

AANBEVELINGEN VOOR DE SELECTIE VAN STALEN VOOR DE SEQUENTIEBEPALING VAN HET VOLLEDIGE GENOOM

RAG subgroep testing – 25 Januari 2021

Opmerking: De huidige aanbevelingen zijn onderhevig aan veranderingen afhankelijk van nieuwe wetenschappelijke gegevens en/of de evolutie van de epidemie.

Voornaamste aanbevelingen:

De strategie bestaat uit drie componenten:

1. Basislijn genoomsurveillance
 - Een aselechte steekproef van 2% van alle stalen die met een RT-PCR positief testen
 - Nooit meer dan 1000/week (is momenteel ongeveer 280/week)
2. Alle positieve stalen van de volgende populaties:
 - Gevaccineerde personen die positief testen minstens twee weken na de vaccinatie
 - Deelnemers aan klinische studies
 - Langdurige chronische infecties
 - Patiënten met immuundeficiëntie
 - Re-infecties (indien het staal van de eerste infectie beschikbaar is)
 - Atypische RT-PCR resultaten, inclusief maar niet beperkt tot S-gen drop out
3. Een selectie van:
 - 20% van positieve stalen in uitbraken in woon-zorg centra die door het outbreak support team als ongewoon beschouwd worden (ongewoon groot OF aanhoudend OF veel ernstige gevallen OF post-vaccinatie OF index geval met mutatie)
 - 5 stalen in uitbraken in woon-zorg centra die niet ongewoon zijn, maar zich in een regio bevinden waar een mutant variant circuleert
 - 20% van positieve stalen in uitbraken in scholen. Momenteel in alle uitbraken, omdat er weinig zijn. Indien toename, enkel in uitbraken die ongewoon zijn.
 - Positieve stalen van reizigers uit een rode zone. In een eerste fase zoveel mogelijk, later een representatief staal.

In alle indicaties worden stalen met een Ct waarde >25 uitgesloten wegens moeilijk te sequencen.

Positieve snelle Ag worden uitgesloten voor de basis surveillance. In andere indicaties worden personen die positief testten met een snelle Ag test uitgenodigd voor een tweede staalafname voor een RT-PCR.

Preselectie met een specifieke PCR test kan momenteel voor de B.1.1.7 variant (detectie van S-gen drop out) in verschillende, maar niet alle, laboratoria. Voor de B.1.351 en B.1.1.28 varianten is er enkel een test beschikbaar in het UZAntwerpen en UZGent.

Omdat de B.1.1.7 variant momenteel veruit de meest voorkomende is en de sequencing capaciteit overbelast dreigt te geraken, wordt aanbevolen een preselectie te doen in de stalen van uitbraken en reizigers, minstens met een PCR test die de S-gen drop out detecteert, en waar mogelijk met de specifieke PCR test die alle mutaties detecteert.

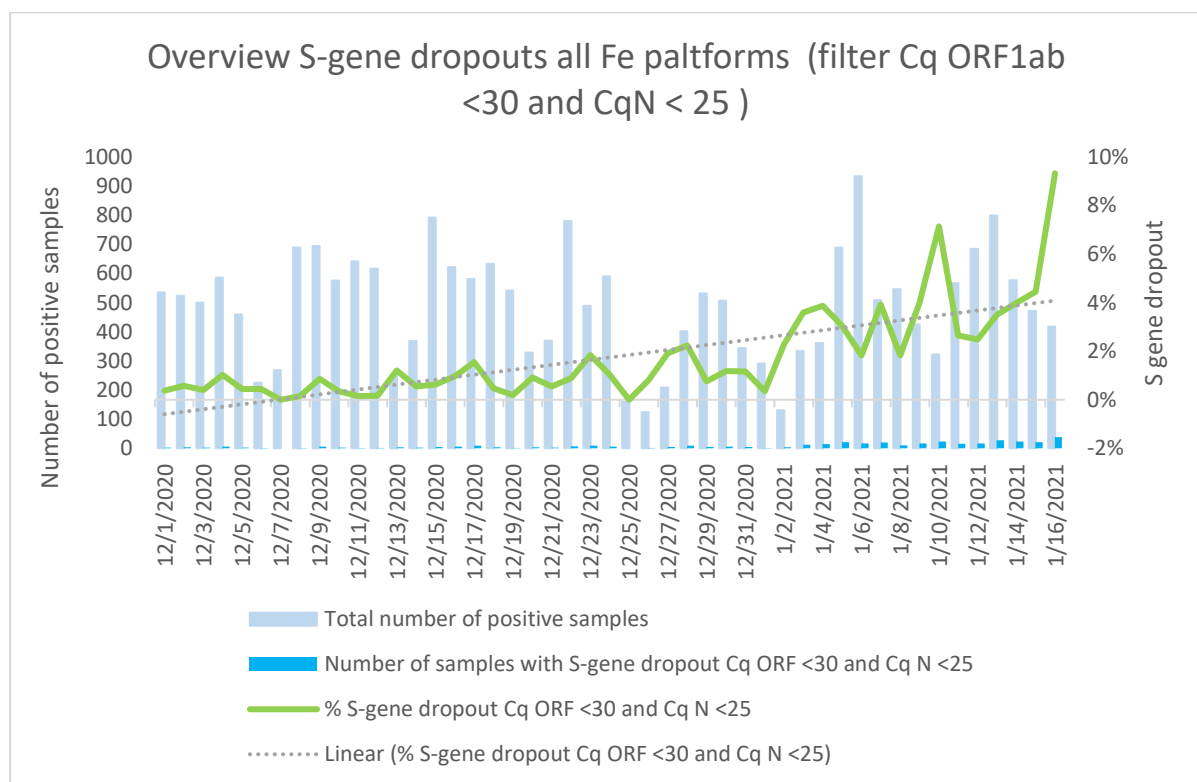
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CONTEXT

In response to the increasing circulation of new SARS-CoV-2 variants, in particular the VOC-202012/01 or 501Y.V1 (B.1.1.7) variant ('UK variant'), but also the 501Y.V2 (B.1.351) variant ('South-African variant') and possibly in the future also the 501Y.V3 (B.1.1.28) variant ('Brazilian variant'), sequencing of samples was reinforced in Belgium.

Information was collected from the laboratories part of the national platform bis on samples with a positive PCR result and presenting with a drop-out of the S-gene, as proxy of a possible B.1.1.7 mutation. This first showed that the proportion of samples with this deletion was increasing over time in all laboratories and a more in-depth analysis of samples presenting with the S-gene drop-out collected by the platform lab UZA/UA since November confirmed that the proportion of samples with S-gene drop-out increased especially after the 21st of December. A more overall in-depth analysis of all platform laboratories shows a sharp increase since the beginning of 2021 (Figure below).



The UZA started a pilot project, with sequencing of all samples received at the Federal Platform Lab UZA/UA after the 2nd of January, presenting with a positive PCR test with S-gene drop out and a high viral load. Extensive contact tracing is carried out around the persons infected with the mutant strain, and the patients will have a second test after 1 week to study the viral load.

A surveillance plan for variant strains is being put in place by the laboratories of the platform-bis, coordinated by the National Reference Center (KULeuven-UZLeuven) and in partnership with Sciensano.

By January 17, a total of 1153 samples have been sequenced, of which 91 where the B.1.1.7 variant and 7 the B.1.351 variant.

INTERNATIONAL RECOMMENDATIONS

Few agencies have developed guidelines for sequencing SARS-CoV-2.

With regard to sample selection, **ECDC** mentions in their technical note of 23 December that *‘the selection will depend on the selected objective and available resources and could include reinfections or vaccine failures. For surveillance purposes, representative strains of virus from different geographic locations and time points, as well as from patients of varied demographics, and across the disease severity spectrum, as well as SARS-CoV-2 variants emerging in animal populations or causing human outbreaks, especially if they are not explained by epidemiological factors, should be selected for sequencing in order to more effectively monitor virus evolution and changes in the virus genome. For resource-limited settings, an event-/risk-based approach may reduce the need and cost for sequencing. If other methods for identifying such variants are available, they should be considered as a complement to sequencing.’*

With regard to preselection by PCR, ECDC recommends in a threat assessment brief of 20 December that multi-target RT-PCR assays that include an S gene target affected by the deletions (and therefore will result in an S gene “drop out”) can be used as a signal for the presence of the 69-70del mutation for further investigation, especially if sequencing capacity is limited.

On January 18, ECDC published an update of the Technical Guidance Sequencing of SARS-CoV-2 in which they state that it is crucial to continue with surveillance of SARS-CoV-2 viruses in community and hospital settings and use random sampling of COVID-19 cases in those settings in order to assess the prevalence of variant viruses in the population and also to detect variant viruses early. In addition to representative sampling of the population, targeted sampling should be applied using the criteria of prioritization established by WHO (see below). In addition to this priority list, virus detections related to travel should be monitored for emergence of variant viruses.

WHO states that samples ideally be sequenced in proportion to true case incidence (Genomic sequencing of SARS-CoV-2 - A guide to implementation for maximum impact on public health - 8 January 2021). It is not possible to give universally appropriate recommendations for SARS-CoV-2 sequencing, as decisions will depend on the outbreak context and questions to be answered.

Where resources to support sequencing are limited, it may be necessary to limit objectives of a sequencing

programme to those activities with high clinical and/or public health potential, and prioritize the sequencing of SARS-CoV-2 to the following indications:

- from individuals vaccinated for SARS-CoV-2 but who later become infected with SARS-CoV-2 despite exhibiting an appropriate immune response to the vaccine;
- in risk settings, such as where there is close human–animal interaction with a large number of animals that are susceptible to SARS-CoV-2 infection, or where there are immunocompromised patients with prolonged shedding, especially when receiving antibody therapy against SARS-CoV-2;
- when there is an unexpected increase or change in SARS-CoV-2 transmissibility and/or virulence;
- when there is suspicion of a change in the performance of diagnostic (antibody, antigen, molecular assays) methods or therapies; and
- during cluster investigations when sequencing can support understanding of transmission events and/or evaluate the efficacy of infection control procedures.

AGREED STRATEGY AND SELECTION CRITERIA

The current foreseen budget allows to sequence up to 1000 samples for surveillance, and an additional 300 specifically for the NRC to perform their reference activities for other indications.

The strategy comprises of (1) baseline genomic surveillance; (2) sequencing of additional priority samples; and (3) a selection of additional samples in specific situations.

Only samples with a Ct value of ≤ 25 will be sequenced, because of quality reasons. Ideally, either the S-gene or polymerase are used as targets, but laboratories routinely using other targets can use these as well.

1. Baseline genomic surveillance

The aim is to sequence 2% (1 of every 50) of all positive samples. Only samples with a Ct value of ≤ 25 can be included, but the 2% should be based on all PCR positives.

The current weekly number of positive tests is approximately 14,000 (2,000/day), which corresponds with approximately 280 samples/week to be sequenced as part of the baseline surveillance. There is currently also a representative sample of 2% of past positive samples being sequenced.

If the number of weekly positive tests would increase, the % to be sequenced will be decreased and the number should never exceed 1000/week.

Ideally, the sequenced samples should have a representative distribution with regards to age and test indications. However, applying a stratified sampling approach will be complicated and it is believed that using a random sampling will ensure sufficient representativeness.

Sequencing of positive rapid Ag tests, such as from ambulatory care, is not possible because the samples are not stored and the buffer used often prohibits sequencing. This will introduce some bias, but it is believed to remain within reasonable limits.

A large network of “sentinel laboratories” will be established. In a first phase, these will be the federal platform laboratories, plus additional labs in provinces with no Platform-bis Labs. In a second phase, additional large laboratories will be added to increase geographical coverage.

2. Additional priority samples to be sequenced on top of baseline surveillance

In addition, all of the samples in the following situations will be sequenced:

- All infections in vaccinated people (>14 days after vaccination)
- All infections in populations with enhanced risk for mutations:
 - Participants clinical trials
 - Long-time chronic infection
 - Immunosuppressed patients
- All reinfections of which the first sample is available.
- All atypical PCR results. These include but are not limited to S-gene drop out. To be considered as a real S-gene drop out, there need to be a high enough viral load for the other genes.

The number of samples with a drop-out of the S-gene has increased and in January sometimes exceeded 100 in one day. Many of these, however, may be from returning travelers or clusters, and therefore overlap with the samples selected from these indications.

For all of these indications it is recommended to use an RT-PCR and not a rapid Ag test.

3. A selection of the following:

- A selection of positive samples of unusual outbreaks in nursing homes

The decision if an outbreak is unusual will be taken by the outbreak support team (OST), based on any of the following criteria:

- Unusually large outbreaks: >20% positive after 1st or 2nd round of testing (14 days after index)
- Outbreaks out of control: Persisting transmission despite good respect of measures – decision after 3rd round of testing
- Large number of severely ill or deceased: +/-10% of residents in a period of 3 weeks
- Outbreaks after vaccination
- Outbreaks in which the index case was confirmed to have a variant strain

The number of samples to sequence per unusual outbreak is 20% of the positive samples. This percentage can be altered, based on the results of the initial samples.

- A selection of positive samples of not unusual outbreaks in nursing homes in regions where new variants are known to circulate

The number of samples to sequence per outbreak is less than for unusual outbreaks and should not exceed 5.

It is difficult to predict the evolution of the number of clusters, in particular considering the roll-out of the vaccination program. Nevertheless, we can expect that in some weeks there might be more than 50 unusual clusters and more than 250 samples to be sequenced.

- A selection of positive samples of outbreaks in schools

The number of outbreaks in schools is currently low, and therefore all outbreaks will be included.

If the number of outbreaks increases, priority will be given to:

- Outbreaks with many positive pupils (Class closure due to outbreak at 1st and 2nd testing round)
- Outbreaks with relatively a lot of symptomatic pupils
- Repeat outbreaks
- Outbreaks in which the index case was confirmed to have a variant strain

The number of samples to sequence per outbreak in a school is 20% of the positive samples. This percentage can be altered, based on the results of the initial samples.

- Returning travellers: in a first phase all positive samples of returning travellers with a Ct value ≤ 25 will be sequenced. This approach will be re-evaluated after a couple of weeks, in order to have a representative picture of the strains circulating in Belgium, without over-representation of travellers. The selection of samples from travellers to be sequenced will occur at the sequencing facilities.

The number of returning travelers to be sequenced will depend on the number of travelers and the indications for which travelers will be tested. In the week of 5-11 January, there were 595 positive tests among returning travelers, although with a decreasing trend. It can be expected that the number will be lower in the coming weeks, but increase again with the school break in February.

People fulfilling the criteria for sequencing that are, according the recommendations, tested with a rapid Ag test (e.g. symptomatic returning travelers with symptoms ≤ 5 days, class

members in a school cluster) and who test positive, will be requested to have a second sample taken to perform sequencing.

Summary expected weekly numbers to be sequenced

Indication	Number per week	Observation
Baseline genomic surveillance	+/-300	Assuming current incidence remains stable
Atypical PCR results	+/-400-500?	To be confirmed Overlap with other indications
Other additional priority samples	?	Expected to be low
Cluster outbreaks	>250	Currently high demand Expected to decrease with vaccination roll-out
Returning travelers	+/-500	Probably with important fluctuations

POSSIBLE PRESELECTION IN RETURNING TRAVELERS AND CLUSTER INVESTIGATIONS BY INDICATIVE PCRS

The routine PCR test does not detect any abnormalities possibly indicating a mutation, such as an S-gene drop-out. The Triple-Gene PCR¹, available at all platform-bis and some other laboratories, detects the S-gene dropout, possibly indicating the 501Y.V1 (B.1.1.7) variant, and also the 'Mink-Cluster5 variant', but not the 501Y.V2 (B.1.351) or 501Y.V3 (B.1.1.28) variants. In addition, several labs are investigating the possibility to have an S-gene specific PCR with their routine platform. PCR tests detecting other mutations are in development. One specific commercial PCR detecting all N501Y mutations (from Roche), is already available and used at UZA and UZGent, and might be introduced in more federal platform labs.

The current sequencing plan does not propose a strategy for preselecting samples to be sequenced, the reason being that currently mutations other than B.1.1.7 (and the Mink-Cluster5 variant) will be missed. However, the current demand risks to exceed the capacity and, considering that currently the B.1.1.7 variant seems the most prevalent one of the new variants, the preselection of samples by the Triple-Gene PCR¹ or other S-gene specific PCR seems to be relevant. A validated specific PCR that detects all 501Y mutations can be used, if available. Priority should be given to samples with an abnormality and a high viral load (≤ 25 Ct).

It is therefore recommended:

- Not doing preselection among the 2% baseline genomic surveillance samples or the priority samples (post-vaccination infections, infections in populations with enhanced risk and reinfections)
- Systematic sequence all atypical PCR results (other than in clusters or travelers) detected at the Platform-bis laboratories, as long as the proportions are not too high.
- Do a preselection in cluster outbreaks and returning travelers, to reduce and target the number of samples to sequence. This can currently be done with a Triple-Gene PCR test or other S-gene specific PCR. A quick short S-gene PCR will be cheaper than the additional TF PCR. If a PCR detecting all 501Y mutations is available, this test can be used.

¹ TaqPathThermoFisher

ORGANIZATIONAL ISSUES TO CONSIDER (TO BE ADDRESSED BY TASK FORCE)

- Nursing homes, health inspectors, occupational physicians and school physicians should be recommended to supply the outbreak-related samples directly to the platform-bis, in order to have the Triple-Gene PCR test directly performed, and in order to do follow-up analyses such as the specific PCR detecting the 501Y mutation, and WGS if indicated.
- Hospitals should be encouraged to forward their COVID19 positive outbreak-related samples towards the platform-bis, to have follow-up analyses done on these samples (such as the Triple-Gene PCR, and if indicated WGS).
- Hospitals are also encouraged to perform the Triple-Gene PCR, or other S-gene specific PCR, themselves, on their positive detected samples, but in that case they should report the evolution in the number of analyzed samples and the number of abnormal PCR results obtained.
- Travel-related samples from country-entry-points as airports and major international train stations should be analyzed by the platform-bis, in order to have the Triple-Gene PCR executed as soon as possible, and to pre-select for sequencing and confirmation of variant type with a minimal TAT.