

Respiratory Viruses in Luxembourg (ReViLux)

Weekly report (Period 12/07/-18/07/2021)

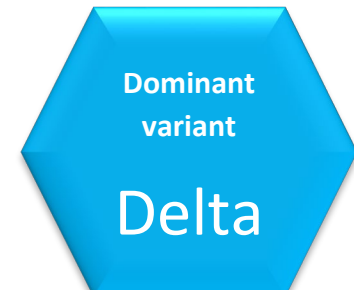
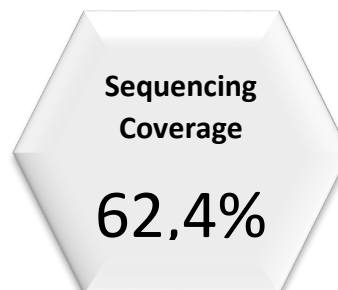
Executive Summary

The Sentinel Surveillance Network identified 2 cases of influenza-like illnesses, which remains below the recommended threshold for the interepidemic season, according to the European Center for Disease Prevention and Control (ECDC) guidelines.

Regarding SARS-CoV-2 genomic surveillance, the Laboratoire national de santé analysed 454 specimens in week 28/2021 (from 727 total cases in the Great Duchy of Luxembourg, 62,4%). This exceeds the minimum coverage (10%) and sample size recommended by the ECDC (which is 45,3% in our current epidemiological situation).

Community case surveillance revealed an increase of Delta variant cases, which have been on an increasing trend over the last five weeks. Currently, the Delta variant remains the dominant variant in Luxembourg.

Target group surveillance showed 49 VOCs within hospitalized cases (51,0% Delta and 46,9% Gamma) and all cluster cases were linked to VOCs (80,6% Delta, 19,4% Gamma). Additionally, 14 reinfection or post-vaccination breakthrough cases linked to VOCs were identified.



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 three 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 dynamic detailed observation of SARS-CoV-2 mutations and variants, but also monitoring specific groups of interest.
- **The COVVAC Serology Project.** It assesses the post-vaccination serological status in Long-term care facilities and its evolution over time.

The ReViLux provides updates on the first two projects.

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 WHO¹ and ECDC² guidance, it focuses on cases of acute respiratory infection (ARI) and influenza-like illness (ILI).

Results of syndromic surveillance during week 28 (12 July 2021 - 18 July 2021) are displayed in **Table 1** and the history of ILI consultations since the 2018-2019 season is shown in **Figure 1**. **The percentage of ILI remains below the threshold for the interepidemic season**, according to the ECDC.

Regarding the virological surveillance, no data is available for week 28.

¹ World Health Organization

² European Centre for Disease Prevention and Control

Table 1. Syndromic surveillance during week 28

	Count	Percent
ARI consultations	37	12,0%
ILI consultation	2	0,6%
Total consultations	309	100%

ARI: Acute Respiratory Infections (acute respiratory symptoms like bronchitis, pharyngitis, rhinitis, pneumonia... with or without fever). ILI: Influenza-Like Illness (acute respiratory symptoms <10 days, fever 38°C, systemic symptoms like myalgia or malaise...).

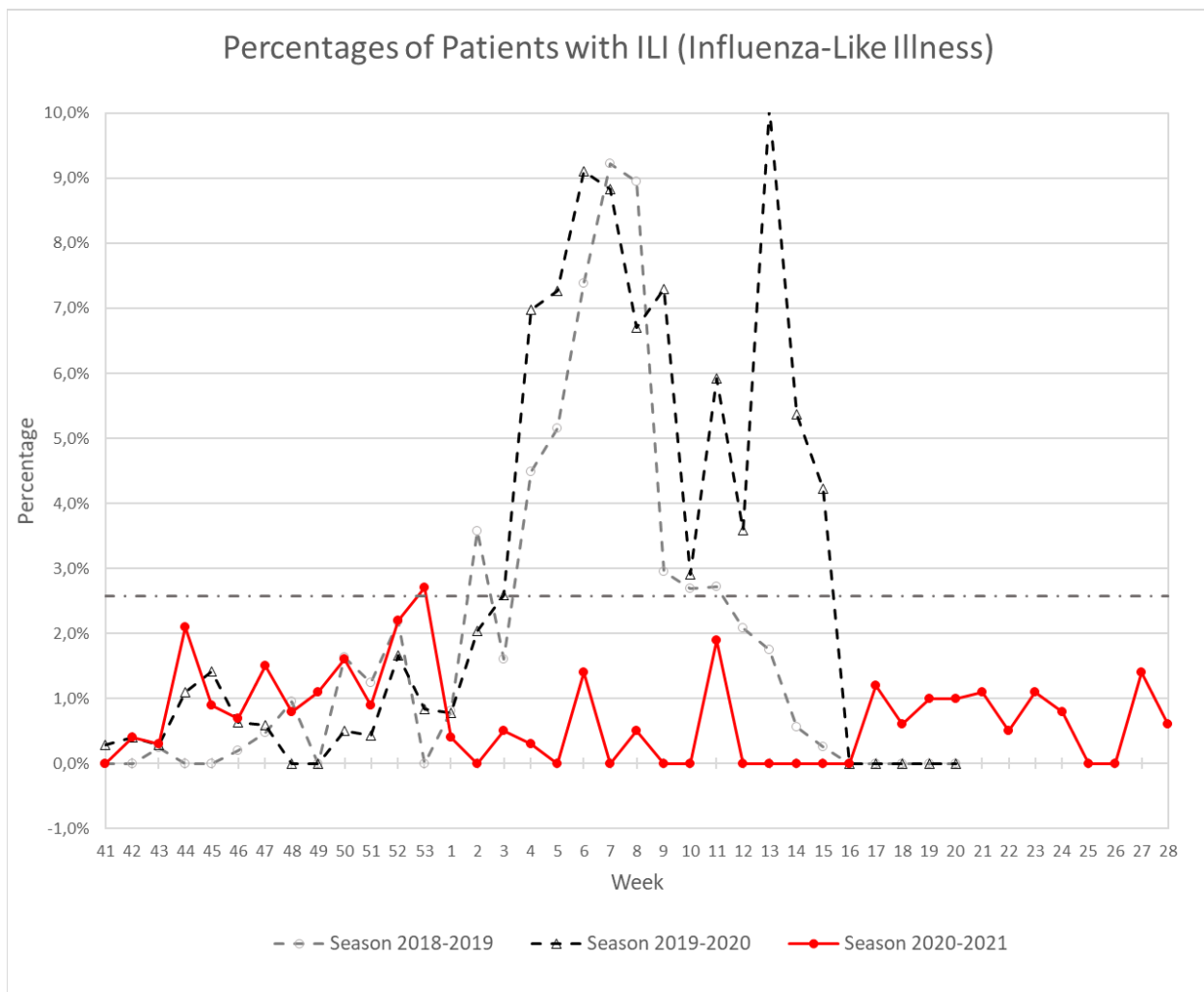


Figure 1. Percentage of patients with influenza-like illness over the epidemiological weeks

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 for (nasopharyngeal or oropharyngeal swabs analysed by RT-PCR) from the national network of laboratories and proceeds as follows:

- 1) Sequencing all specimens from hospitalized cases.
- 2) Sequencing all specimens from reinfection and post-vaccination cases.
- 3) Sequencing all specimens from cluster cases.
- 4) Sequencing a representative sample of community cases.

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

In week 28, 727 new cases were registered in Luxembourg; hence, the minimum sample size required to detect a 2.5% incidence is estimated to be 329 specimens (45,3%). The number of **non-targeted specimens** from Luxembourgish residents successfully sequenced this week was **425 (58,5%), which exceeds the minimum coverage (10%) and minimum sample size (329) recommended by ECDC.**

The LNS shares its sequencing results with GISAID EpiCov database (www.gisaid.org) periodically. SARS-CoV-2 lineages (variants) have been assigned based on Rambaut et al. using Phylogenetic Assignment of Named Global Outbreak LINEages (pangolin) software (v3.1.5, pangoleARN version 2021-06-15). The ReViLux continues to use the Pango nomenclature, in addition to the WHO nomenclature, to allow easier visualization of links between any evolving variants and their ancestor (<https://cov-lineages.org>). See nomenclature equivalences in **Annex 1**.

Sequenced specimens

Last week, the microbial genomics platform at the LNS sequenced 559 specimens, with 475 having been collected in week 28/2021. Among the latter, 31 specimens were reported to be part of a cluster or vaccine failure investigation, and 21 specimens were from non-residents (2 specimens overlapping). The number of non-targeted specimens from Luxembourgish residents successfully sequenced this week was 425 (58,5% coverage of 727 total cases) (see coverage trend in **Figure 2**).

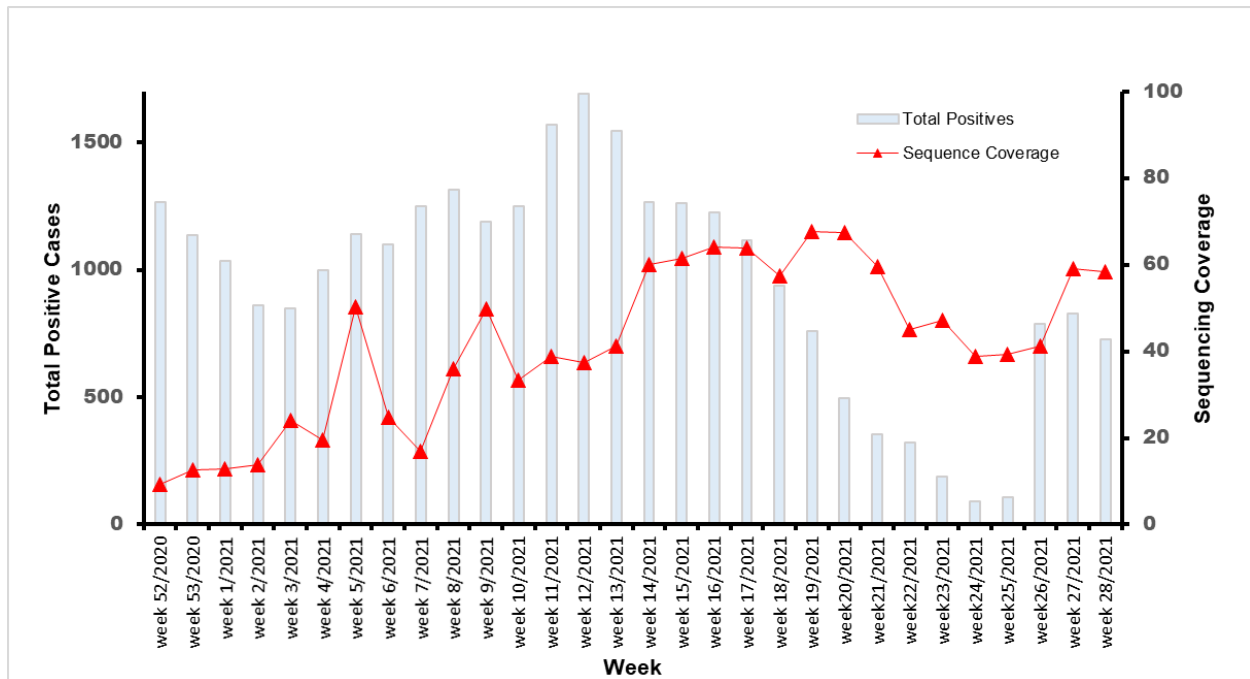


Figure 2. Sequence coverage based on total number of positive cases in Luxembourg in between week 52/2020 and week 28/2021

Circulating lineage detection

The evolution of variants over the weeks is shown in **Figure 3**. Delta variant showed a constant increase, in absolute values, during the last five weeks, and has become again the dominant variant.

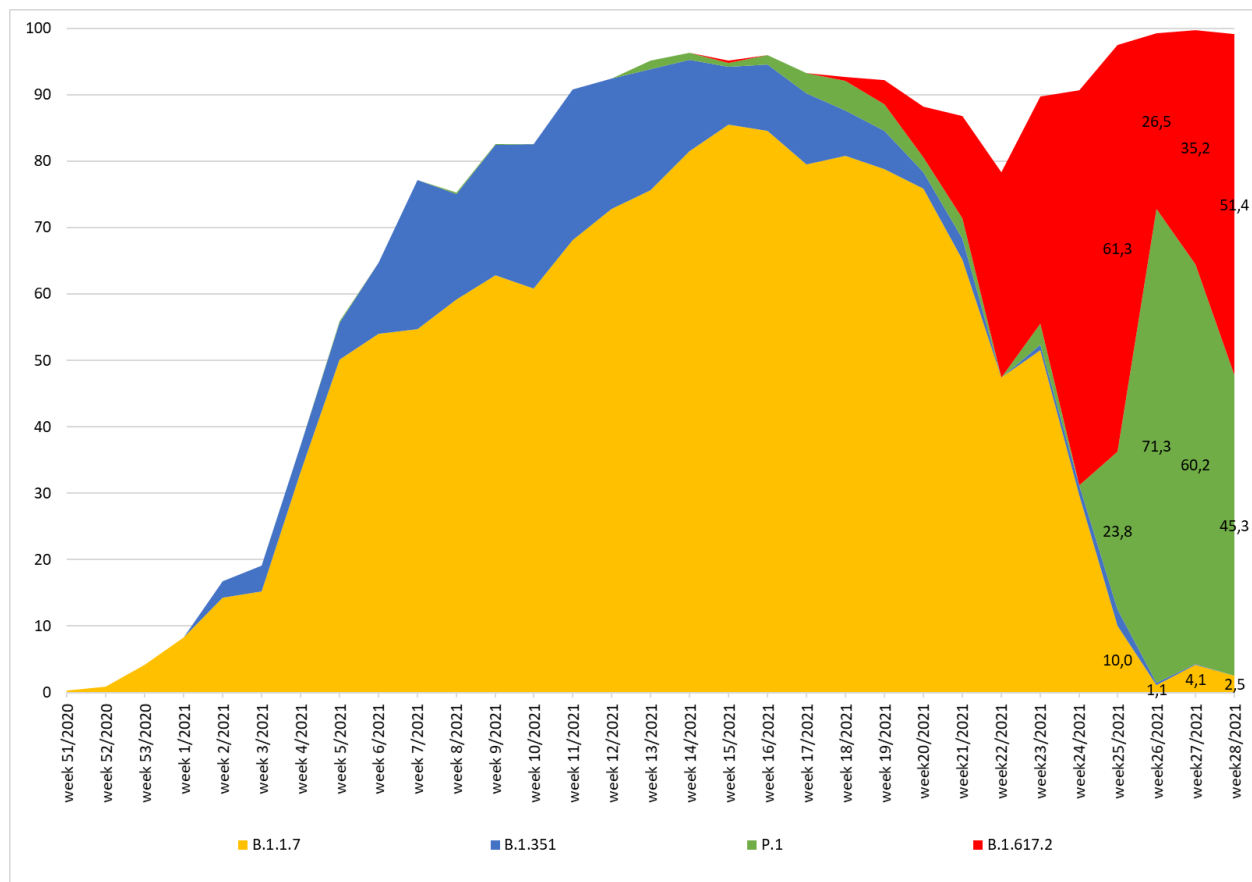


Figure 3. Evolution of variants in Sequencing pool of all specimens including targeted sequencing (clusters, non-residents) since first detection of B.1.1.7 in Luxembourg

In week 28/2021, 5 circulating SARS-CoV-2 variants were detected within our representative sequencing pool, after removal of cluster specimens, and excluding specimens collected from non-residents, as shown in [Figure 4](#). The most prevalent lineages are displayed in [Table 2](#) and information about the lineages is provided in [Annex 2](#).

Table 2. Distribution of SARS-CoV-2 lineages detected within the community (cluster and non-resident cases excluded) in weeks 27 and 28/2021

VOC	Week 28			Week 27		
	N	%	CI	N	%	CI
B.1.1.7 (Alpha)	11	2.6%	1.1 - 4.1	21	4.3%	2.5 - 6.1
B.1.351 (Beta)	0	0.0%	-	1	0.2%	-
P.1 (Gamma)	203	47.8%	43.1 - 52.5	299	61.0%	56.7 - 65.3
B.1.617.2 (Delta)	209	49.2%	44.4 - 54.0	165	33.7%	29.5 - 37.9
Others	2	0.4%	-	4	0.8%	-
Total	425	100.0%		490	100.0%	

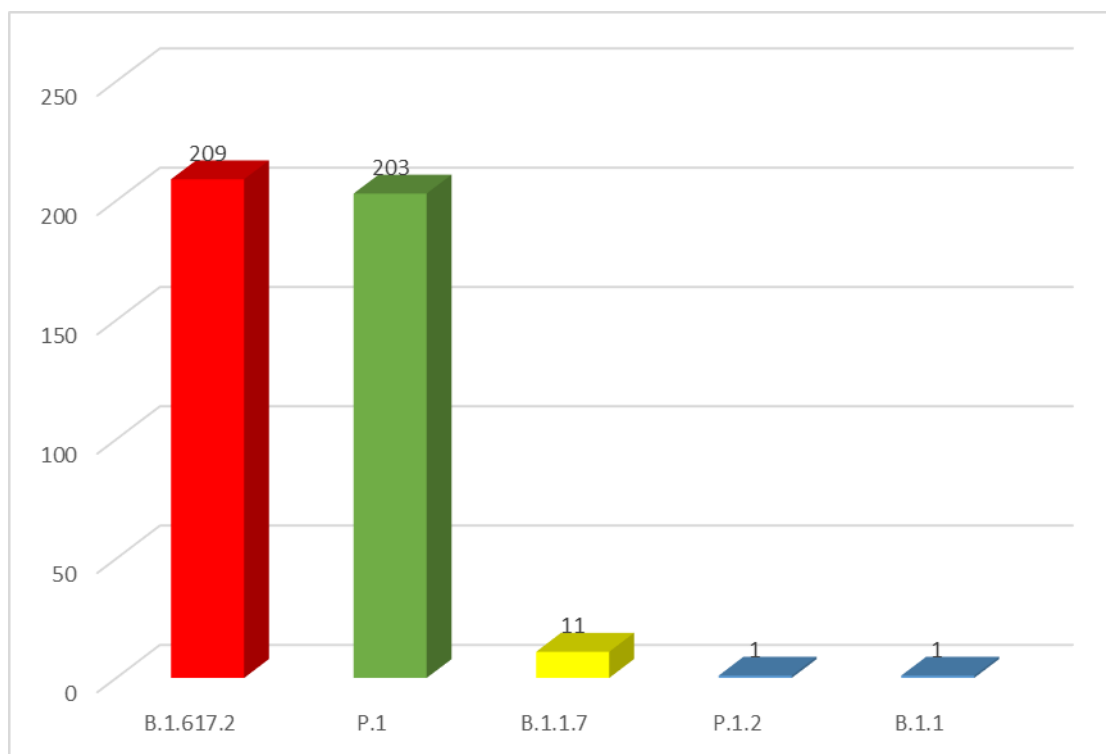


Figure 4. Number of SARS-CoV-2 variants in representative sample for week 28/2021

Mutation surveillance

In addition to the surveillance of SARS-CoV-2 variants, the LNS monitors the occurrence of SARS-CoV-2 mutations assumed to have a clinical and epidemiological relevance. Currently, 13 mutations are being observed, and this list is updated continually.

Table 3 provides the overall frequencies of these mutations, detected in the lineage-assignable genome sequences, analyzed between 1 Sep 2020 and 11 Jul 2021 (N=14977), as well as the frequencies in week 28/2021.

Table 3. List of clinically relevant mutations identified upon week 28/2021 sequencing

Mutation	Gene	Genomic Position in reference	Frequency Overall [%]	Frequency Week 28/2021 [%]	Characteristics	Reference
D614G	S gene	23402	95,3	96,3	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
P323L	ORF1ab	14407	88,3	92,6	Higher severity	Biswas & Mudi 2020
R203K	N gene	28880	58,2	49,5	Fitness advantage for the virus	Leary 2020
G204R	N gene	28883	58,1	49,3	Fitness advantage for the virus	Leary 2020
N501Y	S gene	23063	56,9	46,4	501Y.V1/V2; Improved ACE2 binding affinity/higher transmissibility	Filip Fratev 2020 COVID-19 Genomics Consortium UK, 2020
E484K	S gene	23012	16,2	44,9	501Y.V2 / possible impact on antibody neutralization activity (escape mutation), improved ACE2 binding affinity	Greaney 2020
Y144del	S gene	21991-21993	45,8	3,1	possible impact on antibody binding affinity	Dawood 2020
H69/V70del	S gene	21765-21770	45,2	2,5	possible impact on antibody neutralization activity and reinfection; included in "mink" mutation	Kemp 2020

P681H	S gene	23604	42,9	2,5	immediately adjacent to the furin cleavage site, a known location of biological significance	COVID-19 Genomics Consortium UK, 2020
L37F	Nsp6	11081	3,5	1,9	Favored viral infection, higher severity	Aiewsakun 2020
Q57H	ORF3a	25561	21,8	0,4	Higher severity	Biswas & Mudi 2020
K417N	S gene	22813	7,7	0,2	501Y.V2 / possible impact on antibody binding affinity (escape mutation)	Kemp 2020
N439K	S gene	26143	0,8	0	Improved ACE2 binding affinity	Zhou 2020

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Annexes

Annex 1. SARS-CoV-2 variants naming

The ReViLux continues to use the (Pango) system to allow easier visualisation of links between any evolving variants and their ancestor. Equivalence for most frequently used VOC nomenclatures are shown in Table A1 (adapted from WHO).

Table A1. Variants of concern nomenclature by 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 B.1.351.2 B.1.351.3	GH/501Y.V2	20H (V2)	+S:L18F	South Africa, May-2020	18-Dec-2020
Gamma	P.1 P.1.1 P.1.2	GR/501Y.V3	20J (V3)	+S:681H	Brazil, Nov-2020	11-Jan-2021
Delta	B.1.617.2 AY.1 AY.2 AY.3	G/478K.V1	21A	+S:417N	India, Oct-2020	VOI: 4-Apr-2021 VOC: 11-May-2021

Adapted from WHO - Tracking SARS-CoV-2 variants

Annex 2. Summary of information regarding the most frequently detected variants in the Great Duchy of Luxembourg

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 more transmissible than B.1.1.7 (increasing confidence). Neutralization studies show reductions in cross-neutralizing activity between B.1.1.7 and B.1.351.