Genomic surveillance of SARS-CoV-2 in Belgium

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

Situation update - 26 of January 2021
(4th report for 2021)

Executive summary

Genomic surveillance in Belgium is based on whole genome sequencing (WGS) of a selection of representative samples, complemented with targeted active surveillance initiatives aiming to early detect and precisely monitor the presence of variants of concern (VOCs). Currently, 3.368 sequences of samples collected in Belgium since the start of the epidemic are available on GISAID in open access.

Since the 1st of December 2020, a total of 1.784 sequences have been produced by the sequencing platforms participating to the federal genomic surveillance initiative. 408 501Y.V1 and 54 501Y.V2 VOCs have been identified (increasing trend; numbers are an under-estimation of the current situation). As the 87% of "S dropout" PCR results are now confirmed by sequencing, it is not anymore mandatory that these strains are systematically sequenced to confirm the presence of 501Y.V1. The proportion of presumptive 501Y.V1 is now estimated between 15% and 25% of all positive strains.

Belgium has recently experienced multiple introductions of variants of 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, including in schools and nursery homes. Based on the observed trends, it is currently estimated that VOCs will nearly entirely replace current circulating SARS-CoV-2 strains by early March 2021 (rapid replacement phenomenon).

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.

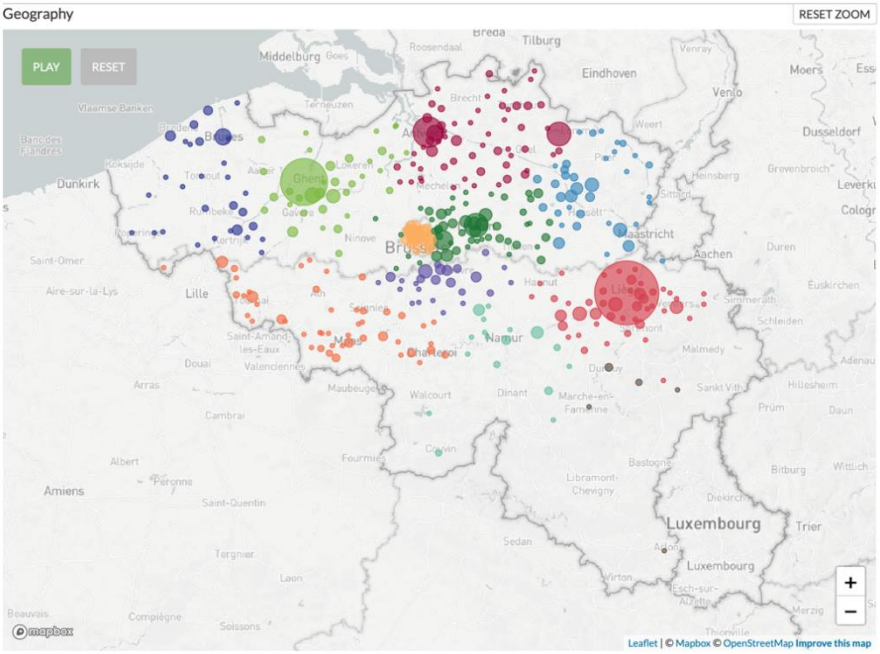
2. Belgian genomic surveillance

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 the federal government has recently supported a scale-up of this network, built upon the federal platform laboratories. During the last week, a few clinical laboratories have independently started submitting a limited number of sequences on GISAID.

A particular attention is currently given to strengthen the baseline surveillance - non biased - sampling and to adequately describe the context when submitting sequences on GISAID. In particular:

- Returning travellers should in the future be screened using alternative methods allowing to detect VOCs. Alternatively, WGS sequences should be reported on GISAID as “active surveillance”
- “S gene dropouts” should in the future be screened using alternative methods allowing to detect VOCs. Alternatively, WGS sequences should be reported on GISAID as “active surveillance”
- Laboratories routinely performing WGS outside the genomic surveillance initiative (ex: clinical labs performing WGS for all or a part of their strains) should report these sequences as “diagnostic samples”

To date, 3.368 sequences originating from Belgian laboratories were uploaded on GISAID and are available in open access. The map hereunder represents the current availability of sequences per province in Belgium.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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, 21 out of 24 pre-selected labs were contacted by the National Reference lab and agreed to refer 5% of their positive samples for the baseline surveillance system. The selection of participating labs 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, with the possibility to increase this coverage if asked or required for public health reasons.

Lab	Type of lab	Region/province	Contacted	Positive answer	Sequencing facility
UZA	Federal platform (including clinical lab)	Antwerp	X	X	Antwerp
UZ Gent	Federal platform (including clinical lab)	East-Flanders	X	X	Ghent
UZ Leuven	Federal platform (including clinical lab)	Flemish Brabant	X	X	Leuven
Liège	Federal platform (including clinical lab)	Wallonia	X	X	Liège
Mons	Federal platform (including clinical lab)	Wallonia	X	X	Ghent => Mons
Namur	Federal platform (including clinical lab)	Wallonia	X	X	Liège => Namur
UCL	Federal platform (including clinical lab)	Brussels	X	X	Leuven => UCL
ULB	Federal platform (including clinical lab)	Brussels	X	X	Leuven => ULB
Medina	Private	West-Flanders	X	X	Ghent
Synlab	Private	Wallonia	X	X	Liège
CMA	Private	Antwerp	X	X	Leuven
AML	Private	Antwerp	X	X	Antwerp
AZ Delta Roeselare	Hospital	West-Flanders	X	Under discussion	Wish to sequence on site
Labo Luc Olivier	Private	Wallonia	X	X	Liège
ASZ Aalst	Hospital	East-Flanders	X	X	Ghent
Medisch labo Bruyland	Private	West-Flanders	X	X	Ghent
ZOL Genk	Hospital	Limburg	X	X	Leuven
LMO-LMC Sint-Truiden	Private	Limburg	X	X	Leuven
AZ Turnhout	Hospital	Antwerp	X	X	Leuven
IFAC Vivalia	Hospital	Wallonia	X	X	Liège
LBS	Private	Brussels	X		
LHUB-ULB	Hospital	Brussels	X	X	Antwerp
CRI	Private	East-Flanders	X		
Eupen	Hospital	German-speaking part	X	X	Liège

Active surveillance aims to promptly identify the introduction of emergence of (possible) variants of concern (VOCs). This surveillance is available for all clinical laboratories and does not systematically require WGS testing. Currently, active surveillance in Belgium focuses on:

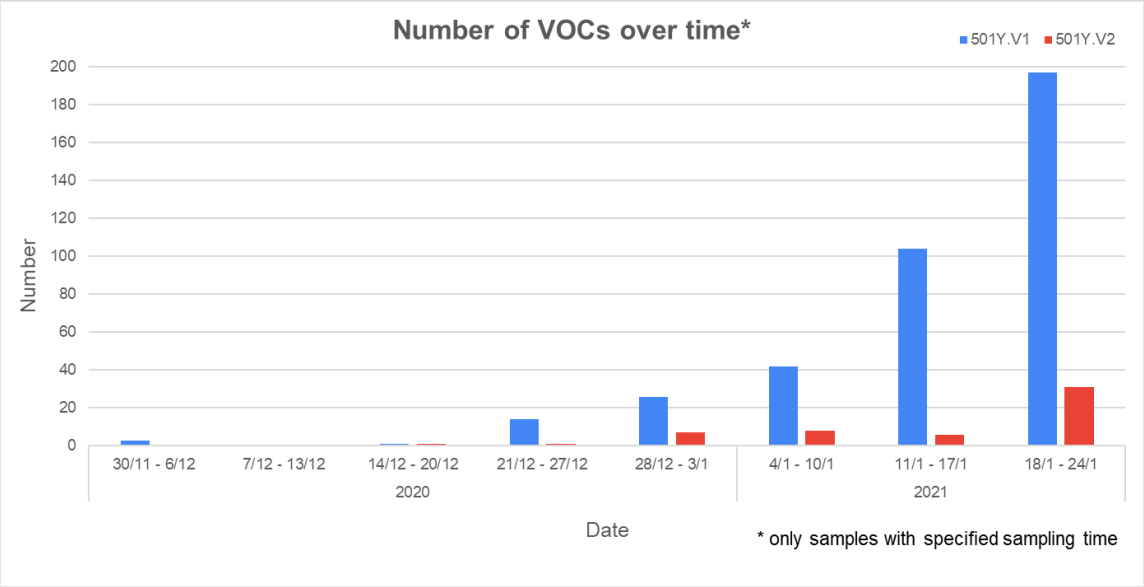
- Systematic screening of VOCs among returning travellers
- Systematic screening of VOCs among atypical PCR or antigen diagnostic test results (including "S dropouts")
- Genetic characterization of a subset of strains in the situation of outbreaks
- Genetic characterization among patients experiencing re-infection or infection after vaccination
- Genetic characterization among patients presenting a higher risk of chronic infection and mutant selection (e.g. immunocompromised, antiviral therapy)

3. Monitoring of variants of concern (VOCs)

Since the 1st of December, 1.784 sequences have been produced by the participating sequencing laboratories. During the week of 18/1/2021, 7 out of 39 WGS analysis performed in the context of baseline surveillance were confirmed 501Y.V1 (20%).

No samples from returning travellers with a positive PCR result were referred for sequencing since 18/1/2021, compared to 102 samples referred during the 4 preceding weeks. In total, 51/102 (50%) of samples referred as "returning travellers with a positive PCR" were VOCs.

The graph below highlights the increasing number of VOCs detected by WGS per week (based on sampling date) since the first VOC was detected in Belgium. Of note, due to the important number of samples, all platform bis laboratories do not systematically confirm anymore by WGS the 501Y.V1 VOC when del69 and 501Y are observed, nor confirm 501Y.V2 when 501Y is present. The figure below therefore underestimates the actual detection of VOCs.



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

Across the 8 laboratories composing the federal testing platform (over 450.000 PCR performed since 1/12/2020), the proportion of "S dropouts" among positive SARS-CoV-2 PCR results has increased over the last 4 weeks, and is between 15% and 25% (last 6 days).

In parallel, the proportion of WGS-confirmed 501Y.V1 among "S dropouts" has increased over the last 4 weeks, and was 87% during the last 2 weeks (259 confirmed among 297 sequenced for "S dropout"). Therefore, although non-501Y.V1 strains harbouring del69 (causing the S dropout) still circulate in Belgium, the amount and the evolution of "S dropout" PCR results can currently be considered as a very good marker of the evolution of the 501Y.V1 in Belgium.

We received the distribution by age and week for "S dropouts" and "non S dropouts" positive PCR results from 3 out of 8 platform bis laboratories. This data supports the introduction of 501Y.V1 in the first week of the year (returning travellers), mainly detected in the adult population. More recently, a higher number of these strains have been detected in younger and older groups, possibly resulting of a combination of factors: higher transmissibility including in nursing homes and the educational sector, and an intensified testing strategy offered in these groups.

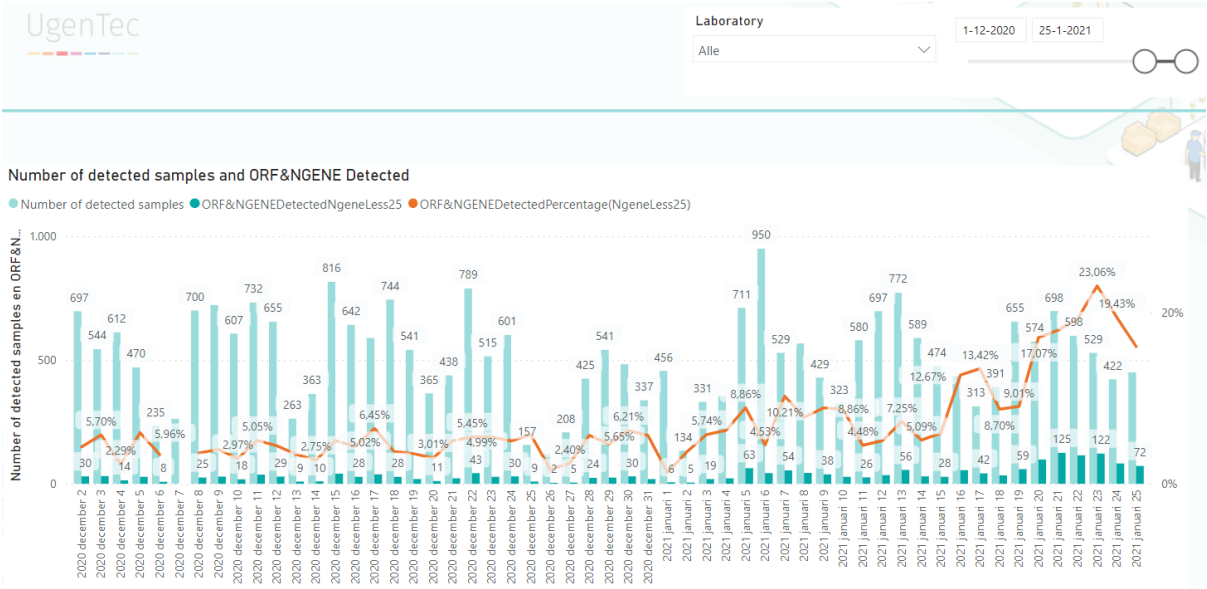
Week	Namur	# S-gene dropouts						# non S-gene dropouts				
		0 – 19 years	20 - 39 years	40 - 59 years	60 – 79 years	80 – 99 years		0 – 19 years	20 - 39 years	40 - 59 years	60 – 79 years	80 – 99 years
28-12 – 3/1		0	1	0	0	0		29	74	49	22	1
4/1 – 10/1		1	6	5	1	4		47	167	105	42	6
11/1 – 17/1		0	3	0	4	8		55	97	87	35	1
18/1 – 24/1		3	4	4	4	15		48	69	91	45	8

Week	Leuven	# S-gene dropouts						# non S-gene dropouts				
		0 – 19 years	20 - 39 years	40 - 59 years	60 – 79 years	80 – 99 years		0 – 19 years	20 - 39 years	40 - 59 years	60 – 79 years	80 – 99 years
28-12 – 3/1		1	2	2	1	0		31	60	48	29	9
4/1 – 10/1		3	1	0	1	0		40	77	60	30	20
11/1 – 17/1		1	10	3	5	2		53	98	62	26	18
18/1 – 24/1		28	9	11	7	7		35	92	56	23	40

Week	Liège	# S-gene dropouts						# non S-gene dropouts				
		0 – 19 years	20 - 39 years	40 - 59 years	60 – 79 years	80 – 99 years		0 – 19 years	20 - 39 years	40 - 59 years	60 – 79 years	80 – 99 years
28-12 – 3/1		0	0	0	0	0		2	5	9	6	3
4/1 – 10/1		0	0	0	0	0		9	28	40	26	47
11/1 – 17/1		0	3	0	0	0		11	20	38	15	28
18/1 – 24/1		2	2	1	0	0		12	12	7	12	24

Week	Total	# S-gene dropouts						# non S-gene dropouts				
		0 – 19 years	20 - 39 years	40 - 59 years	60 – 79 years	80 – 99 years		0 – 19 years	20 - 39 years	40 - 59 years	60 – 79 years	80 – 99 years
28-12 – 3/1		1	3	2	1	0		62	139	106	57	13
4/1 – 10/1		4	7	5	2	4		96	272	205	98	73
11/1 – 17/1		1	16	3	9	10		119	215	187	76	47
18/1 – 24/1		33	15	16	11	22		95	173	154	80	72

For further analysis and projections in the coming weeks, please refer to the **Annex**.



5. Strengthened surveillance of VOCs

Ongoing discussions with INAMI-RIZIV aim to roll-out a strengthened surveillance strategy allowing to better monitor the emergence and spread of the existing (and upcoming) VOCs.

This surveillance system would be built on top of the current genomic surveillance (aim: minimum 2% of unselected positive samples sequenced). This system could include:

- Systematic screening of VOCs among returning travellers (reflex test following a positive SARS-CoV-2 PCR results)
- Systematic or increased screening of 501Y.V2, 501Y.V3 and further emerging VOCs among “non-S dropout” positive SARS-CoV-2 PCR results). This could be done using a rapid PCR-approach for which the cost could be limited (no extra cost for transport, sample reception and RNA extraction)
- Under the condition that the baseline surveillance monitors the association between del69 and 501Y.V1, further confirmation on “S dropout” samples may not be required in the future as “S dropouts” are now very specific for 501Y.V1 variants.

6. **Preliminary results of the transmissibility of novel SARS-CoV-2 Variant of Concern 202012/01 in Belgium** (Tom Wenseleers, Niel Hens, Emmanuel André)

a. Data sources

- sequencing data of S-dropout samples (weeks 49-53 2020, weeks 1-2 2021)
- counts of tests showing S dropout as a share of all positive tests (1-22 Jan 2021)
- to avoid bias in our results, data from UZ Ghent & UZA were excluded, as these labs reported to be heavily involved in pro-active screening of UK variant infection clusters, and data from ULG - FF 3.x were excluded due to low sample size
- COG-UK sequence data (version of 22nd of December, aggregated by NHS region)

b. Methods

The increase over time in share of S-dropout samples that are actually 501Y.V1 are estimated from sequencing data of S-dropout samples using a binomial generalized linear mixed model (GLMM) with sample date included as a covariate and an observation-level random effect included to take into account overdispersion.

The estimated growth rate advantage of the 501Y.V1 variant (i.e. the difference in Malthusian growth rate per day of 501Y.V1 minus that of the wild type variants) is estimated from the S gene dropout data using a binomial GLMM of the proportion of cases that are consistent with being 501Y.V1. This model uses the counts of S dropout samples, multiplied by the estimated probability of being 501Y.V1 (as estimated by a separate binomial GLMM in function of sampling date), as a proportion of the count of all positive tests on a given day. Sample date and laboratory were included as fixed effects and an observation-level random effect was included to take into account overdispersion. A model with or without an interaction effect between laboratory and sample date were both fitted to test if the rate at which 501Y.V1 displaces other strains occurs at the same rate throughout Belgium or not. The growth rate advantage is given by the slope in function of time in this binomial GLMM (Davies et al. [1]).

The estimate transmission advantage (increase in infectiousness in terms of multiplicative effect on the effective reproduction number Rt), assuming an identical generation time, can be shown to be equal to exp(r.T) [1], where T is the mean generation interval (here taken to be 4.7 days, Nishiura et al. 2020 [2]).

c. Preliminary results

<u>Increase over time in share of S-dropout samples that are 501Y.V1</u>

Sequencing results of S-dropout samples show that the share of S-dropout samples that are actually the 501Y.V1 UK SARS-CoV2 variant has been rapidly increasing (Figure 1), with the percentage that is 501Y.V1 among newly diagnosed S dropout samples as of today (26/1/2021) being estimated at 97% [89-99%] 95% CLs, or among new infections (curve shifted ca. 7 days to the left) at 99% [93.7-99.8%] 95% CLs. S-dropout in Belgium can therefore now be used as a reliable proxy for a sample being the 501Y.V1 variant.

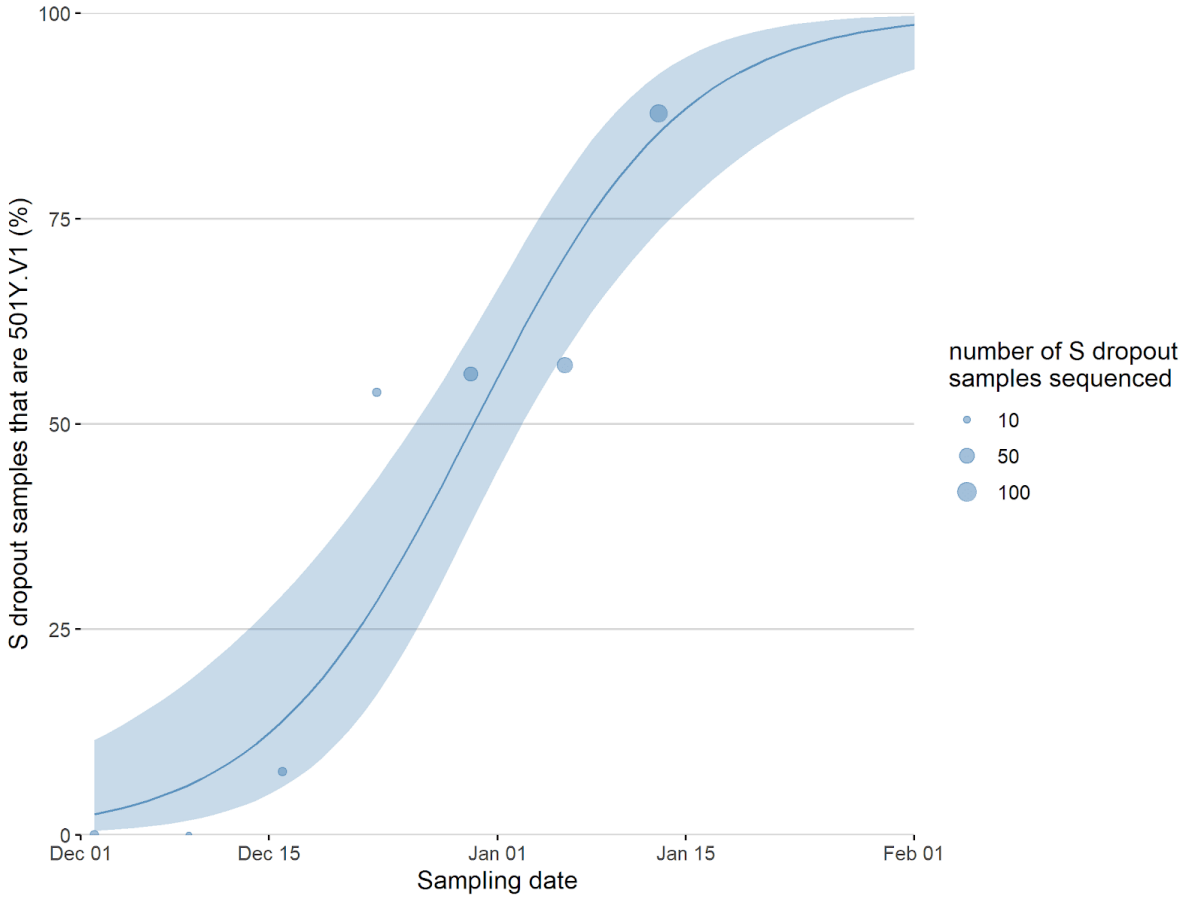


Figure 1. The increase in the proportion of S dropout samples that are actually the 501Y.V1 variant (binomial GLMM with 95% confidence intervals).

Growth rate advantage and increased infectiousness of variant 501Y.V1

A common-slope binomial GLMM fitted the available data best based on the Bayesian Information Criterion (BIC). In addition, in a model with separate-slopes per laboratory (region), there were no significant differences in the inferred slopes of the binomial GLMM in function of time across the different laboratories (Tukey posthoc tests for differences in slopes, calculated using R's emtrends function in the emmeans package, all $p > 0.05$). Hence, we can conclude that the variant 501Y.V1 is displacing other strains at approximately the same rate across the whole of Belgium. The common-slope binomial GLMM had a marginal slope of 0.12 [0.09-0.16] 95% CLs (observation-level random effect variance: 0.45), which implies that the 501Y.V1 variant has a 12% [9-16%] higher growth rate than the previous SARS-CoV2 wild types. This estimate is compatible with other international data, which demonstrate a growth rate advantage of the 501Y.V1 variant of 11% [10-12%] in the UK (Davies et al. Table S1, [1], range 9-15% across different NHS regions), 8% [7-10%] in Denmark (Davies et al. Table S1, [1]), 8% [7-10%] in Portugal (Borges et al., [3]) and 8% [7.5-9.5%] in the US (T. Bedford, pers. comm.).

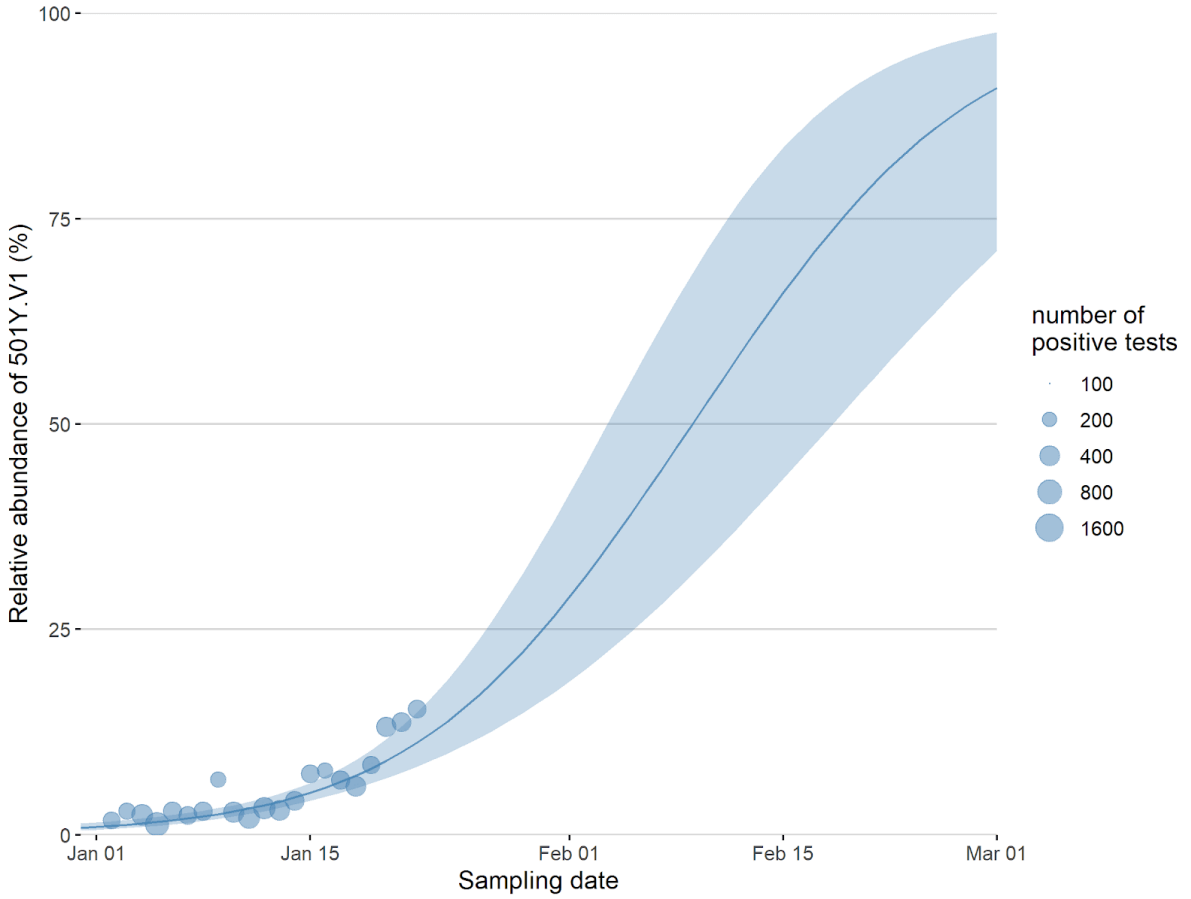


Figure 2. Estimated increase in the relative abundance of the 501Y.V1 variant in Belgium based on S dropout data (mean and 95% confidence intervals, binomial GLMM with random intercept for laboratory and an observation-level random effect to take into account overdispersion, with correction for the expected proportion of true positives). An extrapolation up to the first of March is shown.

If we assume that the 501Y.V1 variant has the same generation as the SARS-CoV2 wild type (which epidemiological models have shown is likely, Davies et al. [1]), and assuming a generation interval of T=4.7 days (following Nishiura et al. 2020 [2]), the estimated growth rate advantage r for Belgium would be expected to have a multiplicative effect on the effective reproduction number Rt of exp(r.T)=1.79 [1.55-2.08] 95% CLs, implying an increased infectiousness of 79% [55-108%] 95% CLs.

Given that the estimated growth trajectory of the 501Y.V1 variant still has relatively broad confidence intervals, we also carried out a combined analysis of the Belgian S-dropout data and the COG-UK sequencing data (data August 1 2020 - December 17 2020), to be able to further narrow down the predictions. In these analyses, we fitted two binomial GLMMs in which we either allowed separate slopes per country or not, using common slopes per region within each country (NHS region for UK or hospitals for Belgium). As before, we also included an observation-level random effect to take into account overdispersion. These analyses show that a model with a constant slope per country and region was most parsimonious, having the lowest BIC value. With such a model, we estimated a growth advantage of the 501Y.V1 variant across the UK and Belgium of 10.6% per day [10-11%] (observation-level random effect variance: 0.39), which with a generation time of 4.7 days would translate to an increased infectiousness of 65% [60-70%]. In the model with separate slopes per country, the growth advantage of the 501Y.V1 variant was 12.5% [9.5-15.5%] for Belgium and

10.6% [9.9-11.2%] for the UK (observation-level random effect variance: 0.38). The differences in slope across both countries, however, were not significant (z ratio=1.23, p = 0.22), implying that we cannot reject the hypothesis that the 501Y.V1 variant is displacing other variants at the same rate in Belgium as in the UK. Model predictions for this model with separate slopes by country are shown in Figure 3.

d. Conclusion

We can conclude that although data on the relative rate of spread of the 501Y.V1 variant is still somewhat limited for Belgium, it is already plain clear that the variant will become the dominant strain in a very short timespan, being projected to reach 90% of all newly diagnosed infections before the end of February. The growth advantage relative to other strains is on the order of 11% per day, or even slightly higher, which would translate to an increased infectiousness of ca. 65%. This increase in infectiousness is entirely in line with estimates that can be made for other countries based on the observed growth advantage there (typically 8-11% per day, cf. above). These figures are worrisome, as they would imply that under the current measures, which causes the Rt value in Belgium to be around 1, the Rt value would likely increase to a value of ca. 1.65 by the time that 501Y.V1 will become the dominant variant (i.e. before the end of February).

We should note that earlier preprints in which the increased infectiousness of 501Y.V1 were estimated do not always use correct procedures and often use differing generation times, which is a major cause of the differences in the estimates obtained [4]. For example, Volz et al. [5] calculated an additive change in the Rt value based on the product of the difference in growth rate and generation time, while the actual relationship is multiplicative [1]. Likewise, Walker et al. [6] analysed ONS S gene dropout data from the UK, but forgot to filter out samples with single-gene amplifications (indicative of random gene dropout due to very low virus titers, e.g. linked to old infections), which resulted in a large underestimation of the incidence (ca. 60% prevalence across England among new infections now vs. >90% shown by the Pillar 2 S gene target failure data) as well as the contagiousness of the 501Y.V1 variant (K. Pouwels, pers. comm.). This is currently being corrected. As shown above, if the same procedure is used to estimate the growth and transmission advantage of the 501Y.V1 variant then highly concordant estimates are obtained across different countries and regions. We therefore believe these conclusions to be reliable and robust.

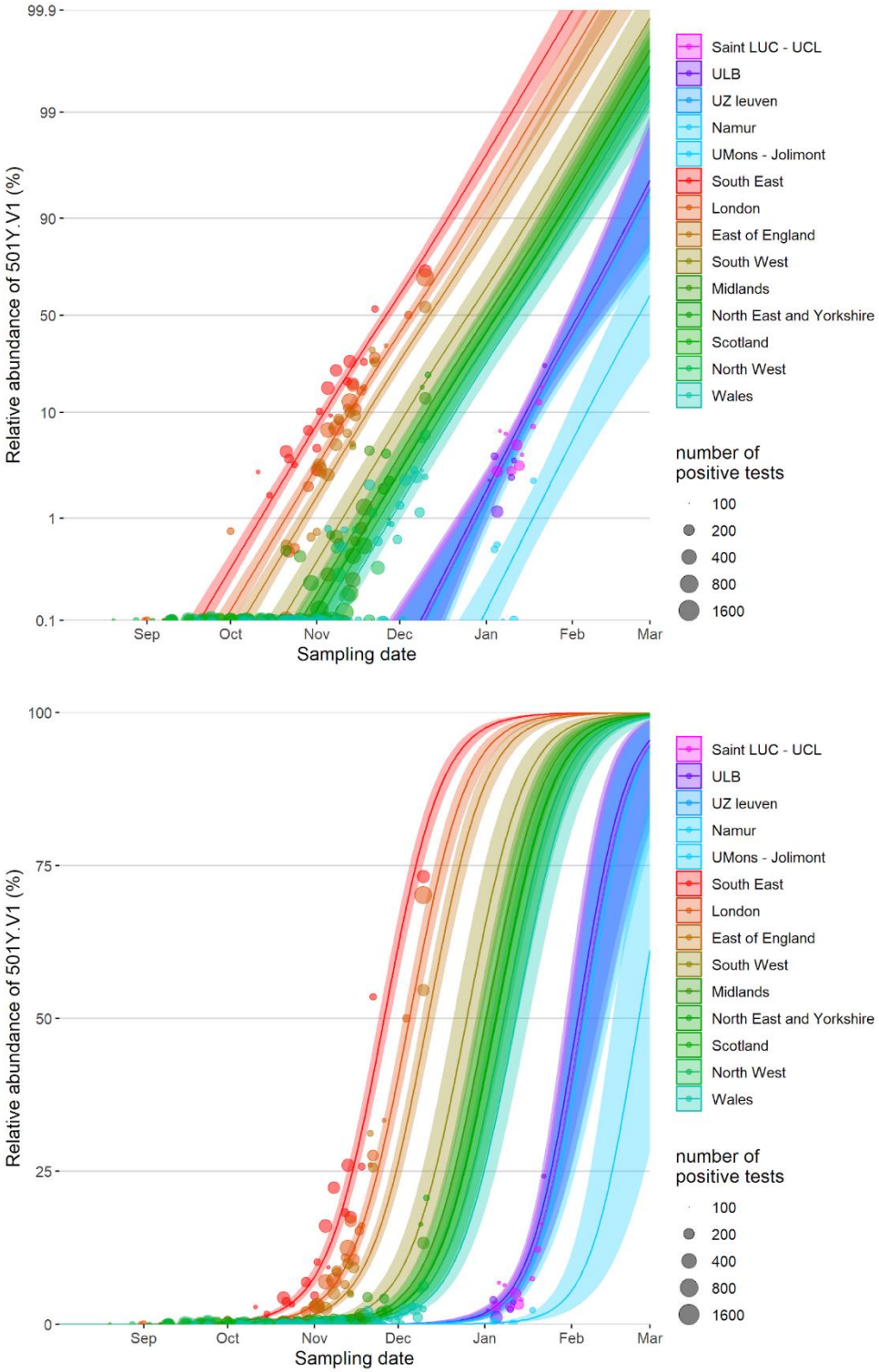


Figure 3. Estimated increase (plus 95% confidence intervals) in the relative abundance of the 501Y.V1 variant in the UK and Belgium, based on a joint analysis of S dropout data for Belgium and COG-UK sequencing data for the UK (binomial GLMM with separate slopes per country, but identical slopes per region within each country). The plots are shown either on a logit link (top) or a backtransformed response scale (bottom). The introduction of the 501Y.V1 variant clearly occurred with a delay compared to the spread in the UK, and also happened somewhat later in Mons.

e. Outlook

To further minimise bias related to the selection of samples tested and sequenced, more data on the reason for testing and whether data pertain to specific outbreaks are needed. Further in depth analyses are only possible with good quality data.

f. References

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