Genomic surveillance of SARS-CoV-2 in Belgium

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

Situation update - 21th of January 2021
(3rd report for 2021)

Executive summary

Genomic surveillance in Belgium is currently based on 3.141 genomic sequences available on GISAID, with a recent acceleration in sequencing capacity. Since the 1st of December 2020, a total of 1.360 sequences have been produced by the participating sequencing platforms. 178 501Y.V1 and 8 501Y.V2 VOCs have been identified (increasing trend).

Belgium has recently experienced multiple introductions of variants of concern (VOCs), particularly since the last days of 2020. The consolidated genomic and epidemiological data are consistent with a rapidly increasing number of events of local transmission. Based on the evolution of atypical PCR results ("S dropouts"), we estimate that VOCs currently represent 10-15% of infectious cases in the country (increasing trend).

Content of this document

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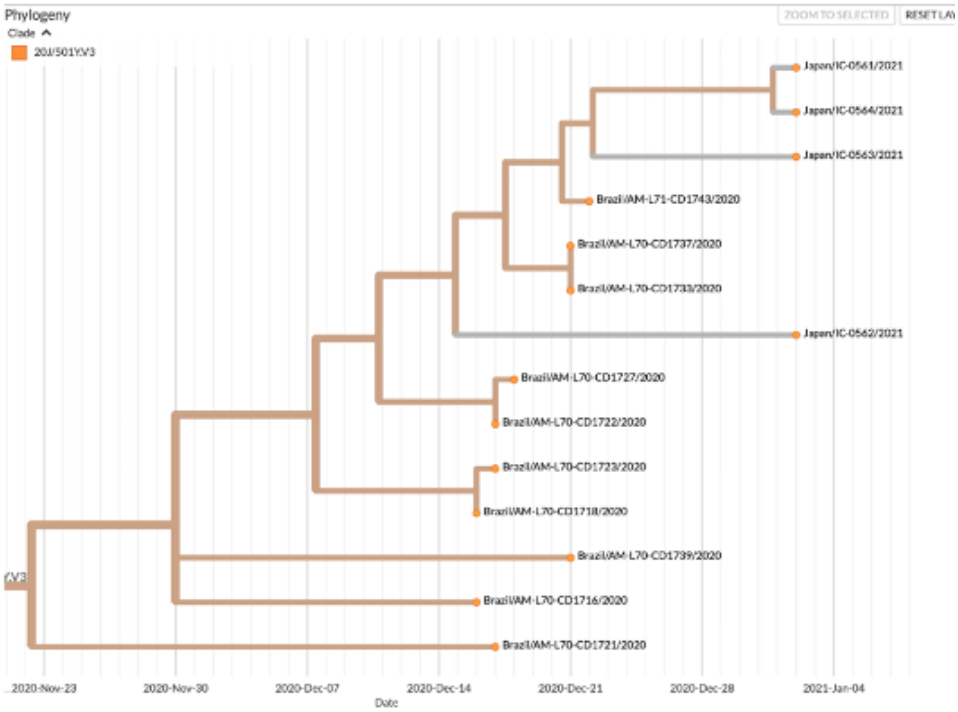
1. International context

Since the end of the year, 3 variants of concern (VOCs) have arisen independently of one another in the United Kingdom (501Y.V1), South Africa (501Y.V2) and Brazil (501Y.V3). These variants harbour a number of mutations and deletions associated with higher infectiousness and immune escape. All 3 variants are spreading internationally, with 501Y.V1 and 501Y.V2 having been detected in Belgium. Other variants harbouring the 501Y mutation have recently been reported (Ohio, US), and therefore, the number of VOCs to be monitored in Belgium might increase in the future.

While the vast majority of sequences publicly available for 501.V1 originate from the UK, the map below illustrates the current spread of this variant all over the world. It is to be noted that many countries do currently not have sequencing facility in place or do not publicly share their sequences. This illustration is therefore illustrative but certainly represents an under-estimation of the spread.

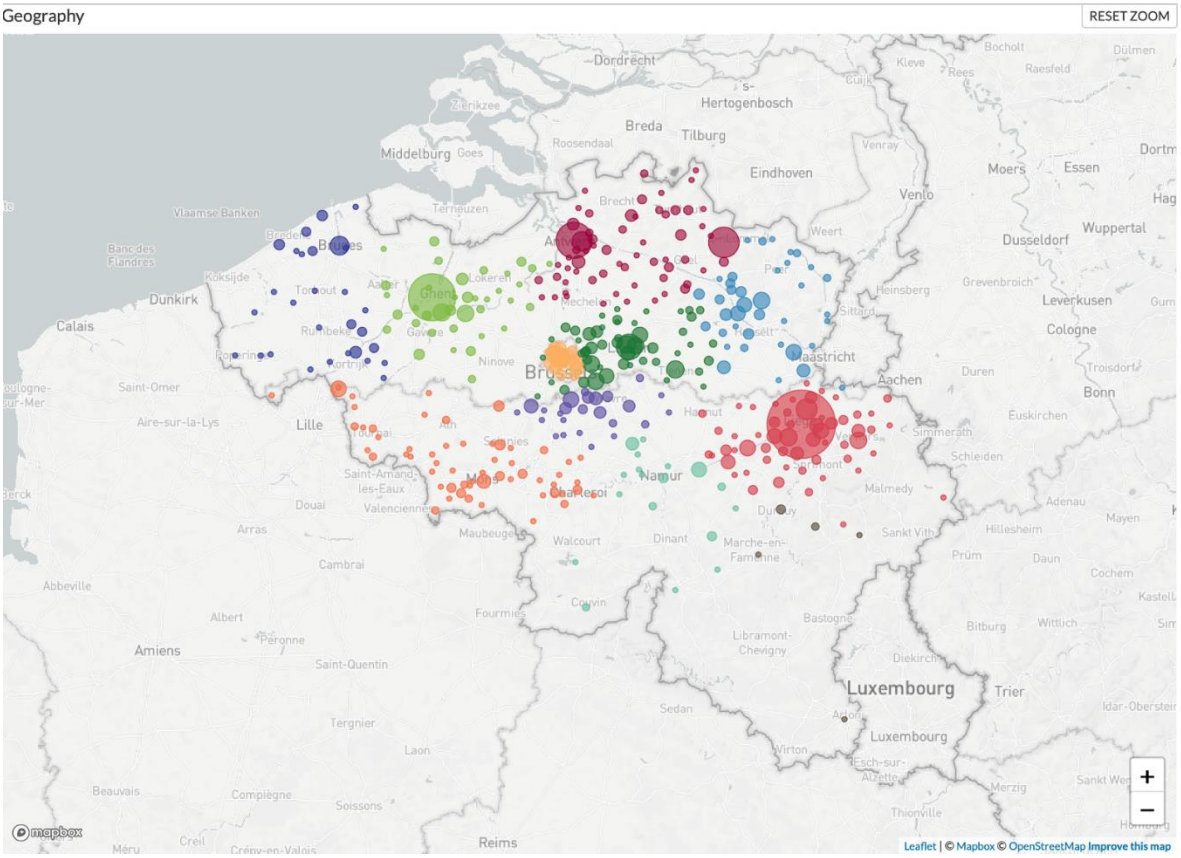
The 501Y.V2 variant seems to have currently a more limited international spread but has already been identified in most European countries that are performing genomic surveillance and is probably already largely spreading in many African countries in the southern part of the continent.

The 501Y.V3 has currently only been identified in Brazil and in Japan.



2. Belgian genomic surveillance

The National Reference Centre hosted at UZ Leuven – KU Leuven has put in place genomic surveillance at the national level since the first introduction of the virus in February 2020. Along the way, other university centres have contributed to this surveillance effort through complementary initiatives, and in the coming weeks, sequencing efforts will continue to increase using the structure of the federal platform laboratories. In total, 3.141 sequences were uploaded on GISAID and are therefore made openly available for national and international surveillance. The map hereunder represents the current availability of sequences per province in Belgium.



Sequencing laboratory	Sequences	% of total
KU Leuven (Rega Institute, National Reference Laboratory)	1486	47,3%
U Liège (GIGA Medical Genomics)	1290	41,1%
U Gent (Onderzoeksgroep Virologie and Lab voor klinische biologie)	303	9,6%
Institute of Tropical Medicine	47	1,5%
ULB (CUB Hopital Erasme Laboratoire d'Anatomie Pathologique)	8	0,3%
U Antwerpen (Laboratory of Medical Microbiology)	7	0,2%
Total	**3141**	

Genomic surveillance in Belgium is based on two arms:

Baseline Surveillance. A representative sampling of the positive cases in Belgium organised with the collaboration of a sentinel network of laboratories, allows to follow over the time the trends in the genetic diversity of circulating strains of SARS-CoV-2. Overall, 24 labs have been pre-selected and contacted by the National Reference lab to refer 5% of their positive samples in this baseline surveillance system. The selection was made to ensure an optimal geographical coverage and a diversity of clinical severity patterns (university hospitals, regional hospitals, GPs and community-based testing centres). The aim is to cover at all times a minimum of 2% of all positive cases in Belgium.

Lab	**Type of lab**	**Region/province**	**Contacted**	**Acceptance to participate confirmed**
UZA	Federal platform (including clinical lab)	Antwerp	X	X
UZ Gent	Federal platform (including clinical lab)	East-Flanders	X	X
UZ Leuven	Federal platform (including clinical lab)	Flemish Brabant	X	X
Liège	Federal platform (including clinical lab)	Wallonia	X	X
Mons	Federal platform (including clinical lab)	Wallonia	X	X
Namur	Federal platform (including clinical lab)	Wallonia	X	X
UCL	Federal platform (including clinical lab)	Brussels	X	X
ULB	Federal platform (including clinical lab)	Brussels	X	X
Medina	Private	West-Flanders	X	X
Synlab	Private	Wallonia	X	X
CMA	Private	Antwerp	X	X
AML	Private	Antwerp	X	
AZ Delta Roeselare	Hospital	West-Flanders	X	
Labo Luc Olivier	Private	Wallonia	X	X
ASZ Aalst	Hospital	East-Flanders	X	
Medisch labo Bruyland	Private	West-Flanders	X	x
ZOL Genk	Hospital	Limburg	X	
LMO-LMC Sint-Truiden	Private	Limburg	X	
AZ Turnhout	Hospital	Antwerp	X	X
IFAC Vivalia	Hospital	Wallonia	X	
LBS	Private	Brussels	X	
LHUB-ULB	Hospital	Brussels	X	
CRI	Private	East-Flanders	X	
Eupen	Hospital	German-speaking part	X	

This baseline surveillance aims to guide public health policies and diagnostic strategies. It is not dedicated to ensuring individual diagnostic needs and should continuously work on avoiding structural bias that van be caused by over-sampling of specific geographical regions, categories of patients or particular atypical PCR results.

Active surveillance aims to promptly identify the introduction of emergence of (possible) variants of concern (VOCs). This surveillance should not systematically be based on whole genome sequencing testing. Currently, active surveillance in Belgium focuses on:

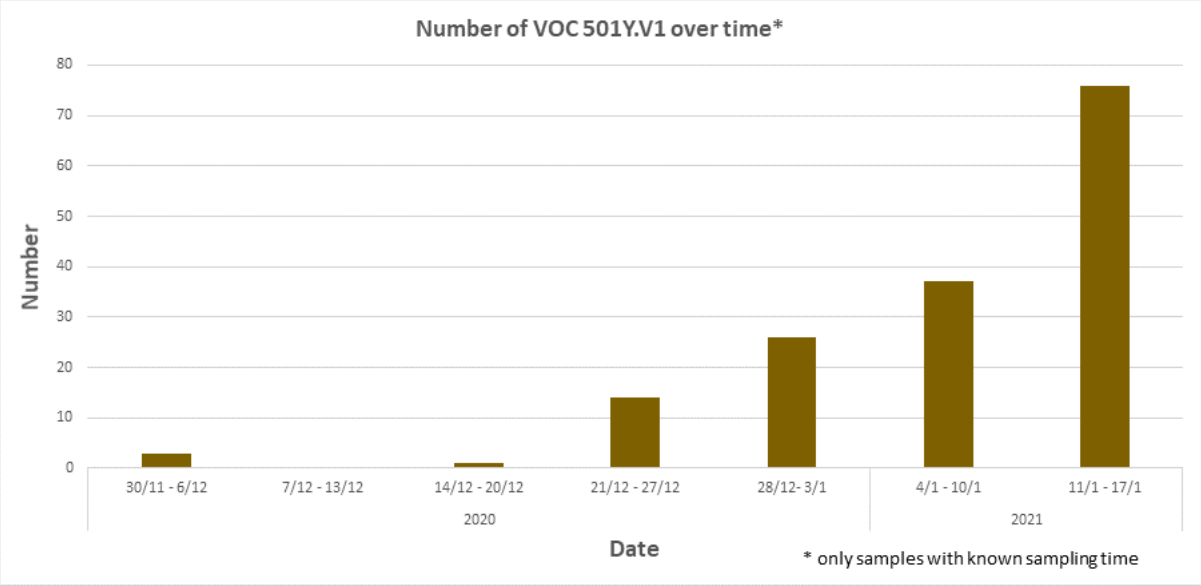
- Systematic screening of travellers returning from areas with (unknown) circulation of VOCs
- Systematic screening of patients experiencing re-infection or infection after vaccination
- Systematic screening of patients with a higher risk of chronic infection and mutant selection (e.g. immunocompromised, antiviral therapy)
- Screening of (atypical) outbreaks
- Screening of atypical PCR or antigen results (including "S dropouts", awaiting for the implementation of a more scalable approach: see point 5)

3. Monitoring of variants of concern (VOCs)

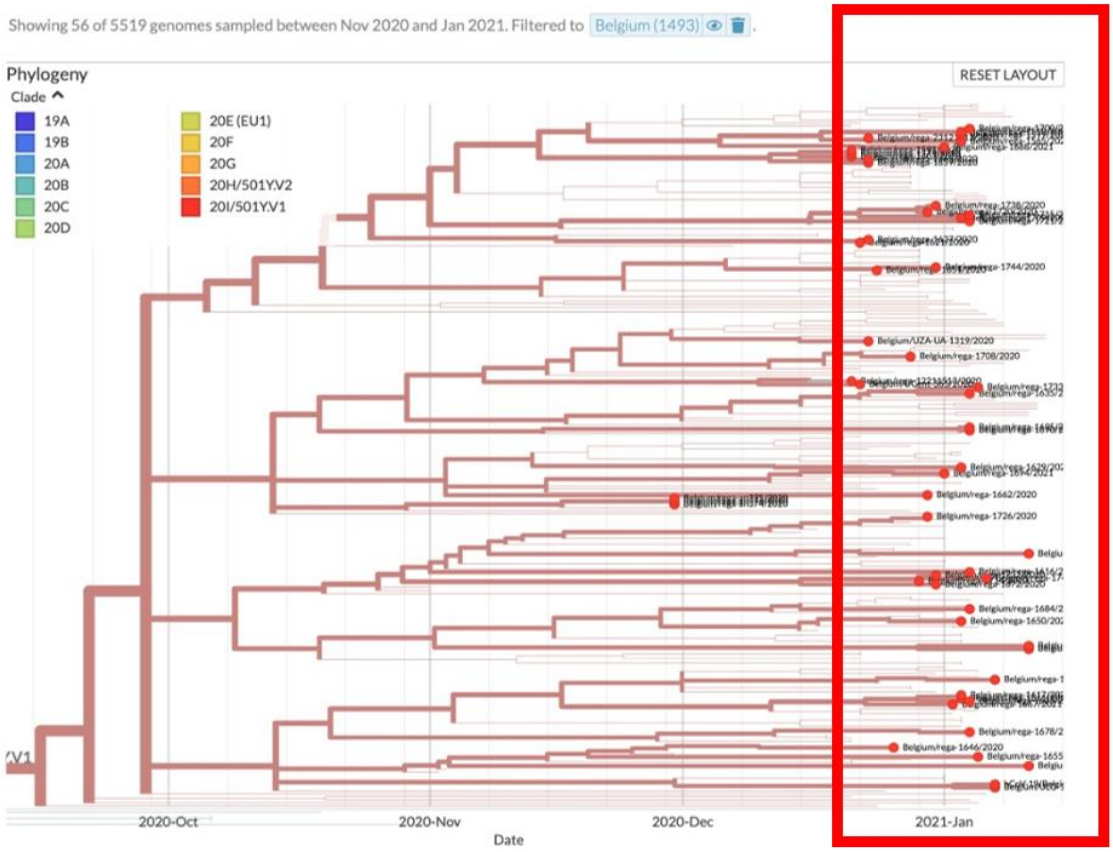
Since the 1st of December, 1360 sequences have been produced by the participating sequencing laboratories.

1 dec - 19 jan	KUL	Gent	Liège	Antwerper	Consortiur
Sequenced	738	277	304	41	1360

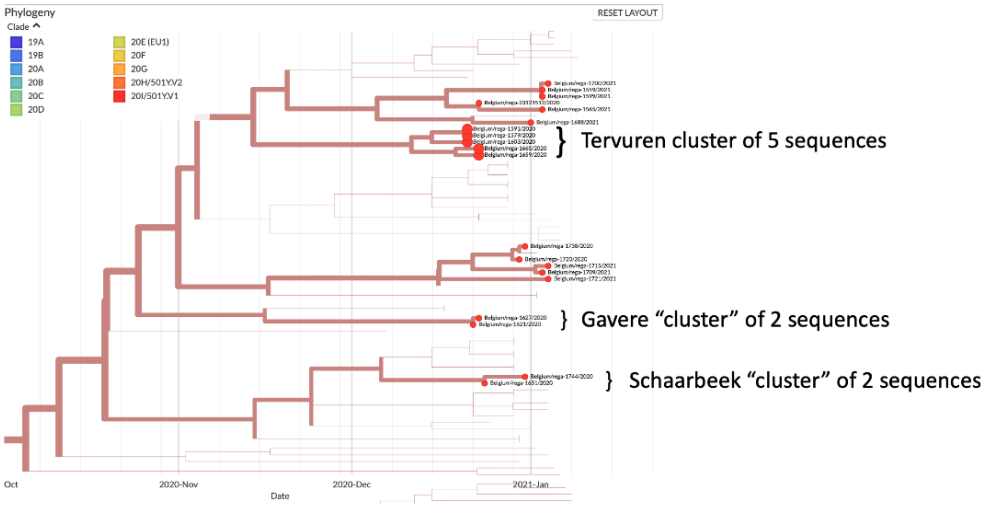
The graph below highlights the increasing number of 501Y.V1 VOCs detected by sequencing per week (based on sampling date) since the first VOC was detected in Belgium.

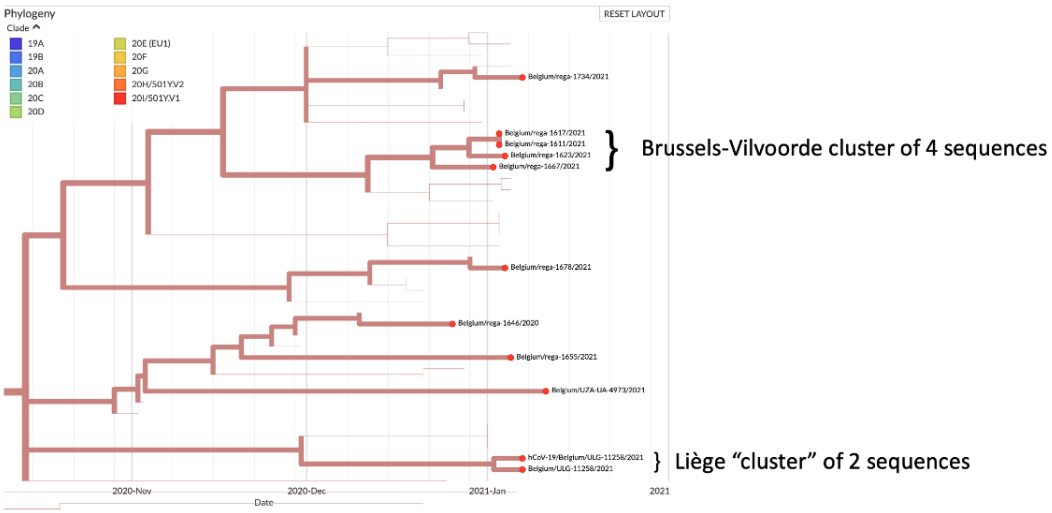


To date, 80 out of 178 501Y.V1 and 6 out of 8 501Y.V2 sequences from Belgium are available for phylogenetic analysis. This analysis shows that apart from an early and isolated cluster of 501Y.V1, all VOCs in the country have been identified from positive samples of patients diagnosed around the Christmas holydays. These data further illustrate that travels have played a determinant role in generating multiple (a minimum of 27 estimated to date) independent introductions of VOCs.



As travels are now self-limiting due to the end of the holiday period and as a result of travel-discouraging communications, we expect that in the coming days the majority of the VOCs identified will be secondary cases and local transmission. Such local transmission events already appear in the analysis below.





It is important to note that most of the 501Y.V1 (UK) variants have currently been identified from patients which are resident from Flanders and Brussels, and much less from Wallonia (see figure below). While this may be due to more limited travels or to travels with less circulating VOCs, the most probable reason for this difference remains under-sampling, as less samples from Wallonia are currently being referred for whole genome sequencing.

The National Reference Laboratory has taken a number of actions to provide a structural solution for this problem:

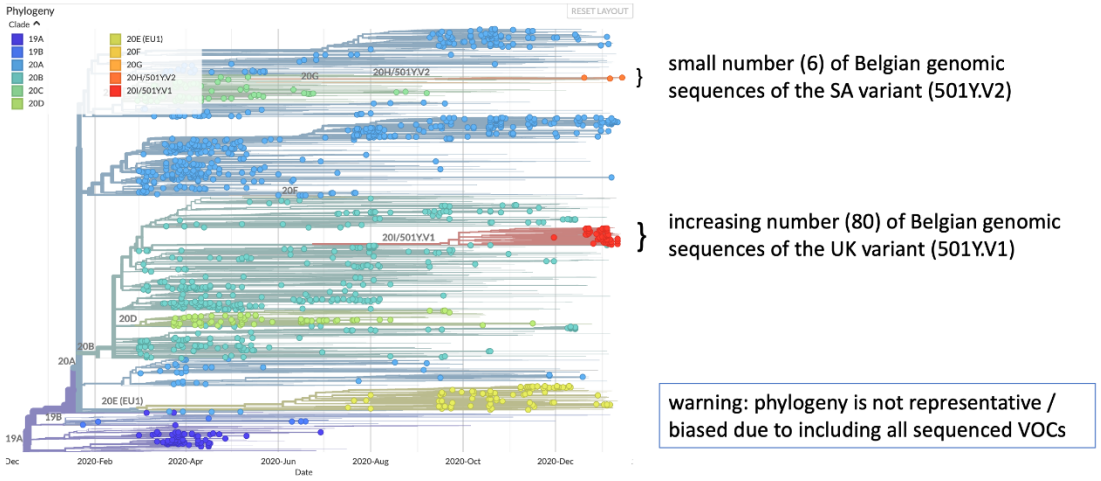
- Active contact with large laboratories in Wallonia to solve practical issues
- A seminar for all clinical laboratories will be given on 28/1/2021 to provide information on genomic surveillance and the diagnosis of VOCs
- Training of staff from the Namur and Mons labs has started at the NRC. These laboratories should therefore be able to start sequencing as soon as they receive their sequencing material on-site.
- In the meantime, other sequencing platforms (in particular KUL and Liège) remain available to provide this service. But samples need to arrive to these platforms.

The 501Y.V1 (UK) is currently the most-detected variant in Belgium. Nevertheless, the 501Y.V2 (South Africa) variant has already been introduced in several independent occasions. Of note, it is currently more challenging to promptly identify 501Y.V2 and 501Y.V3 variants, as these do not generate an “S dropout”, and detection currently relies therefore principally on baseline surveillance and active surveillance of returning travellers.

4. Relative importance of VOCs compared to other circulating strains in Belgium

Two indicators are followed over the time to evaluate the relative importance of VOCs compared to other circulating strains in Belgium.

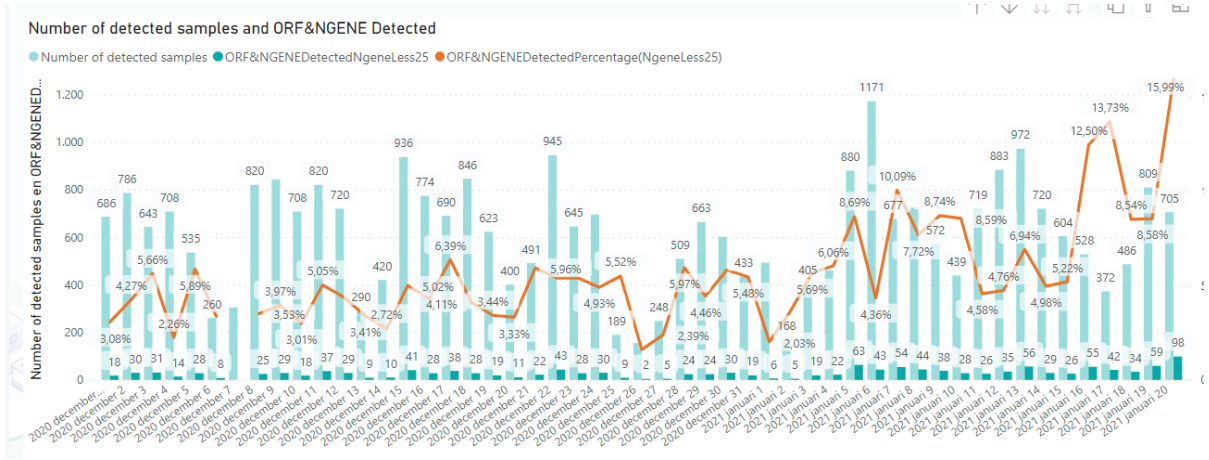
The first indicator is the relative proportion of VOCs among sequences from recent strains. This approach has a limitation as there is currently an important selection bias among the available sequences as patients at high risk for VOCs (principally returning travellers and S dropouts) are prioritized for sequencing. The figure hereunder highlights the recent appearance of VOCs in Belgium, but until we can compensate this over-representation with a corrective baseline sampling, this analysis leads to an over-representation of VOCs compared to other circulating strains.



The second indicator currently followed is the relative proportion of positive PCR tests presenting an "S dropout" in the laboratories of the federal platform (8 laboratories distributed in 7/10 provinces of the country). Although also imperfect, this indicator allows to monitor a trend on a daily basis.

The H69- deletion in the S gene, which generates the "S dropout" profile in the PCR used by the Belgian Federal Platform Bis laboratories, is not only present in the 501Y.V1, but also in non-VOC strains circulating in Belgium since several months. This is the reason why this signal cannot be considered as specific for VOCs, nor highly sensitive, considering that 501Y.V2 and 501Y.V3 do not present this deletion. Of a total of 106 Belgian sequences with the H69- deletion available, 80 (75%) are the 501Y.V1 variant. This proportion is increasing over time as a consequence of the higher transmissibility of the 501Y.V1 variant. Of note, the H69- deletion may, beyond its consequence on the performance of a particular PCR assay used, also have a biological impact as some studies suggest a possible role in immune-escape strategies. These "S dropout but non-VOC" strains have been associated with local outbreaks (Example: in Bilzen in November 2020).

When looking at the proportion of S-gene dropouts (Orf & N genes detected, restricting to results where both genes show a strong signal) against all positive results in the National Platform laboratories, we observe a significant and continuous increase which has started around the 1st January 2021, and thus compatible with the higher number of importations and secondary infections related to travels during the Christmas holydays. Since the 1st January 2021, 801 positive PCRs with an S dropout have been detected by the federal platform laboratories. On January 20, 98 S dropouts were detected out of 705 positive samples (16%).



5. Diagnosis of VOCs in clinical laboratories

The potential public health impact and the rapid increase in VOCs in Belgium require that all positive samples are to be screened for at least the 501Y mutation, and preferably also the 484K mutation of the S gene. INAMI-RIZIV is currently working to integrate such confirmation test in the nomenclature which will allow all clinical laboratories to perform a confirmatory test. This financial support should last as long as the VOCs do not represent >75% of the circulating strains and that the information is required to guide public health policies and interventions. The information of VOC should be transmitted to Sciensano.

Currently, there are different technical options to perform this confirmation:

- Whole Genome Sequencing
- Sequencing of the Receptor binding domain of the S gene (RBD) (KUL protocol[1])
- Targeted commercial PCRs (ex: MolBio : only detects the 501Y mutation, and is therefore suitable for confirmation of a UK variant following an “S dropout” ; other commercial tests).

Performing Whole Genome Sequencing on all samples may be the choice of some clinical laboratories. It is not considered as a requirement, and every laboratory should be given the liberty to choose one of the above techniques based on technical, medical and financial considerations. In any case, these efforts should be considered and analysed in the future apart from the baseline surveillance.

[1] KUL validated SARS-CoV-2 S/RBD gene PCR (387bp/129aa fragment) followed by Sanger sequencing of the amplicon:

F primer 22930-22951
5’ -TAGGAAGTCTAATCTCAAACC-3’

R primer 23297-23317
5’-AGAATCTCAAGTGTCTGTGG-3’