Genomic surveillance of SARS-CoV-2 in Belgium

Report of the National Reference Laboratory (UZ Leuven & KU Leuven)

Situation update – 5th of February 2021
(report 2021_08)

Rapid communication to clinical laboratories

The 501Y.V1 (B.1.1.7) variant of concern (VOC) presents a panel of mutations and deletions, mainly present in the S gene (spike protein). As a consequence of genetic variations inherent to virus variants, the performance of some PCR results may be altered. Currently, the NRC is not informed of diagnostic assays that would provide a false negative result.

During the second half of December 2020, the scientific community was informed that the 69-70del present in 501Y.V1 generated a **"dropout" of the S gene** target present in the Applied Biosystems **TaqPath COVID-19 Combo Kit**, which contains 3 gene targets. Since a number of weeks, as 501Y.V1 widely circulates in Belgium, the "S dropout" signal of this test can be considered as a reliable indicator of the presence of this VOC.

More recently, it has been observed that 501Y.V1 generates a **delayed Ct or a dropout of the N gene** target present in the **Seegene Allplex COV2/FLUA/FLUB/RSV kit**. This seems to be caused by the D3L mutation present in the N gene, a mutation which is associated with 501Y.V1 and no other viral populations currently circulating in Belgium. While further confirmation work is being conducted, we invite clinical laboratories to consider as "probable VOC – 501Y.V1/B.1.17" samples presenting such results and to refer these samples to the National Reference Laboratory for further confirmation. The proportion of samples presenting this particularity can in the meantime by used as a reliable proxy for following the epidemiological evolution of 501Y.V1 at a local level.

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1. International context

Since the end of the year, 4 variants of concern (VOCs) have arisen independently of one another in the United Kingdom (501Y.V1 / B.1.17), South Africa and Brazil. These variants harbour a number of mutations and deletions associated with higher infectiousness and immune escape. All variants are spreading internationally, with 501Y.V1 being the most prevalent to date in Belgium.

2. Belgian genomic surveillance

The National Reference Centre has put in place genomic surveillance at the national level since the first introduction of the virus in February 2020.

Genomic surveillance in Belgium is currently principally based on:

- Baseline surveillance: whole genome sequencing of a random selection of positive samples referred by a network of 24 sentinel laboratories
- Active surveillance: intensified research of VOCs in specific groups including returning travellers, patients presenting chronic infection, re-infection and infection after vaccination and atypical PCR results (not limited to the S-gene dropout).

3. Delayed Ct value of the N target in presence of 501Y.V1 with the Seegene Allplex COV2/FLU/RSV kit

CHU Charleroi has observed delayed Ct values of the N gene among 43/350 (12,3%) positive SARS-CoV-2 clinical samples collected since the 1st of January 2021. To date, 14 of these samples have been sequenced at the national reference laboratory. Among these, 13 (93%) were confirmed as 501Y.V1 and presented the N:D3L mutation. One sample was typed as 20A, but presented the N:D3L mutation.

PCRCOV_N	**26.26 Cycle**
PCRCOV_RdRp	**18.62 Cycle**
PCRCOV_S	**18.25 Cycle**

Figure 1: Typical delayed Ct value of the N target in presence of 501Y.V1 with the Seegene Allplex COV2/FLU/RSV kit

4. Presence of N:D3L mutation in Belgium

Using Nextstrain, we looked for the N:D3L mutation among Belgian sequences available on GISAID. Of the 193 available 501Y.V1 strains, all but one (shown in green) are harbouring this mutation. This one sequence presents a mutation reported as N:D3Q.

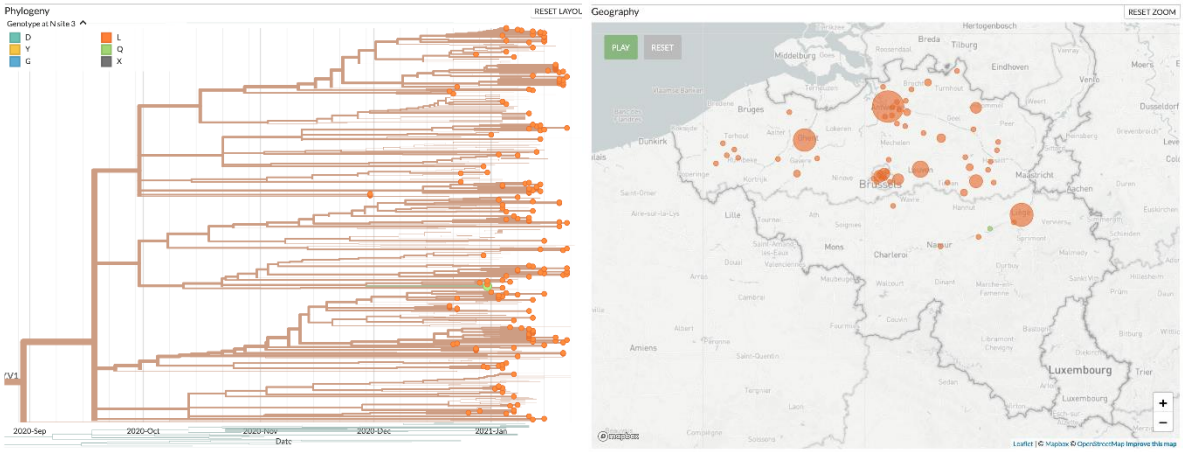

Figure 2: N:D3L mutation is observed among Belgian sequences mainly (all but one of the 193 available) among 501Y.V1 strains

On the other hand, apart from the available 501Y.V1 sequences, one single sequence is harbouring the N:D3L mutation (the single sequence in orange on the left figure below).

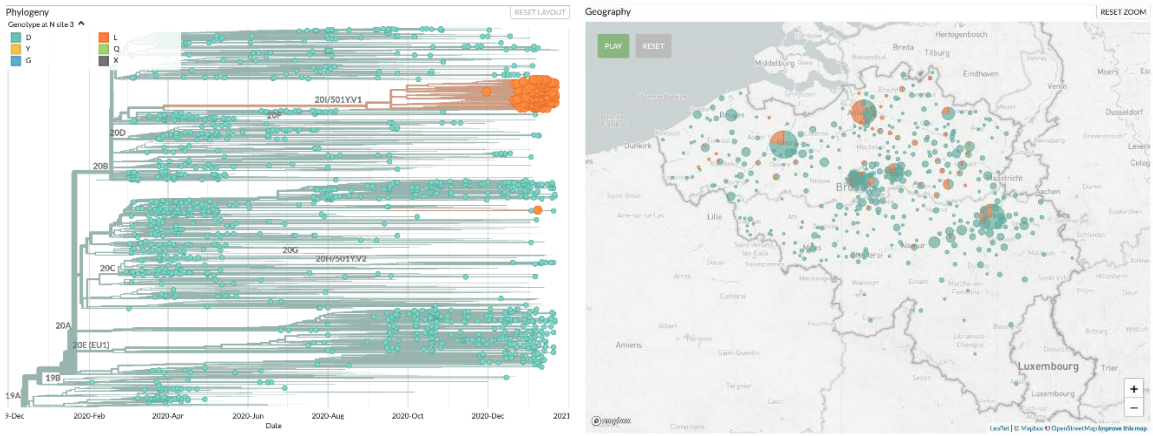

Figure 3: Apart from the 501Y.V1 sequences from Belgium, one non-VOC sequence harbours the N:D3L mutation

5. Impact for 501Y.V1 (B.1.1.7) surveillance in Belgium

In the current epidemiological situation as observed in Belgium, a delayed N Ct or an N dropout observed with the Seegene Allplex COV2/FLUA/FLUB/RSV kit can be considered as a reliable indicator of the 501Y.V1 variant of concern. We invite other labs using this test to be attentive to this signal and to refer abnormal PCR results to the National Reference Lab.