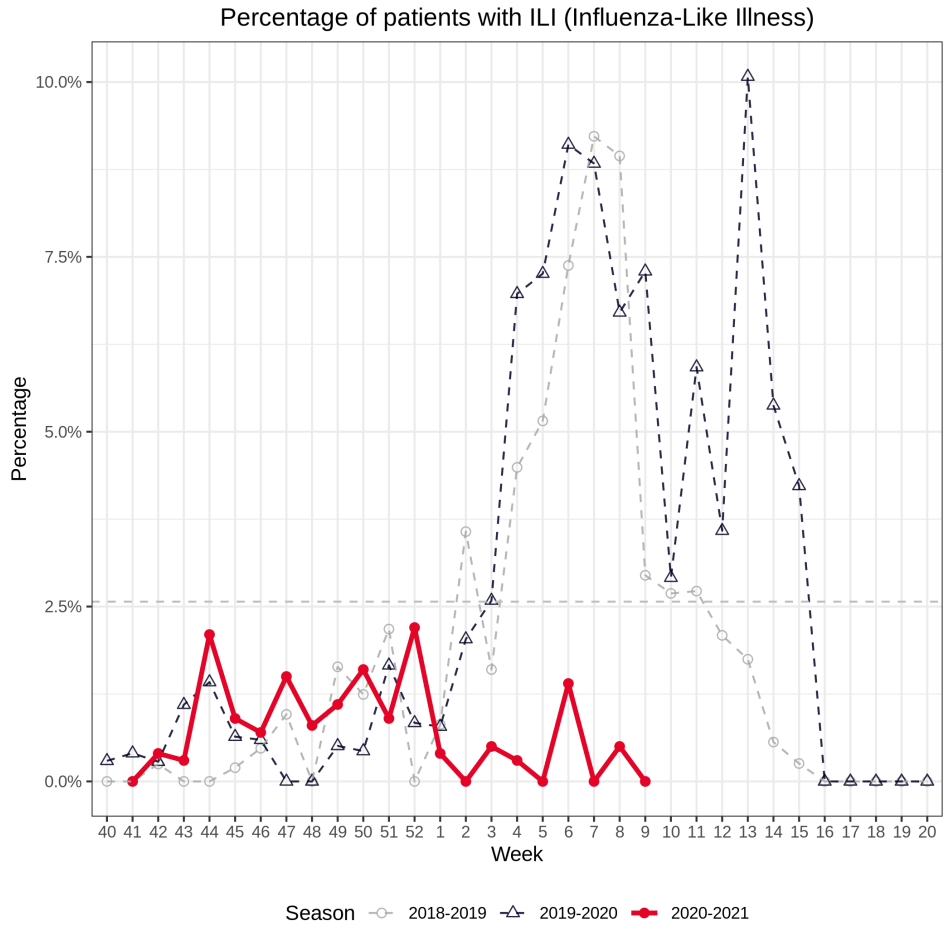

Figure 1 Percentage of patients with ILI over the epidemiological weeks

1. **ILI**: - Influenza-Like Illness: Acute respiratory symptoms <10 days, Fever 38◦C, systematic symptoms (myalgia, malaise, …)
2. **ARI**: - Acute Respiratory Infection: Acute respiratory symptoms (bronchitis, pharyngitis, rhinitis, pneumonia…) with or without fever.

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Virological Surveillance

Patients presenting with ILI and ARI at the Covid Consultation Centre (CCC) in Luxembourg were tested using a respiratory virus panel (ADV = Adenovirus, FLU A = Influenza A, FLU B = Influenza B, HRV = Human Rhinovirus, MPV = Human metapneumovirus, PIV = Parainfluenza virus, RSV = Respiratory Syncytial Virus, SARS-CoV-2 = Severe acute respiratory syndrome coronavirus 2).

The SAR-COV-2 was the most prevalent respiratory virus detected in the "Sentinel" network, with 33 positive cases (19%). The wave of Human Rhinovirus (HRV) continued in week 9/2021, with 16 positive cases in 174 tests (9%). Sporadic cases of other respiratory viruses were identified in week 9, including 2 cases of PIV (1%), 2 cases of ADV (1%) and 1 case of MPV (0,5%). No cases of Influenza A/B were detected, indicating absence of circulation of Influenza viruses in Luxembourg, as shown in Figure 2.

In Luxembourg, we have tested 174 specimens from the Sentinel surveillance network, as compared to 1026 specimens tested in Europe, in the week 09/2021. Three of these 1026 specimens, tested for influenza viruses, were positive. The influenza epidemic in the European Region has usually reached its peak by this point of the year but, despite widespread and regular testing for influenza, reported influenza activity still remains at a very low level, likely due to the impact of the various public health and social measures, implemented to reduce transmission of SARS-CoV-2 (Source: FluNews Europe).

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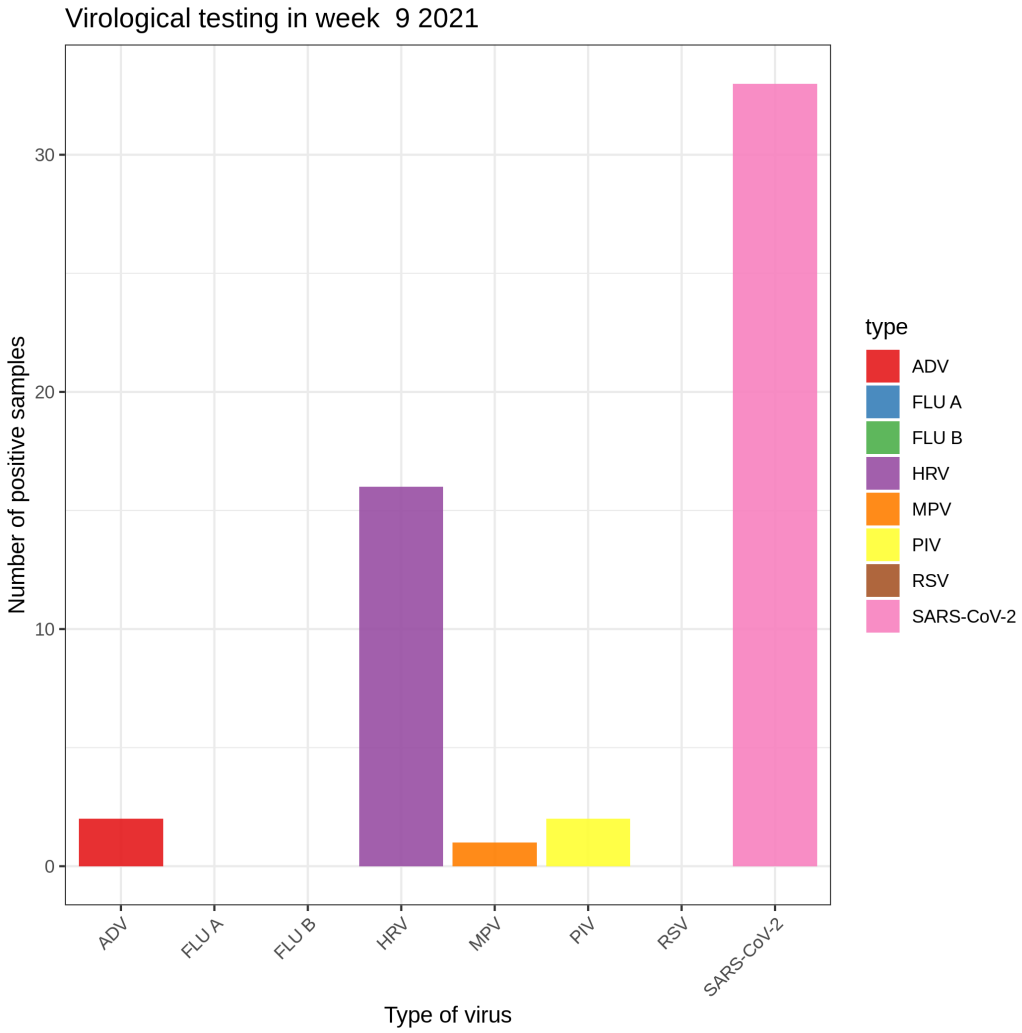

Figure 2 Positive cases of respiratory viruses in the CCC specimens

SARS-CoV-2 Genomic Surveillance

The current sequencing strategy

The National Reference Laboratory for Acute Respiratory Infections at LNS continues to improve the representativeness of the pool of sequenced specimens to reach real-time epidemiology, by implementing the following weekly sequencing activities:

1) Sequencing specimens from all hospitalized positive cases
2) Sequencing specimens from all positive cases from Airport testing program
3) Sequencing specimens from all outbreaks and identified clusters
4) Systematic sequencing of specimens from reinfections and post-vaccination-infections
5) Population sequencing of specimens from representative regions and age groups, to follow the evolution of the different variants in the Luxembourg population.

The representative sequencing sample was based on the minimum number of specimens required to extrapolate prevalence of VOC variants with error rate of 5%. The representative sample was estimated based on the number of positive cases in Luxembourg for week 9 (1191). The minimum sample size required to detect prevalence of B.1.1.7 (63%) with an error margin of 5% was estimated to be **276** specimens. The calculation was based on a sample size calculation tool that uses the expected prevalence of the variant in the total population. (Population Proportion - Sample Size - Select Statistical Consultants (select-statistics.co.uk). This number represented a coverage of 23.1% which exceeds the minimum coverage recommended by ECDC (10%). Hence our sequencing results this week are representative of the circulating variants in Luxembourg.

This week, the Health Inspection provided a set of 300 randomly selected cases. Between this list and our sequencing pool, there was an overlap of 91 specimens. This set of 91 specimens represents an alternative representative sample for the purposes of the weekly genomic surveillance. We will call this sample "*randomized sample*".

The starting material used for sequencing is respiratory specimens (nasopharyngeal or oropharyngeal swabs) that have already been tested positive by RT PCR.

The LNS sequencing data sharing strategy includes sharing of the sequencing data with GISAID EpiCov database (www.gsaid.org) on a periodic basis.

Sequenced specimens

Last week the microbial genomics platform at the LNS sequenced 475 specimens. Out of these, 411 specimens were collected in week 9/2021. This represents 34% of the new infections reported in Luxembourg in week 9/2021. Among the 475 specimens, 70 specimens were reported to be part of a cluster or outbreak investigation.

The population sequencing coverage in week 9/2021 was 34% (Figure 3). Based on statistical inference, the frequency of the reported variants in Week 9/2021 is **representative** of the circulating variants in Luxembourg.

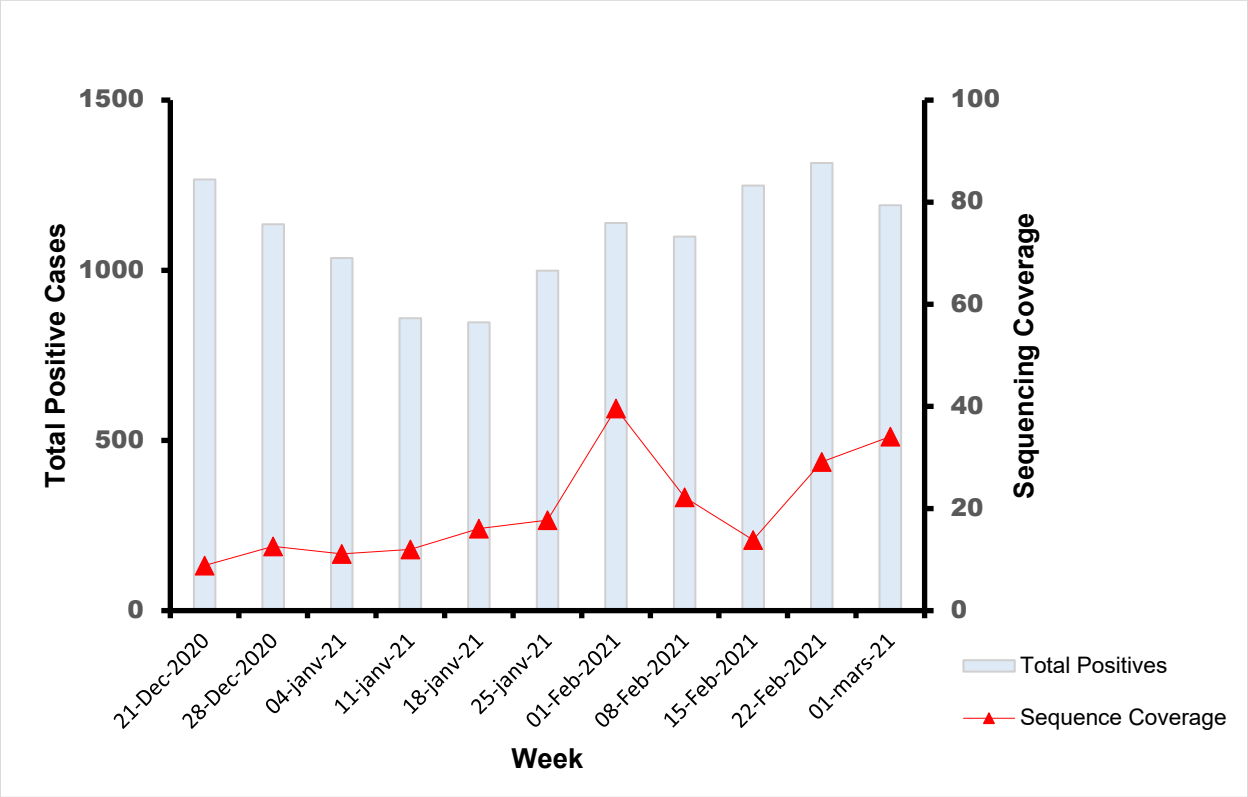

Figure3 Sequence coverage based on total number of positive cases in Luxembourg in week 9/2021

Circulating lineage detection

Lineages (variants) have been assigned based on Rambaut *et al* by means of Phylogenetic Assignment of Named Global Outbeak LINeages (pangolin) software (v2.3, pangoLEARN version 2021-02-21).

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The lineage nomenclature system that we use is the one proposed by Rambaut et al. that focuses on actively circulating virus lineages (https://cov-lineages.org).

In the sampling period of week 9/2021, 20 circulating SARS-CoV-2 variants were detected within our sequencing pool, as shown in Figure 4a. The most prevalent lineage was B.1.1.7 (62,8%, CI 58,1% - 67,5%), followed by B.1.351 (18.5%, CI 14,7% - 22,2%) and B.1.1.29 (9%, CI 6,2% - 11,8%).

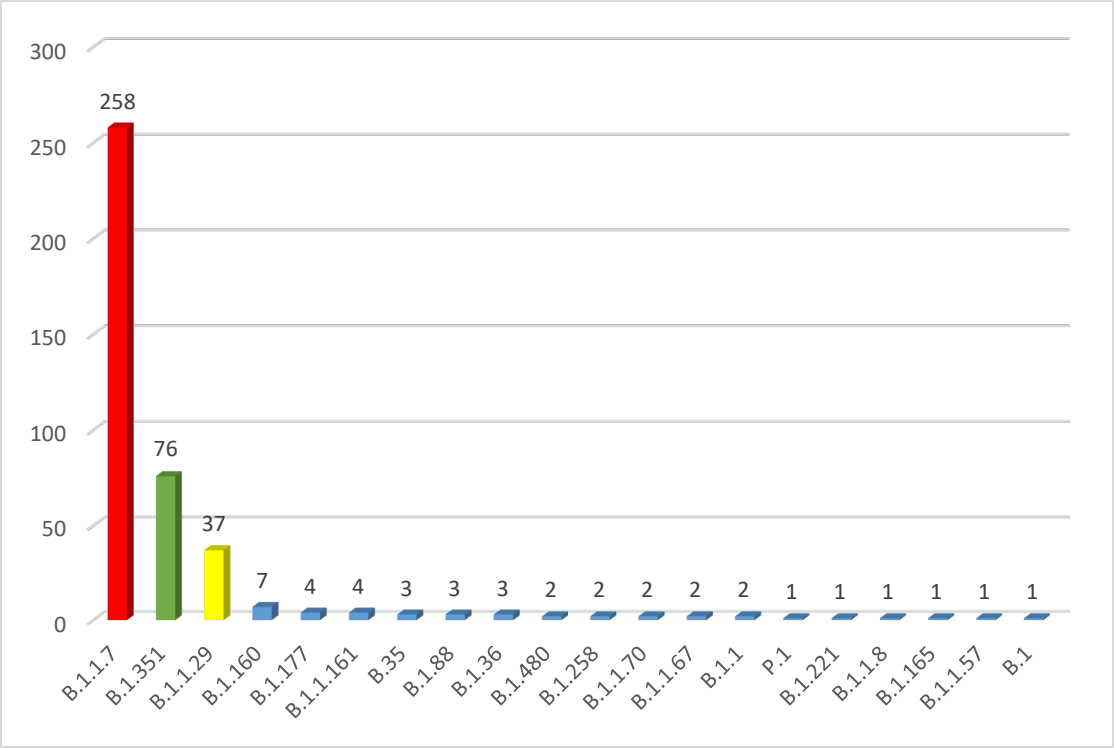

Figure 4a Number of SARS-CoV-2 variants in Luxembourg in the sequencing pool at LNS in Week 9/2021

The "*randomized sample*" of 91 specimens included 10 variants, again with the main three variants being B.1.1.7 (58,2%, CI 48,1% - 68,3%), B.1.351 (19,8%, CI 11,6% - 28%), followed by B.1.1.29 (12,1, CI 5,9% - 19,5%), as shown in Figure 4b.

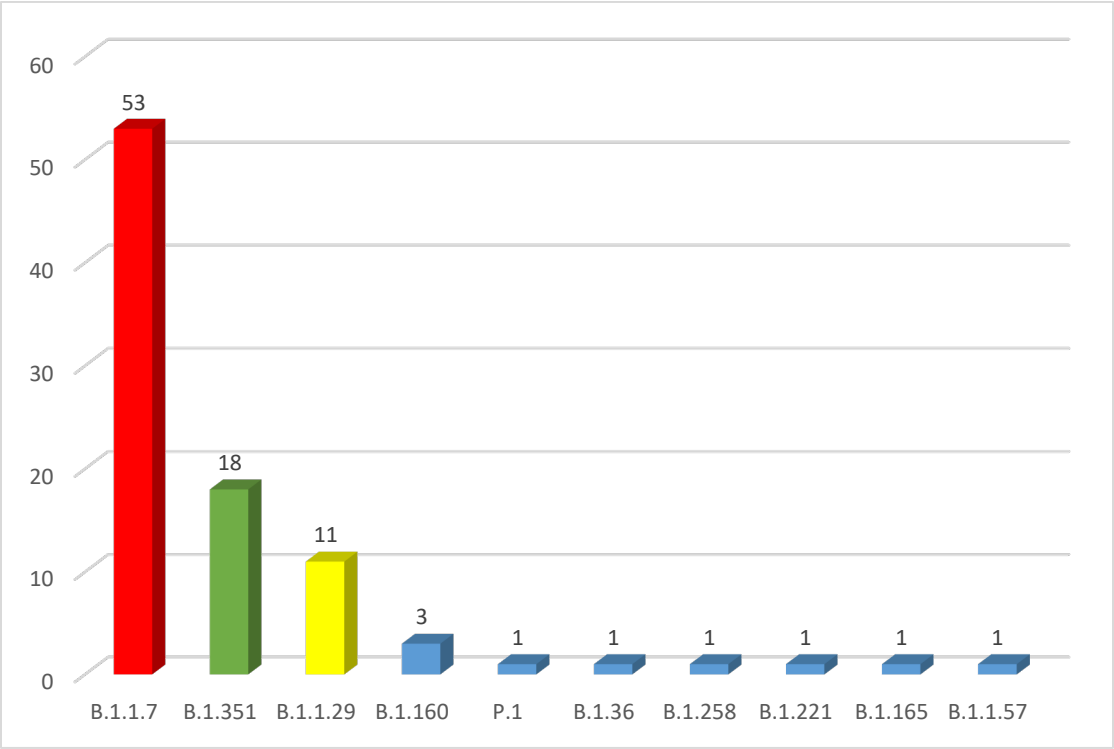

Figure 4b Number of SARS-CoV-2 variants in representative sample for Week 9/2021

Variants of Concern tracker (B.1.1.7, B.1.351, P.1, A.23.1 and B.1.525)

Among specimens collected within the week 9/2021, 258 cases of the B.1.1.7 variant have been detected, representing 62,7% of the specimens in the week's sequencing pool (by comparison, the week 8/2021 pool, which also included specimens from previous weeks' collections, had shown 63,7% of this variant). The total case count of sequenced variant B.1.1.7 was 1090 by week 9/2021. The earliest collection date for this variant remains 19/DEC/2020 and the latest is 07/MAR/2021.

In the collection period of week 9/2021, 76 cases of the South African variant B.1.351 have been detected, representing 18,5% of the specimens in the week's sequencing pool (by comparison, the week 8/2021 pool, which also included specimens from previous weeks' collections, had shown 17,2% of this variant). The total case count of sequenced variant B.1.351 was 246 by week 9/2021. The earliest collection date for this variant remains 11/JAN/2021 and the latest is 07/MAR/2021.

In week 9/2021, an additional case of the Brazilian variant P.1 was detected, therefore the total case count of variant P.1 was 3 by week 9/2021 (latest collection date 05/MAR/2021).

No additional cases have been detected for lineage B.1.525, and by now no specimen in Luxembourg corresponded to the A.23.1 variant (Figure 5).

Lineage B.1.1.7 is characterized by several spike protein mutations, including N501Y, H69/V70del and P861H. The variant seems to have a considerable epidemiological impact, as it has a higher transmissibility rate.

Lineage B.1.351 holds numerous spike protein mutations, of which three are located in the receptor binding domain (K417N, E484K and N501Y), and are therefore relevant for antibody binding. As for B.1.1.7, a higher transmissibility rate and viral loads seem to be associated with this variant. Due to the K417N and E484K mutations, an impact on vaccination efficacy and possibility of reinfection is subject to scientific investigation.

Lineage P.1 (descendent of B.1.1.28), initially found in the Amazon region, has a similar mutation profile as the South African variant, including E484K and N501Y. Concerns are, as for the South African variant, higher transmissibility and a decreased protection by neutralizing antibodies.

Lineage B.1.525 carries several mutations of biological significance, including E484K, Q677H and F888L. It does not carry N501Y, but a set of deletions similar to the B.1.1.7 variant.

Of note, additional specimens are in the pipeline for sequencing, so that numbers may change with increasing representativeness of circulating variants and proportions.

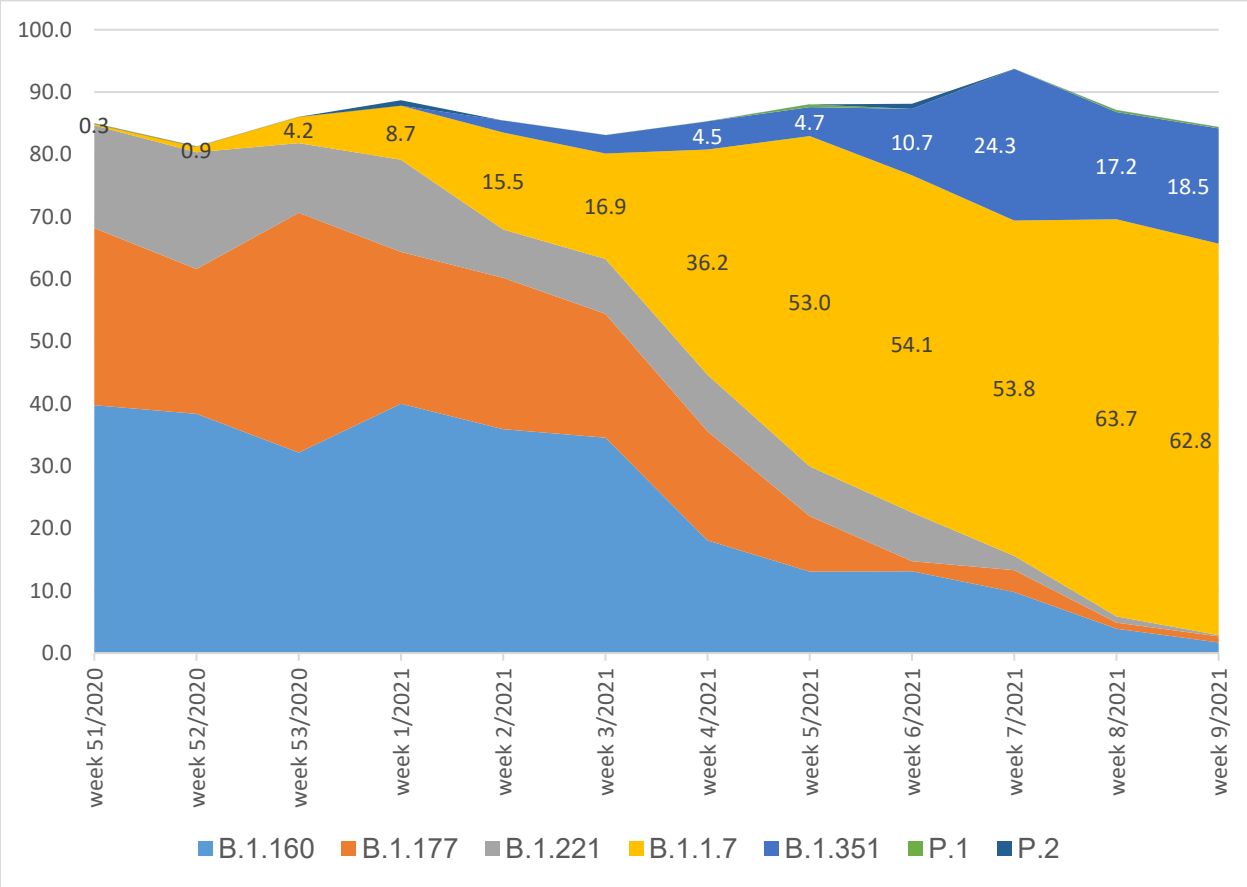

Figure 5 Evolution of variants in Sequencing pool of all specimens including Targeted sequencing (cluster/contact tracing/ non residents) since first detection of B.1.1.7 in Luxembourg

Clinically relevant mutations

Currently the LNS genomic surveillance program - independently from lineage calling - notes the occurrence of 13 different known SARS-CoV-2 mutations, assumed to have clinical and epidemiological relevance. The list of observed mutations is being updated continually, based on the appearance and prevalence of SARS-CoV-2 variants.

The following table provides the overall frequencies of these mutations, detected in the lineage-assignable genome sequences, analyzed between 01/SEP/2020 and 07/MAR/2021 (N=5542), as well as the frequencies in week 9/2021.

Mutation	Gene	Genomic Position in reference	Frequency Overall [%]	Frequency Week 9/2021 [%]	Characteristics	Reference
L37F	Nsp6	11081	7,6	2,7	Favored viral infection, higher severity	Aiewsakun 2020
P323L	ORF1ab	14407	91,4	89,8	Higher severity	Biswas & Mudi 2020
H69/V70del	S gene	21765-21770	20,6	75	possible impact on antibody neutralization activity and reinfection; included in "mink" mutation	Kemp 2020
Y144del	S gene	21991-21993	19,6	60,3	possible impact on antibody binding affinity	Dawood 2020
K417N	S gene	22813	4,4	18,2	501Y.V2 / possible impact on antibody binding affinity (escape mutation)	Kemp 2020
E484K	S gene	23012	4,6	18,2	501Y.V2 / possible impact on antibody neutralization activity (escape mutation), improved ACE2 binding affinity	Greaney 2020
N501Y	S gene	23063	22,7	75	501Y.V1/V2; Improved ACE2 binding affinity/higher transmissibility	Filip Fratev 2020 COVID-19 Genomics Consortium UK, 2020
D614G	S gene	23402	95,7	91,5	Higher infectivity, higher case fatality rate, higher transmission; replaced original Wuhan strain, became globally dominant form of the virus	Eaaswarkhanth 2020 Becerra-Flores 2020, Hu 2020, Plante 2020
P681H	S gene	23604	20,2	54,2	immediately adjacent to the furin cleavage site, a known location of biological significance	COVID-19 Genomics Consortium UK, 2020
Q57H	ORF3a	25561	37,7	22,1	Higher severity	Biswas & Mudi 2020
N439K	S gene	26143	2,1	0,5	Improved ACE2 binding affinity	Zhou 2020
R203K	N gene	28880	28,1	75,7	Fitness advantage for the virus	Leary 2020
G204R	N gene	28883	28,1	75,4	Fitness advantage for the virus	Leary 2020

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Conclusion

The ReViLux data are communicated as support to the understanding of respiratory virus transmission dynamics, including introduction of new variants, to the evaluation of the impact of response measures, to the contact tracing and the investigation of clusters.

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A Rambaut et al. A dynamic nomenclature proposal for SARS-CoV-2 lineages to assist genomic epidemiology. Nat Microbiol 2020;5:1403-1407

https://github.com/cov-lineages/pangolin

Y Guangchuang et al. ggtree: an R package for visualization and annotation of phylogenetic trees with their covariates and other associated data. Methods in Ecology and Evolution 2017;8:28-36

For more information on lineages visit: https://cov-lineages.org
For more information and statistics on Covid-19 infections in Luxembourg visit: https://covid19.public.lu/en.html

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