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 26/04/-02/05/2021)

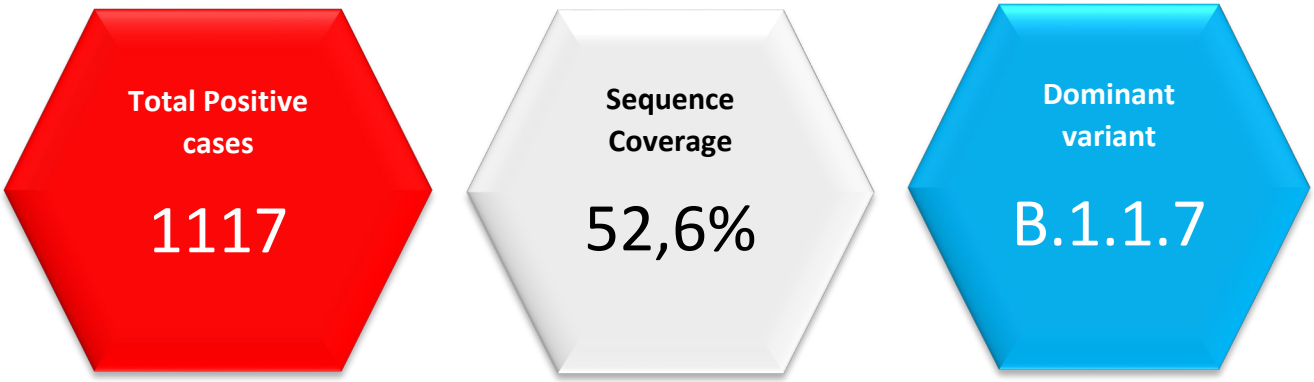
Executive Summary

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 17/2021, the overall frequency of the SARS-CoV-2 B.1.1.7 variant in all sequenced specimens decreased to 80,8% (CI 77,6% - 84%, p<0,05). For the SARS-CoV-2 B.1.351 variant, we found an overall frequency of 10,7% (CI 8,2% - 13,2%, p<0,05) within the sequenced sample, while for P.1, the frequency was 2,6% (CI 1,3% - 3,9%, p<0,05).

The representative sample was estimated, based on the number of positive cases in Luxembourg for week 17 (1117). The minimum sample size required to detect prevalence of B.1.1.7 (85%) reported in week 16, with an error margin of 5%, was estimated to be **167** specimens. This number corresponds to a coverage of 14,9 %, which exceeds the minimum coverage recommended by ECDC (10%). The sequencing results of week 16 are representative of the circulating variants in Luxembourg with a margin of error of 5%.

The total number of sequences performed this week was 678, with 588 specimens (deduplicated) having been collected in the time frame of week 17/2021. The sequencing coverage this week was 52,6% from all positive cases in Luxembourg.



Clinical Surveillance

The "Sentinel" surveillance network reported 324 consultations in week 17 (26/APR/2021 – 02/MAY/2021). There were 4 cases of ILI[1], corresponding to 1,2% of the consultations, as shown in Figure 1. The number of consultations for ARI[2] was 23, which represents 7,1% of the consultations.

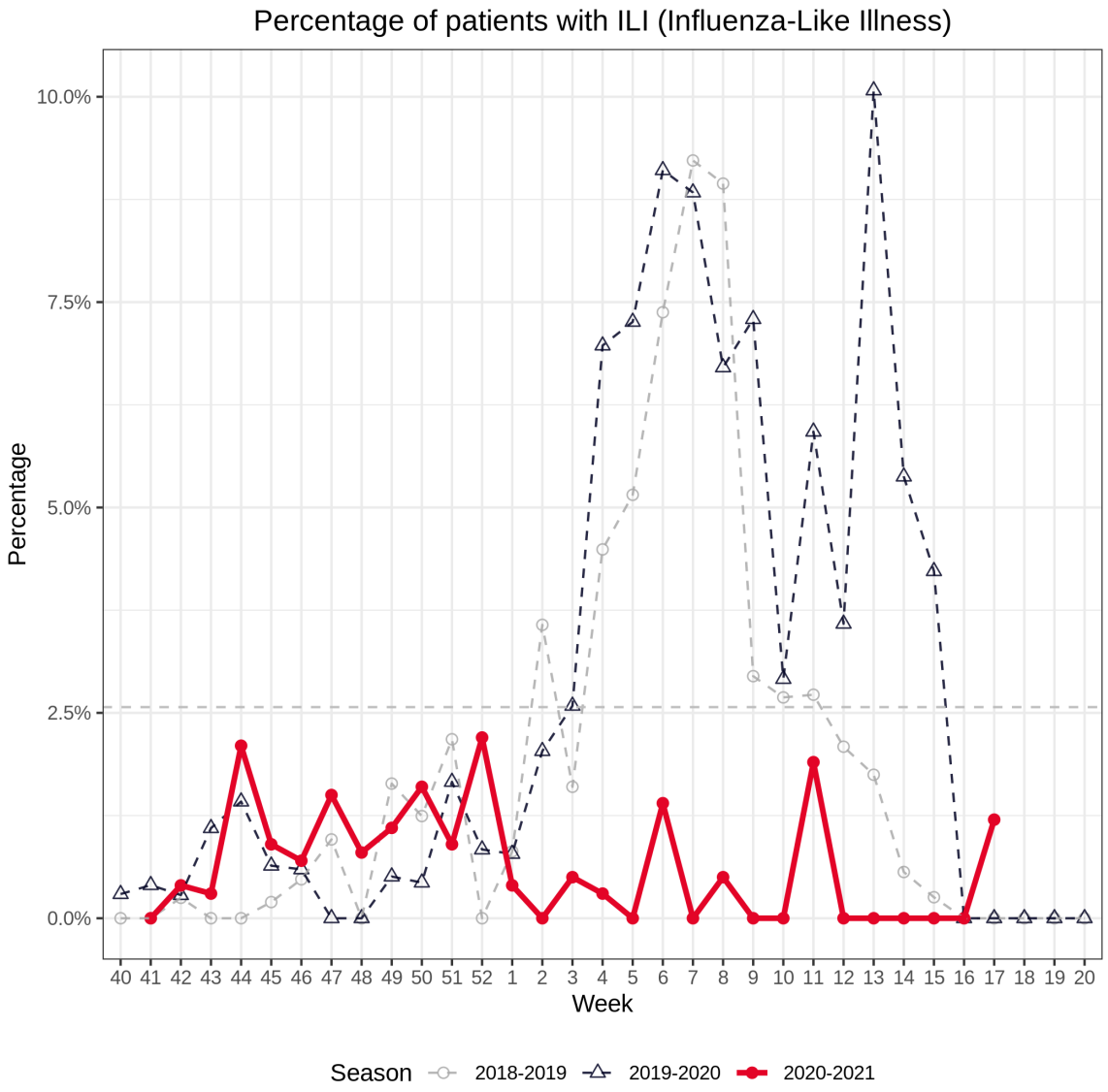


Figure 1 Percentage of patients with ILI over the epidemiological weeks.

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 SARS-COV-2 was the most prevalent respiratory virus detected in the "Sentinel" network, with 37 positive cases (28,9%). The wave of Human Rhinovirus (HRV) continued in week 17/2021, with 9 positive cases in 128 tests (7%). Sporadic cases of other respiratory viruses continued to appear, such as 2 cases of PIV and RSV (each 1,5%) and 1 case of ADV. No case of Influenza A/B was detected. (c.f. Figure 2).

In Luxembourg, we have tested 128 samples from the Sentinel surveillance network, as compared to 822 specimens tested in Europe, in the week 17/2021. None of these 822 specimens tested positive for Influenza type A/B virus (Source: FluNews Europe).

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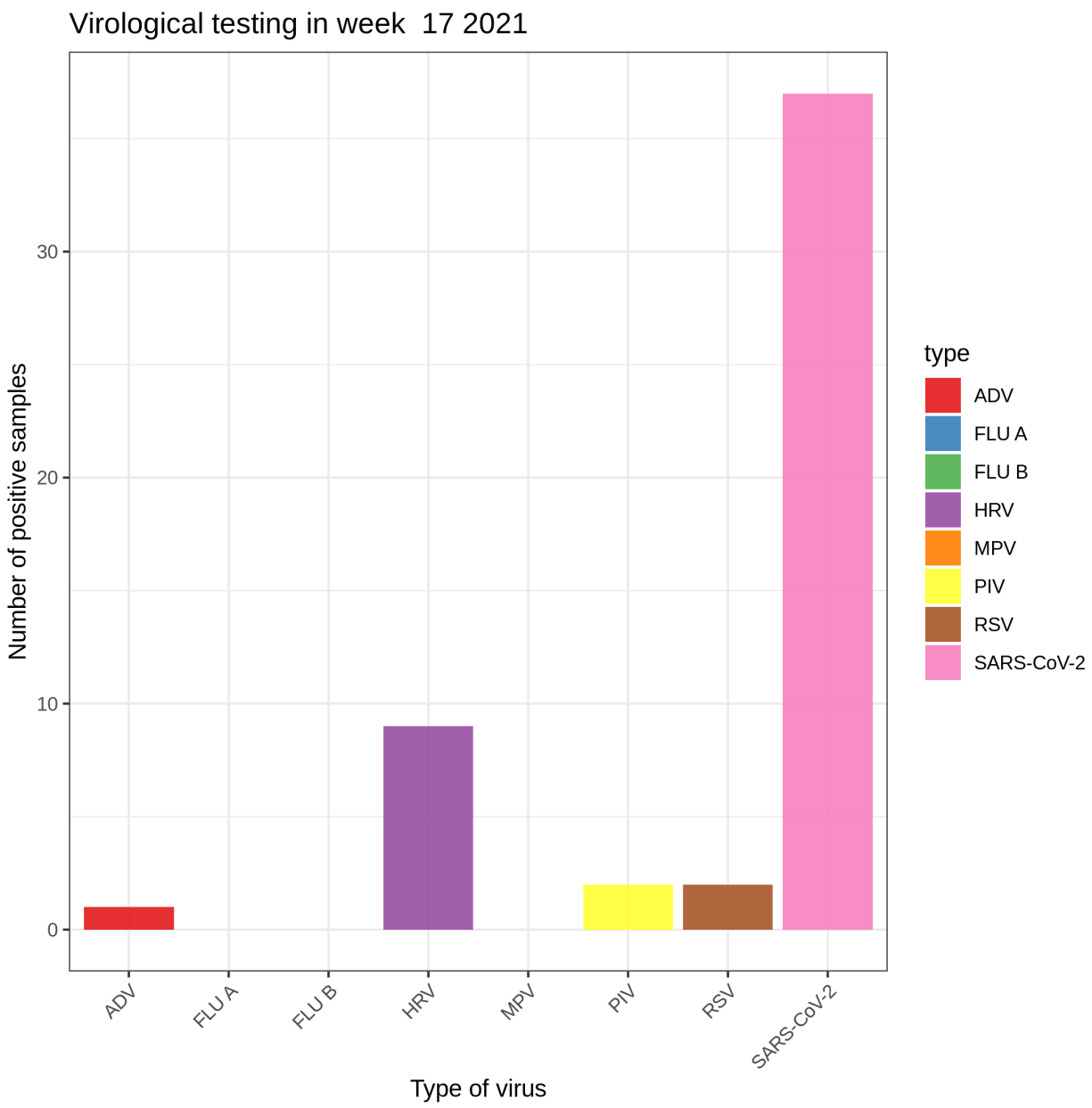

Figure 2 Positive cases of respiratory viruses in the CCC specimens (week 17)

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 in week 17 (1117). The minimum sample size required to detect prevalence of B.1.1.7 (85%) reported in week 16 with an error margin of 5% was estimated to be **167** 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 14,9% which exceeds the minimum coverage recommended by ECDC (10%). The number of non-targeted specimens from Luxembourgish residents sequenced this week was **498**. Therefore, our sequencing results this week are representative of the circulating variants in Luxembourg.

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

In parallel, we continue to evaluate commercial PCR screening, using the same starting material, to detect the Variants of Concern (VOCs), with specific PCR. This will enable a faster investigation time of any outbreak and allow us to screen 100% of positive cases referred to LNS, including those that do not pass the quality criteria for sequencing. Specimens that are not eligible for sequencing can thus be used in a PCR to detect the presence of variants of concern.
The LNS sequencing data sharing strategy includes sharing of the sequencing data with GISAID EpiCov database (www.gisaid.org) on a periodic basis.

Sequenced specimens

Last week the microbial genomics platform at the LNS sequenced 678 specimens, with 588 collected in week 17/2021. This represents 52,6% of the new infections reported in Luxembourg in week 17/2021. Among these 588 specimens, 60 specimens were reported to be part of a cluster or outbreak investigation, and 40 specimens were from non-residents (10 specimen overlapping). This leads to 498 specimens, collected in week 17, and being the representative population sequencing sample. In the population representative sample of residents, the frequency of B.1.1.7 and B.1.351 was 82,1% and 10,2% respectively, while the frequency of P.1 was 2,2%.

The population sequencing coverage in week 17/2021 was 52,6% (Figure 3). Based on statistical inference, the frequency of the reported variants in week 17/2021 is **representative** of the circulating variants in Luxembourg with a margin of error of **5%**.

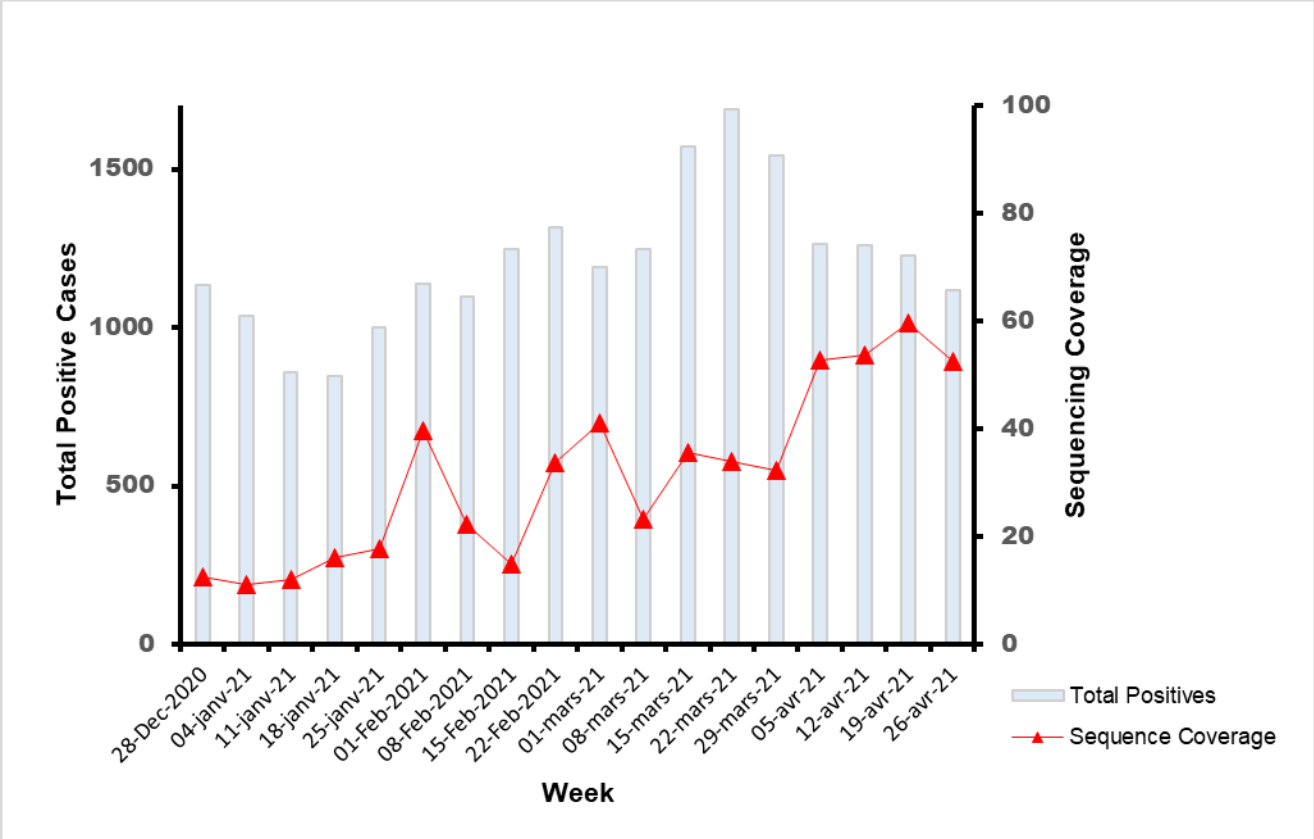

Figure 3 Sequence coverage based on total number of positive cases in Luxembourg in week 17/2021. Data have been updated back to week 16/2021.

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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 (v2.4.2, pangoLEARN version 2021-04-28).

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 week 17/2021, in the population representative sample, after removal of cluster specimens, and excluding specimens collected from non-residents, there were 13 circulating SARS-CoV-2 variants, with the main three variants being B.1.1.7 (82,1%, CI 78,7% - 85,5%), B.1.351 (10,2%, CI 7,5% - 12,9%), followed by P.1 (2,2%, CI 0,9% - 3,5%), as shown in Figure 4.

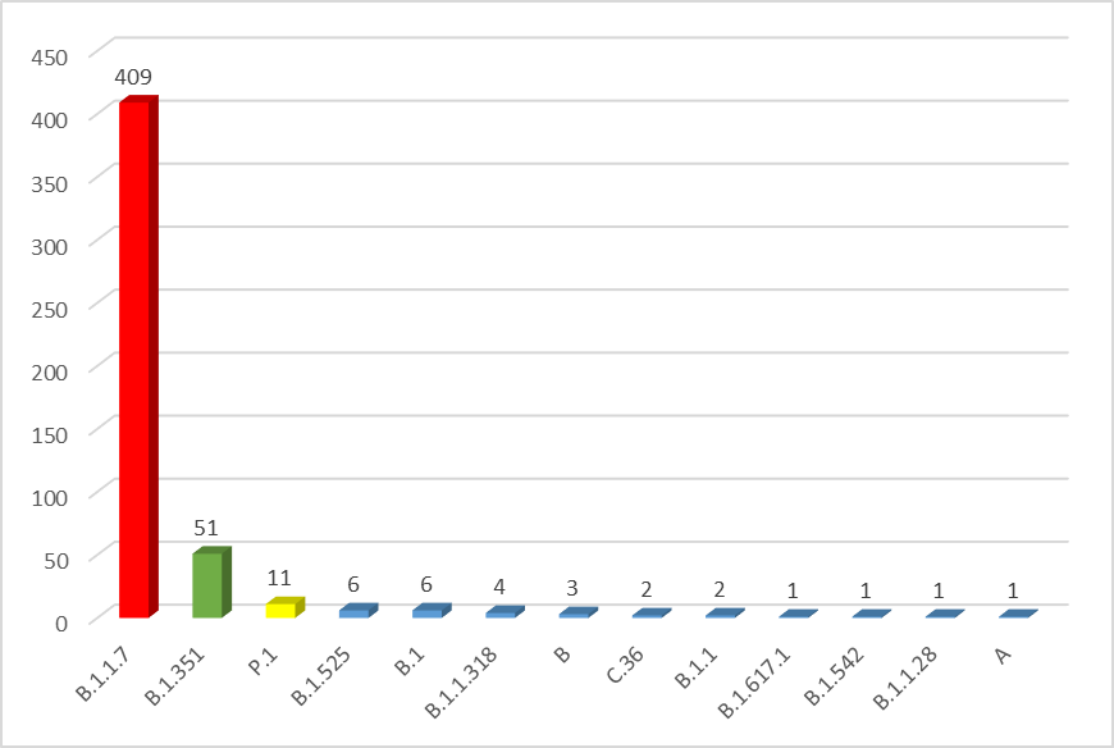

Figure 4 Number of SARS-CoV-2 variants in representative sample for week 17/2021

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Variants of Concern tracker (B.1.1.7, B.1.351, P.1, A.23.1, B.1.525, B.1.617.2)

Among specimens collected within the week 17/2021, 475 cases of the B.1.1.7 variant have been detected, representing 80,8% of the specimens in the week's sequencing pool (by comparison, the week 16/2021 pool had shown a frequency of 85,4% of this variant, including additional specimens having been sequenced from previous weeks). The total case count of sequenced variant B.1.1.7 was 4704 by week 17/2021. The earliest collection date for this variant remains 19/DEC/2020 and the latest is 02/05/2021.

In the collection period of week 17/2021, 63 cases of the South African variant B.1.351 have been detected, representing 10,7% of the specimens in the week's sequencing pool (by comparison, the week 16/2021 pool had shown 9,9% of this variant, including additional specimens having been sequenced from previous weeks). The total case count of sequenced variant B.1.351 was 933 by week 17/2021. The earliest collection date for this variant remains 11/JAN/2021 and the latest is 02/MAY/2021.

In week 17/2021, 10 additional cases of B.1.525 have been detected, and 15 new cases of the Brazilian variant P.1. Thus, the case count by week 17 for B.1.525 is 25 (latest sampling date 30/APR/2021) and for P.1 the case count is 49 (latest sampling date 30/APR/2021).

In week 17/2021, 1 additional case of the B.1.617 subtype B.1.617.1 has been detected (latest sampling date 26/APR/2021). The case count by week 17 for B.1.617.1 is six, and for B.1.617.2 one. Since May 6th 2021, B.1.617.2 has been escalated by Public Health Engand from a variant under investigation to a variant of concern.

By now, no case of A.23.1 has been detected in Luxembourg (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.

Lineage B.1.617 is a variant first detected in India and was designated "Under Investigation" on 1st April 2021 by Public Health England. It contains a number of spike mutations associated with antigenic escape or found in other variants of concern, including L452R, E484Q and P681R. Subtype B.1.617.2 does not carry S:E484Q and seems to be at least as transmissible as B.1.1.7 (with moderate confidence).

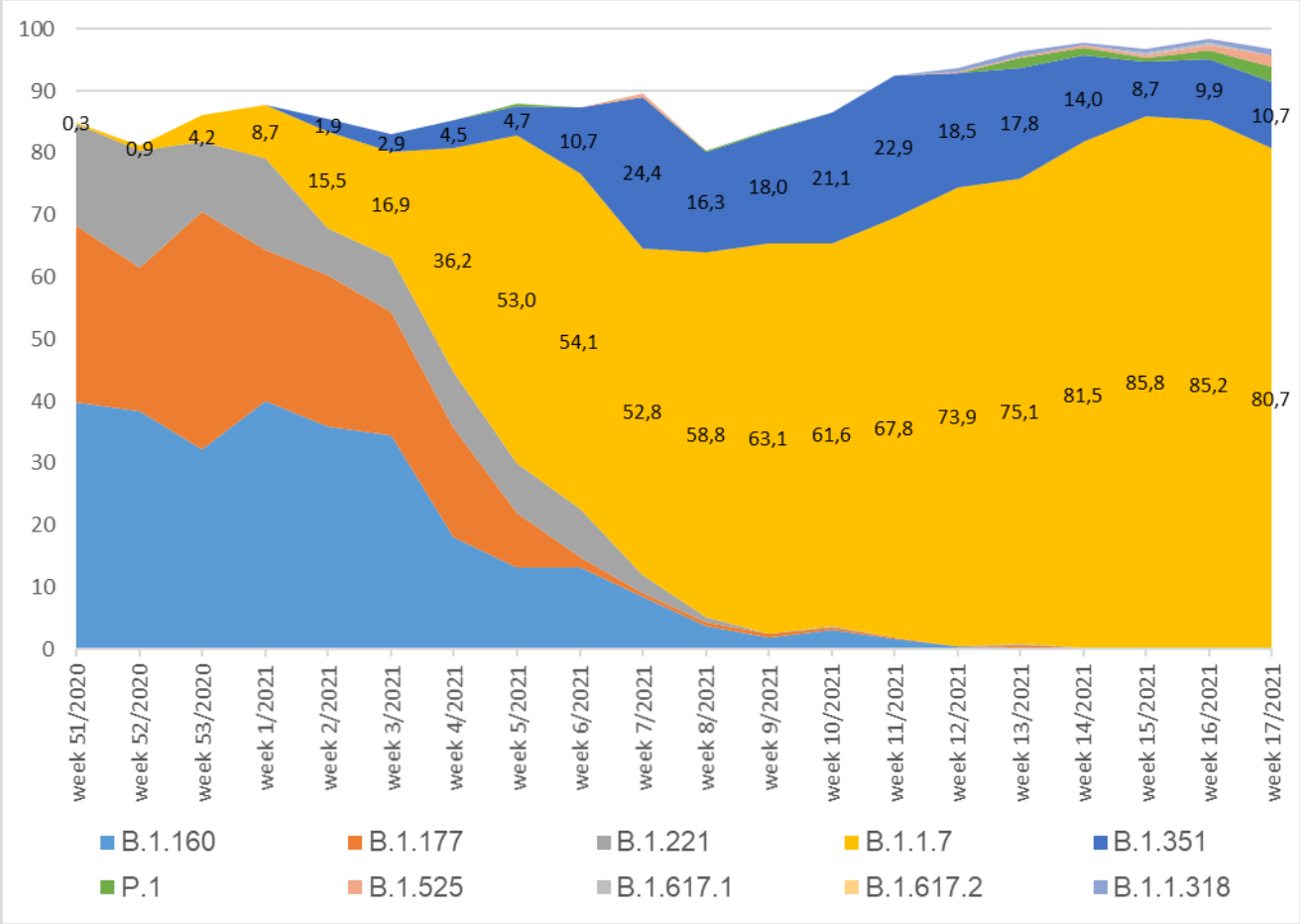

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Figure 5 Evolution of variants in Sequencing pool of all specimens including targeted sequencing (cluster/contact tracing/ non residents) since first detection of B.1.1.7 in Luxembourg

By week 17/2021, 97,5 % of detected variants in Luxembourg are declared as either Variants of Concern (VOC – B.1.1.7, B.1.351, P.1, B.1.617.2) or Variants Under Investigation (VUI – B.1.525, B.1.1.318, B.1.617.1).

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 02/MAY/2021 (N=10333), as well as the frequencies in week 17/2021.

Mutation	Gene	Genomic Position in reference	Frequency Overall [%]	Frequency Week 17/2021 [%]	Characteristics	Reference
L37F	Nsp6	11081	4,0	1,5	Favored viral infection, higher severity	Aiewsakun 2020
P323L	ORF1ab	14407	88,3	81,2	Higher severity	Biswas & Mudi 2020
H69/V70del	S gene	21765-21770	45,2	78,4	possible impact on antibody neutralization activity and reinfection; included in "mink" mutation	Kemp 2020
Y144del	S gene	21991-21993	45,5	81,2	possible impact on antibody binding affinity	Dawood 2020
K417N	S gene	22813	9,2	11,1	501Y.V2 / possible impact on antibody binding affinity (escape mutation)	Kemp 2020
E484K	S gene	23012	10,4	18,0	501Y.V2 / possible impact on antibody neutralization activity (escape mutation), improved ACE2 binding affinity	Greaney 2020
N501Y	S gene	23063	51,6	84,6	501Y.V1/V2; Improved ACE2 binding affinity/higher transmissibility	Filip Fratev 2020 COVID-19 Genomics Consortium UK, 2020

D614G	S gene	23402	95,1	92,1	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	43,3	66,4	immediately adjacent to the furin cleavage site, a known location of biological significance	COVID-19 Genomics Consortium UK, 2020
Q57H	ORF3a	25561	28,2	12,1	Higher severity	Biswas & Mudi 2020
N439K	S gene	26143	1,1	0	Improved ACE2 binding affinity	Zhou 2020
R203K	N gene	28880	52,4	84,9	Fitness advantage for the virus	Leary 2020
G204R	N gene	28883	52,4	84,9	Fitness advantage for the virus	Leary 2020

References

Genomic sequencing of SARS-CoV-2. A guide to implementation for maximum impact on public health. WHO, 8 January 2021.

COVID-19 data portal. 2020 (https://www.covid19dataportal.org/sequences)

J Hadfield et al. Nextstrain: real-time tracking of pathogen evolution. Bioinformatics 2018;34:4121-4123

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

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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