Genomic surveillance of SARS-CoV-2 in Belgium

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

Situation update – 23th of February 2021
(report 2021_13)

Executive summary

Genomic surveillance in Belgium is organised around 3 different arms aiming to monitor the emergence and the further spread of specific viral populations (variants of concern or VOCs) which may impact disease control and/or vaccination strategies.

Through baseline surveillance, an unbiased selection of positive samples from 24 sentinel labs (selected based on geographical dispersion and diversity of clinical patterns) are analysed in designated sequencing platforms. Currently, 6.341 Belgian sequences are available on GISAID. During weeks 6,7 and the first days of week 8, 670 samples have been sequenced as part of the baseline surveillance, among which 292 were 20I/501Y.V1 (43,6%), 34 were 20H/501Y.V2 (5%) and 8 were 20J/501Y.V3 (1,2%).

The majority of new infections occurring in Belgium are now caused by a VOC. Collectively, these VOCs are now driving the epidemic in Belgium and could be the cause of an upcoming rise in daily infections.

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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 (20I/501Y.V1), South Africa (20H/501Y.V2) and Brazil (20J/501Y.V3 or P.1 and P.2). These variants harbour several mutations and deletions associated with higher infectiousness and immune escape. All variants are spreading internationally, with 3 VOCs having been detected to date in Belgium (1.876 for 20I/501Y.V1, 255 for 20H/501Y.V2 and 19 for 20J/501Y.V3).

2. Baseline surveillance and proportion of VOCs among new infections in Belgium

Since support was offered by the federal government at the end of December 2020, both the temporal coverage (number of sequencing analyses performed per week) and geographical coverage (residence of the patients sampled) have improved significantly. Currently, 6.341 Belgian sequences are available on GISAID.

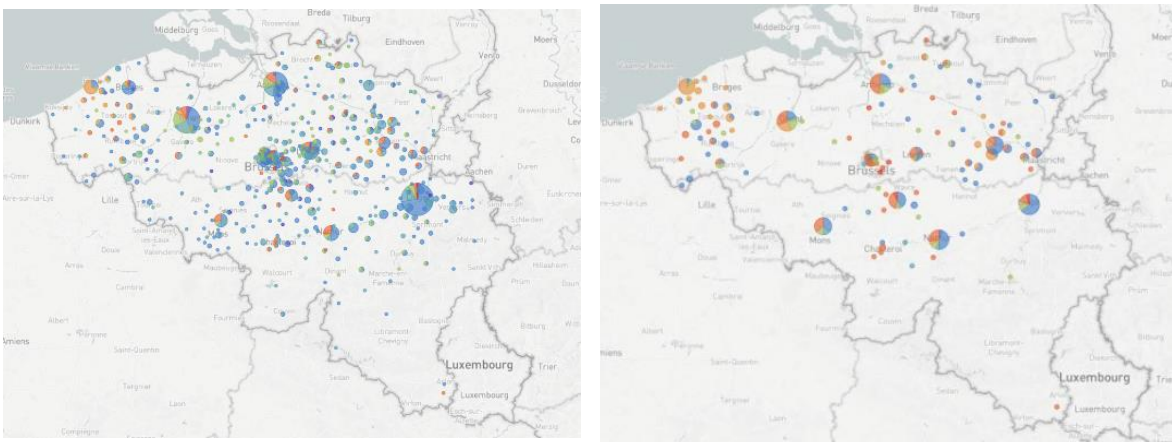


Figure 1: Representation of the geographical coverage of the genomic surveillance network in Belgium since February 2020 (left) and 1st of January 2021 (right).

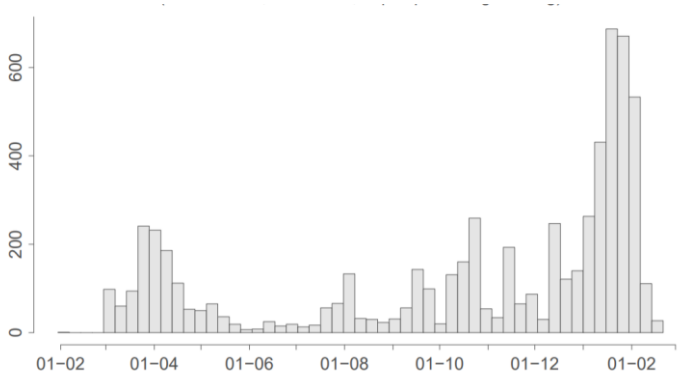


Figure 2: Number of sequences deposited on GISAID per week of sampling since the start of the outbreak in Belgium.

Follow-up of 501Y.V1 (B.1.1.7) is performed using an additional indicator, which is the “S dropout” signal detected among positive COVID-19 PCRs reported by the 8 federal platform laboratories. In order to obtain the best view on the number of recent infections actively contributing to transmission, we consider for the daily follow-up only positive samples for which the N gene has a Cq value under 25. By excluding for this analysis the samples with a Cq value between 25 and 30, we avoid to include possibly older infections and possible false positive S dropout signals that can occur when the signal is close to the limit of detection.

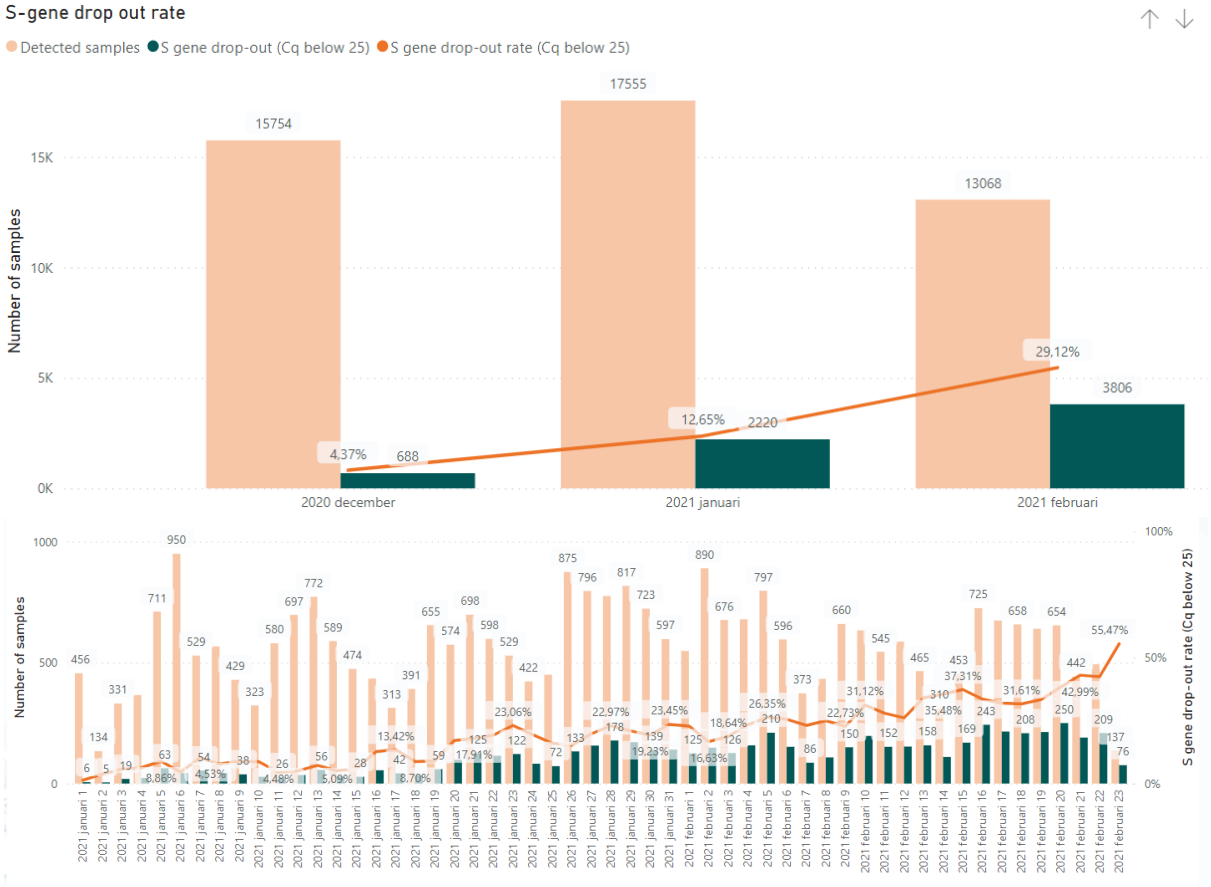


Figure 3: Monthly (figure above) and daily (figure below) evolution of the proportion of infectious samples detected among all positive tests diagnosed in the federal platform laboratories (Presence of the S dropout signal and Cq <25). Based on these figures, we estimate that over 40% of the people infected one week ago were infected with a 501Y.V1 variant. This phenomenon is observed in all regions of the country.

During weeks 6,7 and the first days of week 8, 670 samples have been sequenced as part of the baseline surveillance, among which 292 were 20I/501Y.V1 (43,6%), 34 were 20H/501Y.V2 (5%) and 8 were 20J/501Y.V3 (1,2%). Based on these figures representing the situation of patients having been infected two to three weeks ago, we estimate that the majority of the people infected at the date of this report are infected with one of the 3 VOCs currently circulating in Belgium.

An estimate of the actual proportion of currently diagnosed samples that are 501Y.V1 can be obtained using a binomial GLMM fitted to the S dropout data (Figure 4). This analysis estimates that 70% [67-72%] 95% CLs of all currently diagnosed infections and 80% [78-83%] of all new infections (at time of infection, counted 1 week before diagnosis) could now (on the 23d of February) be 501Y.V1. This is

only slightly lower than the ca. 90% that was estimated in our report of the 28th of January, which used the data from the 1st to the 22nd of January. The growth advantage of 501Y.V1 compared to all other strains is estimated to be 8.8% [8.4-9.3%] per day, which assuming a generation time of 4.7 days, would translate to a transmissibility advantage of 51% [48-55%]. Several factors may explain the slight reduction in the initially estimated growth advantage, including various sampling biases (e.g. a shift from active surveillance to more random baseline surveillance) as well as potentially the invasion of other variants with a growth advantage, such as 501Y.V2 & 501Y.V3

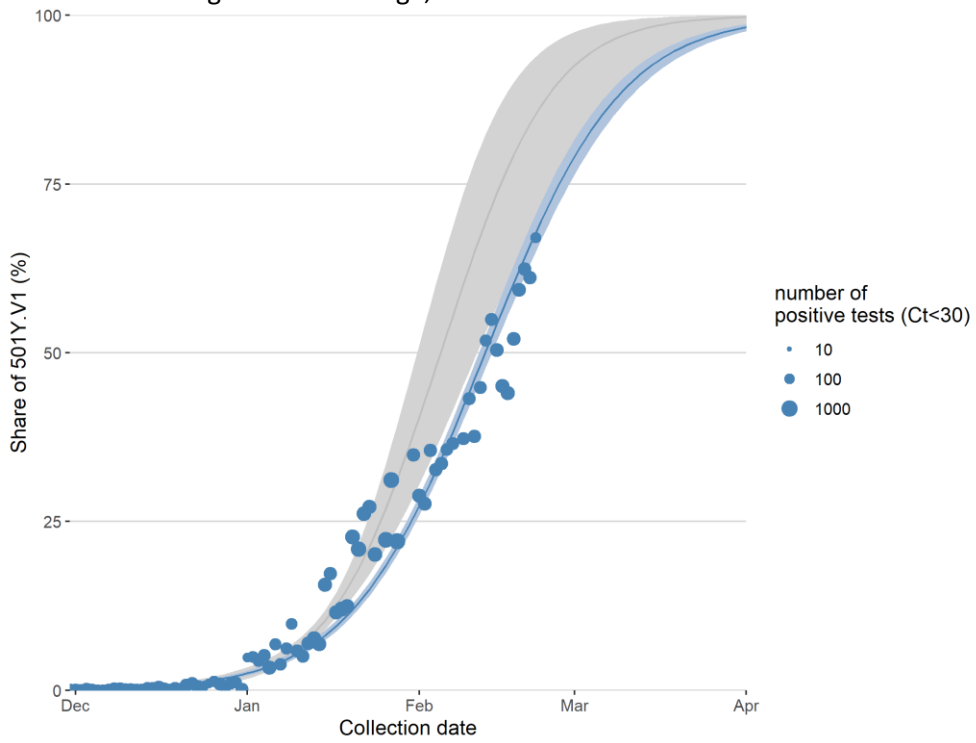


Figure 4: Estimated increase in the relative abundance of the 501Y.V1 variant in Belgium based on S dropout data (mean and 95% confidence intervals, binomial GLMM with random intercept for laboratory and an observation-level random effect to take into account overdispersion, with correction for the expected proportion of true positives, estimated from an independent binomial GLMM fitted to sequencing data of S dropout samples; currently 99.9% of all S dropout samples are indeed 501Y.V1). An extrapolation up to the first of Aprils is shown. Shown in grey is the fit we made in our report of the 28th of January, using the data from the 1st of January until the 22nd of January only. In this analysis, only tests with Ct values for the N and ORF1ab genes < 30 were included. Code available at

https://github.com/tomwenseleers/newcovid_belgium/blob/main/analysis_update_20210223.R

We can further observe that the increase in the share of 501Y.V1 among newly diagnosed infections occurs at approximately the same rate in different regions in Belgium (Figure 5), although initial emergence of 501Y.V1 is inferred to have occurred significantly earlier than average in Brussels and Antwerp, and at significantly later than average in Namur, Leuven and Mons.

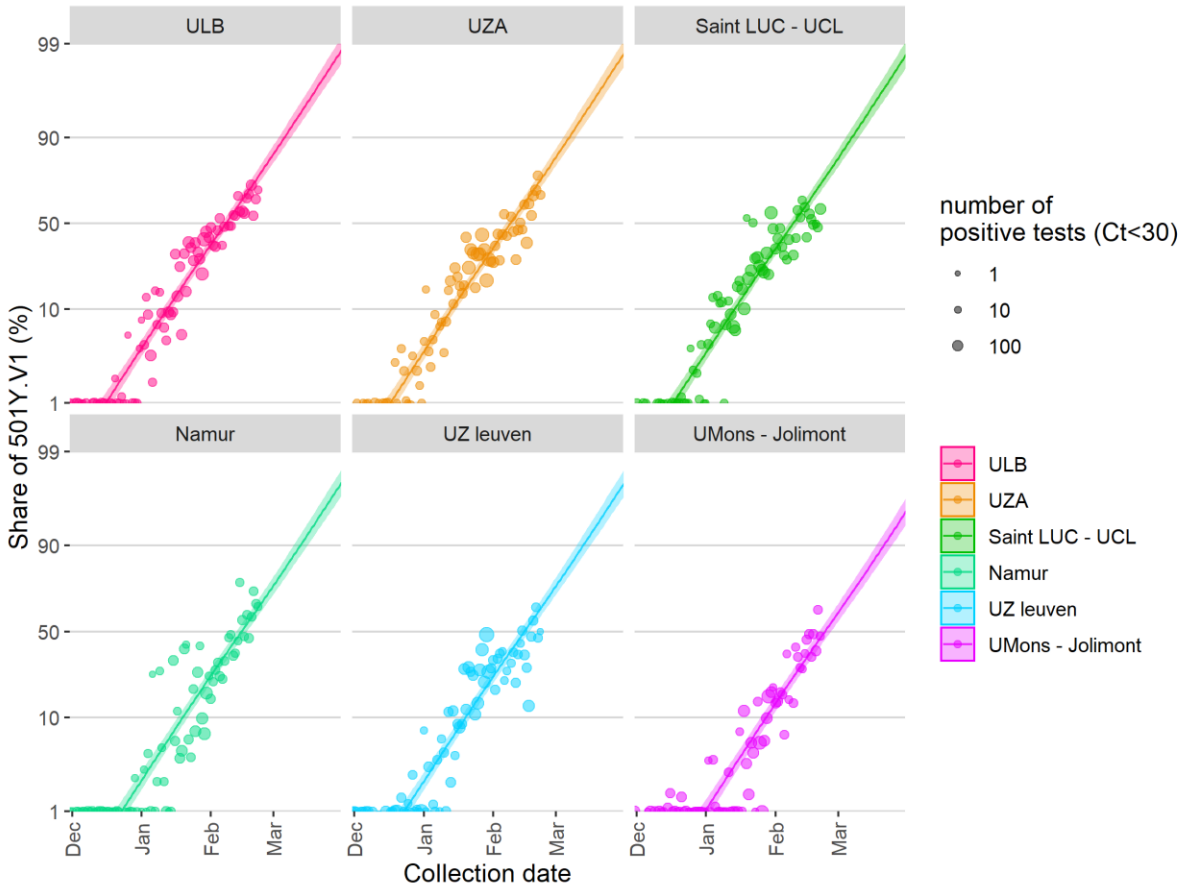


Figure 5: Average share of 501Y.V1 among newly diagnosed infections inferred from a binomial GLMM (cf. Figure 4), plotted separately for different regions in Belgium (predicted means and 95% confidence intervals).

Due to these observed increases in the share of the 501Y.V1 variant among newly diagnosed infections, and its increased transmissibility of ca. 50%, variant 501Y.V1 has now become a main determinant in driving up the effective reproduction number (Re) of the SARS-CoV2 virus in Belgium. Using the fitted proportion of the 501Y.V1 variant among new infections, combined with the estimated transmission advantage of 51% and overall Re values estimated from the Sciensano case and testing data, we can readily estimate the Re values of the 501Y.V1 strain and the other circulating variants separately (Figure 6). This follows from the fact that the overall Re value is at any time an average of the Re values of the individual variants, weighted by their frequency. In Figure 6 we calculated the Re values from the Sciensano case data based on the intrinsic growth rate in number of cases, calculated using a gamma distributed generation time of 4.7 days with a SD of 2.9 days, and with the growth rate calculated as the first derivative of a binomial generalized additive model fit (taking into account weekday, a 32 df cubic spline in function of time of diagnosis, and a 5 df cubic spline in function of number of tests performed). In contrast to the Re analyses that are routinely reported, this analysis corrects for variable testing intensity. It can be observed that this analysis estimates the overall Re value (at time of diagnosis) to already be well above 1, at 1.20 [1.18-1.22]. In addition, while the Re value of the other variants is estimated to be much below 1 (0.85 [0.84-0.87]), the Re of the 501Y.V1 strain is estimated to lie at 1.30 [1.27-1.32]. We expect that the overall Re value would still rise a little further, almost approaching that of the 501Y.V1 strain as 501Y.V1 would further displace the other variants.

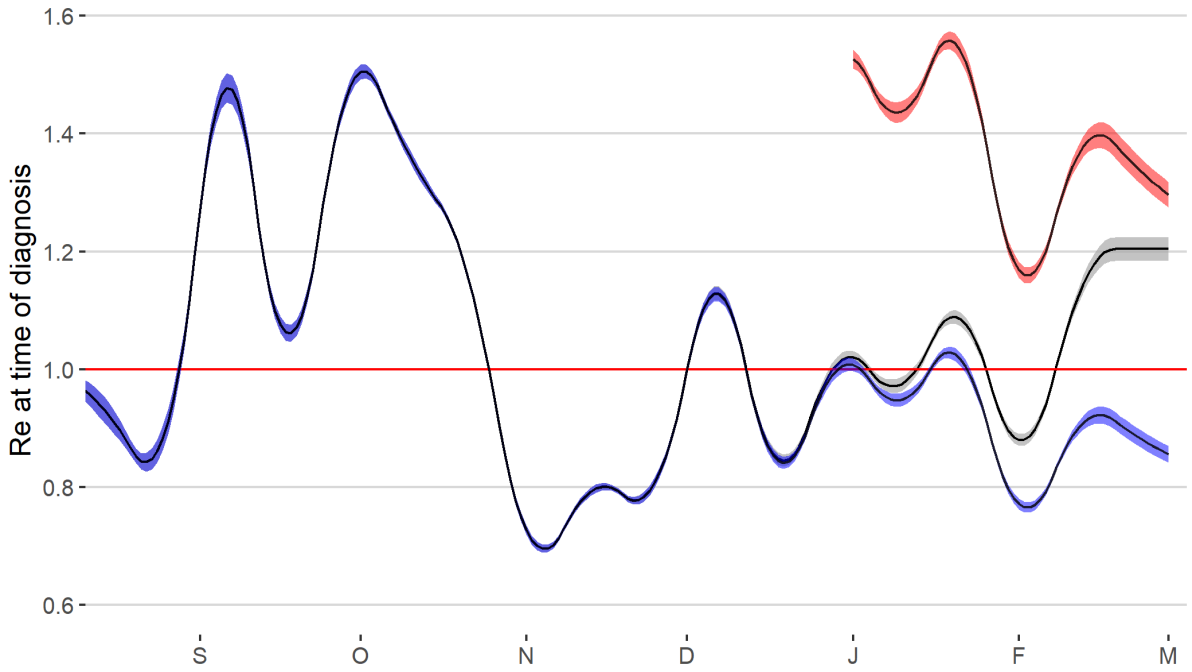


Figure 6: The evolution of the basic reproduction number Re in Belgium (black line) is now heavily influenced by the spread of the highly contagious 501Y.V1 strain (red line), which emerged in Belgium in the middle of December 2020, and which at any time is estimated to have an Re value that is 1.51 times higher than that of the other circulating variants (blue line) (shaded areas = 95% confidence intervals). Overall, the average Re values, as well as the Re values of the 501Y.V1 strain and the other circulating strains, are heavily influenced by changes in behaviour, the implementation of non-pharmaceutical interventions, and so these tend to fluctuate quite a bit. Re values beyond the 19th of February have been extrapolated, and the flat trend shown may not be reliable.

Aside from the 501Y.V1 variant, baseline sequencing also inferred the presence of two other variants of concern, 501Y.V2 and 501Y.V2. During weeks 6,7 and 8, 670 samples have been sequenced as part of the baseline surveillance, and of these 292 were 20I/501Y.V1 (43,6%), 34 were 20H/501Y.V2 (5%) and 8 were 20J/501Y.V3 (1,2%). If we would fit a multinomial model to these data, we can estimate that among new lab diagnoses today 62% [57-66%], 6% [4-8%] and 2% [0-4%] would be by 501Y.V1, 501Y.V2 and 501Y.V3, and 70% [63-76%], 6% [3-9%] and 3% [0-8%] of all new infections (one week before diagnosis) (Figure 7).

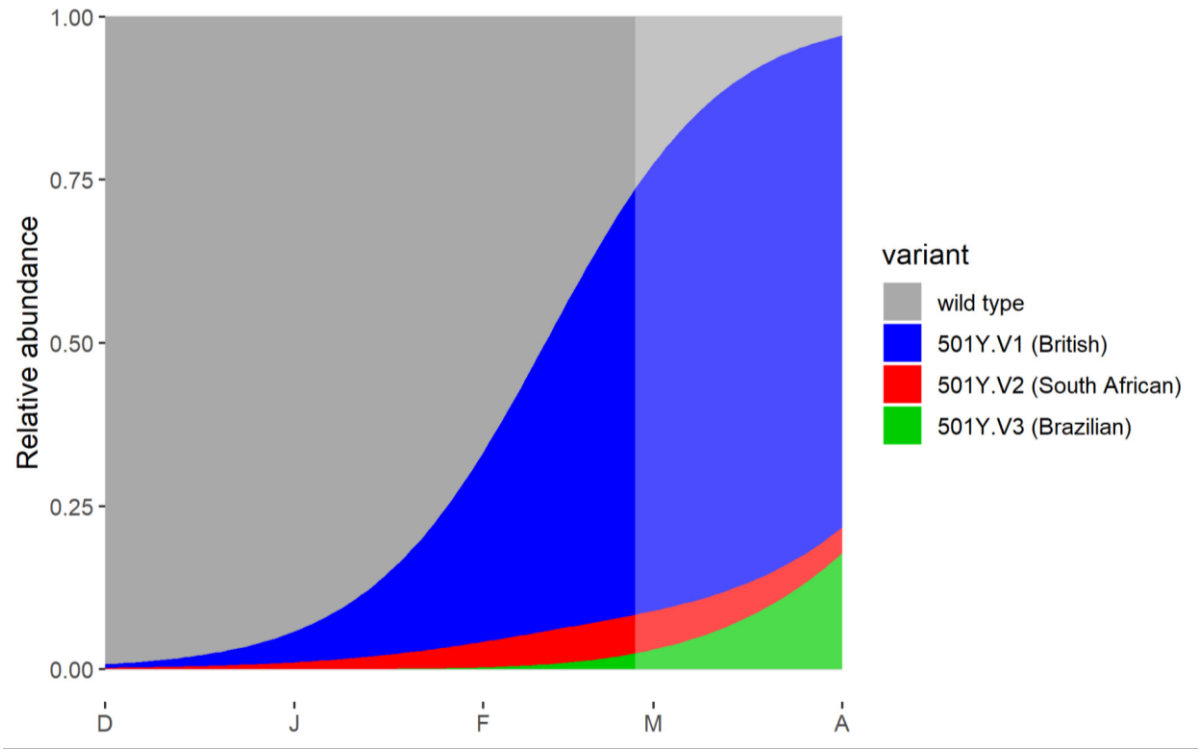


Figure 7: Spread of VOCs in Belgium over time and projections for the upcoming weeks based on a multinomial fit to the baseline sequencing data. A wide and temporary utilization of a reflex PCR positively detecting the 501Y.V2 (Red) and 501Y.V3 (Green) mutants would allow to initiate targeted interventions. Considering that these two VOCs are less susceptible to vaccination, a larger vaccination coverage will be required to mitigate their impact on the general epidemic trends. Temporary interventions targeting these VOCs such as reflex test and highly active contact tracing would contribute to compensate the selection pressure that will be caused during stepwise vaccination rollout.

3. Temporary utility (if rapid implementation) of a reflex VOC PCR

Since the start of the COVID-19 pandemic, viral mutants have continuously emerged as a consequence of high level SARS-CoV-2 circulation. In a first phase, non-pharmaceutical interventions such as contact-restriction policies, have led to the selection of more transmissible variants. In a second phase, the virus is put under pressure to be able to evolve in populations with a partial herd immunity, and experiencing a stepwise rollout of vaccination.

During the upcoming months, a period characterized by incomplete immune protection, partial immunity status will probably become a major driver of selection for variants better adapted to escape human immunity. This situation should in a later stage become less problematic when the overall immunity level of the society, achieved through vaccination, will reach a sufficient level to balance the partial immune escape mechanisms of these variants. To date, a limited number of VOCs have been described, and controlling the spread of mutants harbouring an immune escape mechanisms (in particular S:E484K) at least during the vaccination rollout period.

Performing a reflex PCR on all (or a significant proportion) of positive samples would allow to rapidly detect and subsequently contain community clusters of transmission related to such VOCs. Considering the financially advantageous conditions currently offered to clinical laboratories for diagnostic PCR tests, this reflex PCR complementing a positive result could eventually be offered at no (or reduced) cost for the public health budget during a limited period of time. The implementation of such PCR should be considered as necessary as long as VOCs harbouring the S:E484K mutation remain a minority of the circulating strains and as long as the health inspectors can handle the workload related to the specific interventions required.

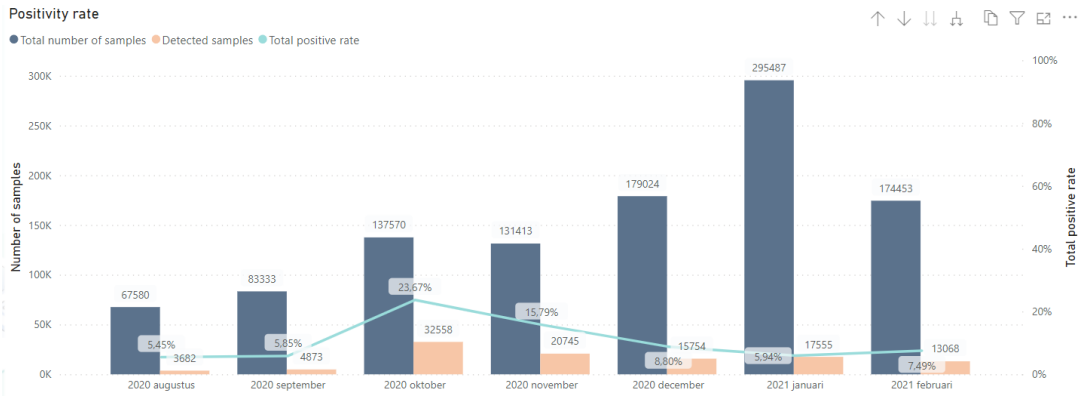
We selected a combination of biologically relevant mutations located in the receptor binding domain of the Spike gene that would allow to detect and discriminate the currently described VOCs. This list was shared with – among others - Applied Biosystems™ which designed the TaqMan® SARS-CoV2 Mutation Assays. In this assay, RNA is first reverse transcribed and then amplified in a 1-step RT-PCR reaction using sequence specific primers to amplify the region of interest. The reverse primer in the assay is used to initiate reverse transcription of the SARS-CoV-2 genomic RNA sequences. Each assay allows detection of a single mutation using two TaqMan® minor groove binder (MGB) probes with nonfluorescent quenchers (NFQ). The mutation is detected by a FAM™-labeled probe, and the reference sequence is detected by a VIC™-labeled probe. Cluster plot analysis is performed in Design and Analysis software v2.5.0.

The assay allowed to detect and subsequently characterize VOCs in a panel of 13 strains characterized by whole genome sequencing. The panel included four non-VOC strains, four 501Y.V1, four 501Y.V2 and one 501Y.V3 samples.

	S:K417N	S:K417T	S:E484K	S:N501Y	S:A570D
20I/501Y.V1	NO	NO	Possible	YES	YES
20H/501Y.V2	YES	NO	YES	YES	NO
20J/501Y.P1	NO	YES	YES	YES	NO
20J/501Y.P2	NO	NO	YES	YES	NO

4. Positivity rate in federal platform laboratories

The positivity rate among samples tested can be seen as the reflection of the level of saturation of laboratories in comparison with the current need. The positivity rate has increased from January to February, but is still below 10%.



The proportion of positive samples presenting a very high viral load (Cq < 15) can be seen as the number of patients diagnosed during the first days of infection. This proportion tends to increase when the tracing is efficient in identifying transmission events, but can also be observed in the early weeks of a resurgence. This rate has increased from January to February, and is for the month of February at the level observed in September 2020, a few weeks before the second wave. This proportion has reached 30% during the last week, a proportion comparable with the month of October 2020, at the start of the second wave.