AANBEVELINGEN VOOR DE SELECTIE VAN STALEN VOOR DE SEQUENTIEBEPALING VAN HET VOLLEDIGE GENOOM IN HET KADER VAN SURVEILLANCE - UPDATE

RAG subgroep testing –15 Maart 2021

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

Voornaamste wijzigingen:

1. Basislijn genoomsurveillance
   - Het % stalen voor de aselecte steekproef wordt opgetrokken naar 5 tot 10% van alle stalen die met een RT-PCR positief testen.
   - Dit % kan indien nodig aangepast worden (door het NRC en Sciensano) in functie van de evolutie van de epidemie.

2. Actieve surveillance in specifieke populaties:
   - Gevaccineerde personen:
     - De post-vaccinatie periode wordt gereduceerd naar positief testen minstens één week na volledige vaccinatie.
     - Stalen van positieve testen na een eerste van twee dosissen worden niet systematisch gesequenced. Wel kunnen deze gescreend worden met een reflex PCR test.
   - Deelnemers aan klinische COVID-19 studies (geen wijzigingen)
   - Langdurige chronische infecties (geen wijzigingen)
   - Patiënten met immuundeficiëntie (geen wijzigingen)
   - Re-infecties
     - Het criterium dat het staal van de eerste infectie beschikbaar moet zijn vervalt. Wel moet de eerste infectie voldoende gedocumenteerd zijn (liefst met beschikbare sequentie).
     - Indien het aantal re-infecties zou oplopen, kan screening met een reflex PCR test overwogen worden.
   - Atypische RT-PCR resultaten
     - S-gen drop out is niet langer een indicatie voor sequencing.
     - De indicatie is nu alle stalen waarin abnormale relatieve kwantitatieve waarden (Ct-waarden) worden gedetecteerd in een PCR met verschillende targets, en die in België nog niet vaak werden beschreven.

3. Een selectie van stalen in ongewone uitbraken:
    - De criteria om als ongewoon beschouwd te worden (ongewoon groot OF aanhoudend OF veel ernstige gevallen OF post-vaccinatie OF index geval met mutatie) worden behouden. Wel komen nu ook uitbraken in andere collectiviteiten dan woon-zorg centra en scholen in aanmerking.
    - Het % stalen dat gesequenced dient te worden, zal bepaald worden door de instantie die de uitbraak onderzoekt (gezondheidsinspecteur, verantwoordelijke arts of ziekenhuisdienst). De regel is dat niet meer dan 20% gesequenced wordt, tenzij er redenen zijn om dit % te verhogen (bv. om de dynamiek van de uitbraak beter te begrijpen).
4. Een selectie van stalen in terugkerende reizigers
    - Momenteel is het nog steeds haalbaar om alle positieve stalen van reizigers uit een rode zone te sequensen.
    - Indien het aantal substantieel zou verhogen (bv. na het opheffen van de reisrestricties) zal een limiet van maximum 500 stalen per week gehanteerd worden.
    - Het is belangrijk dat in de bepaling van rode zones, het criterium van een mogelijke introductie van nieuwe mutaties in rekening gebracht wordt.
5. Ad-hoc indicaties
    - Naast de bovenvermelde indicaties, kan sequencing ook uitzonderlijk aangevraagd worden door de gezondheidsinspecteur indien daar geldige redenen voor zijn.

In alle indicaties worden stalen met een lage virale lading uitgesloten wegens moeilijk te sequencen. De voorgestelde limiet is >=10^4 RNA copies/mL, of <= de overeenkomstige Ct waarde voor de specifieke PCR test.

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.

De volgende personen hebben deelgenomen aan dit advies:

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CONTEXT

In response to the increasing circulation of new SARS-CoV-2 variants of concern, whole-genome, sequencing of positive SARS-CoV-2 samples was reinforced in Belgium. In January 2021, a RAG advice was formulated on indications for sequencing positive RT-PCR samples[1]. The context is changing rapidly, with an increasing proportion of new variants, in particular the 501Y.V1 (B.1.1.7) variant ('UK variant'), but also the 501Y.V2 (B.1.351) variant ('South-African variant') and the 501Y.V3, P1 and P2, (B.1.1.28) variant ('Brazilian variant'). According the latest genomic surveillance report (9 March 2021), the 501Y.V1 variant represented 57.6%, the 501Y.V2 variant 5.4% and the 501Y.V3 variant 1.8% of all samples analyzed in the baseline surveillance during the week of 22-28 February, and 47.7%, 15.6%, and 2,8%, respectively, during the week of 1-6 March (although that data from the latter week were still incomplete)[2]. Indications for sequencing need to follow these new evolutions and an update was therefore requested.

SEQUENCING CAPACITY

Initially, sequencing capacity needs were calculated with the possibility of scaling up in case of an epidemic outbreak. The calculation was done per province to ensure sufficient coverage of the entire territory and because the regional health authorities, with whom there is intense collaboration to contain clusters and/or outbreaks, are organized at the provincial level. A necessary capacity of +/- 1,150 analyses per week was proposed for the basic surveillance (100 samples per million inhabitants or +/- 7% of the current +/- 16,000 positive samples), whereby upscaling to +/- 2,300 samples must be feasible in the event of increased incidence and/or frequent outbreaks, i.e. doubling the capacity (+/- 7% of +/- 32,000 positive samples). If the number of positive samples should increase further, the percentage of samples to be sequenced can decrease to 5% or lower and an additional buffer capacity of 1,500 additional samples can be realized by the NRC at all times.

PROPOSED STRATEGY AND INDICATIONS

The strategy comprises of (1) baseline genomic surveillance and (2) active surveillance, that consists of (a) sequencing of additional priority samples; (b) a selection of samples in unusual outbreaks; and (c) samples of travelers returning from a red zone.

For quality reasons and to put the focus on contagious infections, only samples with a sufficient high viral load should be sequenced. It is recommended to only send samples for sequencing with >=10^4 RNA copies/mL. Labs that do not have the ability to measure copies/mL should use the corresponding Ct-value threshold defined by the calibration curves Ct-values/viral RNA copies that have been made based on the analysis of standardized samples of the NRC[3].

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[1] See: 20210125_RAG_Selection of samples for sequencing_NL.pdf (sciensano.be) and 20210125_RAG_Selection of samples for sequencing_FR.pdf (sciensano.be)

[2] See: genomic_surveillance_update_210309.pdf (uzleuven.be)

[3] See : 20201208_Advice RAG Interpretation and reporting of COVID PCR results.pdf (sciensano.be)

1. Baseline genomic surveillance

For basic surveillance, a network of sentinel laboratories was created by the NRC and Sciensano. The "sentinel laboratories platform" provides samples for genome analysis. The choice of these sentinel laboratories takes into account geographical distribution, population representativeness and volumes of routine diagnostic analysis to ensure that the genomic follow-up of at least 5% to 10% of all positive cases nationally is always covered by this randomly selected but representative sample.

The exact % of positive samples to be sequenced will be continuously evaluated by NRC and Sciensano and, if necessary, will be adjusted to ensure a correct coverage as described above. Also the clinical labs included in the sentinel laboratory platform may evolve to continuously ensure a representative geographical distribution.

Of particular concern are people with a severe form of COVID-19 (hospitalized patients, and in particular patients at the ICU). To monitor sequencing outcomes in this sub-population, the information on disease severity needs to be added to the collected information, through a link with the Health Data database.

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 fully vaccinated people (>7 days after full vaccination)
    - The time period between full vaccination and sufficient immunity differs between vaccines. Some vaccines, such as Comirnaty (Pfizer/BioNTech), provide already complete immunity as early as 7 days after the last dose. While others may need up to 14 days to achieve complete immunity, it is proposed that for simplicity all samples of people who test positive for COVID-19 more than 7 days after complete vaccination are sequenced.
    - It is recommended not to systematically sequence samples of people who test positive after a first dose of a vaccine for which two or more doses are required, because the number of infections after the first and before the second dose is too high (more than 2000 infections >7 days after a first dose already identified). A subset of these infections can be sequenced after screening with a reflex PCR, detecting atypical PCR results (see below).
- All infections in populations with enhanced risk for mutations:
    - Patients with long-time chronic infection
    - Immunosuppressed patients
    - Participants of clinical trials for specific COVID treatments

  In addition to its usefulness for epidemiological surveillance, the sequencing results of patients with these conditions might also sometimes be useful for clinical management. The turn-around-time will therefore be kept as short as possible.
- All reinfections of which the first infection has been properly documented. Ideally, but not necessarily, this includes the sequence. If the number of reinfections is high, screening with a reflex PCR, detecting atypical PCR results (see below), could be considered.
- Infections with specific atypical PCR results.
  The 501Y.V1 variant has become predominant and a systematic sequencing of all PCR results with S-gene drop out (which is closely correlated with the 501Y.V1 variant), without other abnormalities, is no longer indicated.

Samples in which abnormal relative quantitative values (Ct-values) are obtained in a PCR using different targets, and that were not yet frequently described in Belgium, might indicate new genetic modifications and should therefore be sequenced.

- Other, ad-hoc, indications
  In addition of the above, it can be decided case-by-case to perform sequencing on a positive sample for other reasons. This decision will be made by the health inspector.

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

3. A selection of samples in unusual outbreaks

In all outbreaks with an unexpected course, positive PCR samples can be sequenced. The decision to consider an outbreak as having an unexpected course is made in consultation with the regional health authorities, collectivity physicians or the hospital hygiene department. Criteria that should be taken into considerations include:

- Unusually large outbreaks
- Outbreaks out of control (persisting transmission despite good respect of measures)
- Large number of severely ill or deceased
- Outbreaks after vaccination has been completed (regardless of the coverage that was achieved)
- Outbreaks in which the index case was confirmed to have an immune escape VOC

In principle, the number of samples to sequence should not exceed 20% of all positive samples. These samples have to be representative of the total, for example with regards to age or location (classes in a school, rooms in a company…). However, depending on the objective of the sequencing, the regional health authorities, collectivity physicians or the hospital hygiene department might decide to sequence more samples, for example to document the dynamics of the outbreak. Alternatively, a screening with a reflex PCR could be done to identify the samples to sequence.

4. A selection of travellers from a red zone

There are currently approximately 23,000 travellers returning from a red zone each week, of which approximately 9,000 have at least one test result. The combined positivity rate (positive on either the first or second test) is 3.5%, corresponding with approximately 300 positive cases per week. Testing all positive samples with a high viral load of returning travellers is therefore currently possible. This number is, however, expected to increase substantially once the borders reopen. In that event, a maximum of 500 representative positive samples/week of travellers will be sequenced. It is important that in the definition of red zones, the criteria of the likelihood of emerging mutations is taken into consideration.

People who test positive and in whom sequencing is indicated, and who had been, according the recommendations, tested with a rapid Ag test (e.g. class members in a school cluster), will be requested to have a second sample taken to perform sequencing.

INTERNATIONAL RECOMMENDATIONS

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

The last update from ECDC specifically on sequencing of SARS-CoV-2 is from January 18 (Technical Guidance Sequencing of SARS-CoV-2).

However, the ECDC published a rapid risk assessment about increased circulation of variants of concern and vaccine rollout in the EU/EEA on February 15, and a technical report on Methods for the detection and identification of SARS-CoV-2 variants on March 3 2021.

Both documents highlight the importance of assessing the level of circulation of known VOCs in the community. To accurately estimate and monitor prevalence of the VOCs, ECDC recommends **sequencing at least 500 random/representative samples** per country per week. The European commission recommends a sequencing rate of **5 – 10 % of positive samples**.

In parallel, the **testing strategy should include coverage of vaccine break-through infections, reinfections, prolonged/chronic infections, severe infections, zoonotic infections and outbreaks**, especially when the focus will shift to the detection of new variants.

Depending on the available resources, WGS sequencing can be done for additional objectives, like outbreak analyses, **phylodynamic analyses** and other research studies.

Key messages ECDC:

- Whole SARS-CoV-2 genome sequencing, or at least whole or partial S-gene, should be used to confirm infection with a specific variant.
- For the early detection and prevalence calculation of VOCs (i.e. B.1.1.7/501Y.V1, B.1.351/501Y.V2, P.1/501Y.V3), alternative methods, such as diagnostic screening PCR-based assays can also be used.
- Sequencing should be used to confirm at least a subset of the viruses, when PCR-based methods are used.
- Sample and method selection are key and will depend on the objectives, e.g. for assessing the circulation of the different SARS-CoV-2 variants using representative samples from the community, genetic characterisation to monitor the virus evolution, and informing vaccine composition decisions or outbreak analyses.
- Assay validation should be carried out to ensure that the laboratory testing system is performing adequately for the circulating viruses.
- SARS-CoV-2 consensus sequences are strongly recommended to be submitted to GISAID.
- Detection of novel VOCs or outbreaks of currently circulating VOCs should be reported immediately through the EWRS, while VOC detections should be reported to TESSy weekly.

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.