AANBEVELINGEN VOOR DE SELECTIE VAN STALEN VOOR DE SEQUENTIEBEPALING VAN HET VOLLEDIGE GENOOM IN HET KADER VAN SURVEILLANCE - UPDATE AUGUST 2021

RAG subgroep testing –30 Augustus 2021

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

Opgepast: het RMG heeft de aanbeveling inzake surveillance in gehospitaliseerde patiënten enkel goedgekeurd onder voorwaarde dat een maximum bepaald wordt voor het aantal wekelijkse stalen dat voor deze indicatie kan gesequenced worden

Voornaamste wijzigingen:

Actieve surveillance in specifieke populaties:

- Stalen van infecties in volledig gevaccineerde personen hoeven niet langer systematisch allemaal gesequenced te worden. Enkel stalen van infecties in volledig gevaccineerde personen met een ernstig ziekteverloop (hospitalisatie) dienen nog systematisch gesequenced te worden.
- Ook stalen van infecties in volledig gevaccineerde bewoners van woon-zorg centra worden best systematisch gesequenced.
- De criteria om een cluster uitbraak als ongewoon te beschouwen worden licht gewijzigd:
    - Uitbraken in een volledig gevaccineerde populatie worden slechts als ongewoon beschouwd indien minstens 90% van de populatie volledig gevaccineerd is.
    - Uitbraken waarbij het index geval met een 'variant of concern' (VOC) geïnfecteerd is, wordt vervangen door 'uitbraken waarbij het index geval met een atypische variant geïnfecteerd is'.

Surveillance in gehospitaliseerde patiënten:

- Om een uitgebreidere en meer representatieve steekproef van stalen in gehospitaliseerde patiënten te bekomen wordt aanbevolen om de stalen van alle gehospitaliseerde patiënten in een select aantal hospitalen systematisch te sequensen.

Rapportering van sequencing indicaties

- Er wordt aanbevolen om de rapportering van de indicatie voor dewelke sequencing werd aangevraagd te optimaliseren en het aantal ontbrekende gegevens en rapportagefouten te verminderen. Een oplossing dient gevonden te worden voor het rapporteren van patiënten met meer dan één indicatie.

Type test te gebruiken in volledig gevaccineerde personen:

- De aanbeveling om volledig gevaccineerde personen steeds met een RT-PCR te testen vervalt. In volledig gevaccineerde patiënten met symptomen <=5 dagen is een snelle Ag test een aanvaardbaar alternatief. Volledig gevaccineerde patiënten die hospitalisatie vereisen worden best nog steeds met een RT-PCR getest.

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 and the advice was updated in March 2021[1]. The current recommended indications are summarized in Annex 1. Since then, the vaccination program has successfully been rolled out and more and more people are fully vaccinated. By August 22, 2021, 68.2% of the Belgian population and 82.4 % of the adult population (>=18 years old) had been fully vaccinated. As a consequence, COVID-19 infections in fully vaccinated people (breakthrough cases or BTC) represent an increasing proportion of all new infections and, combined with the still increasing incidence, their absolute number has become substantially large (see below). The increasing number puts a high pressure on the sequencing capacity, especially because most breakthrough infections were sequenced at one lab, namely the National Reference Center (NRC). To relieve the NRC, laboratories have been requested to send breakthrough infections to other labs with sequencing capacity, but the pressure remains high. The question was therefore raised if it is still necessary/ useful to systematically sequence all breakthrough infections.

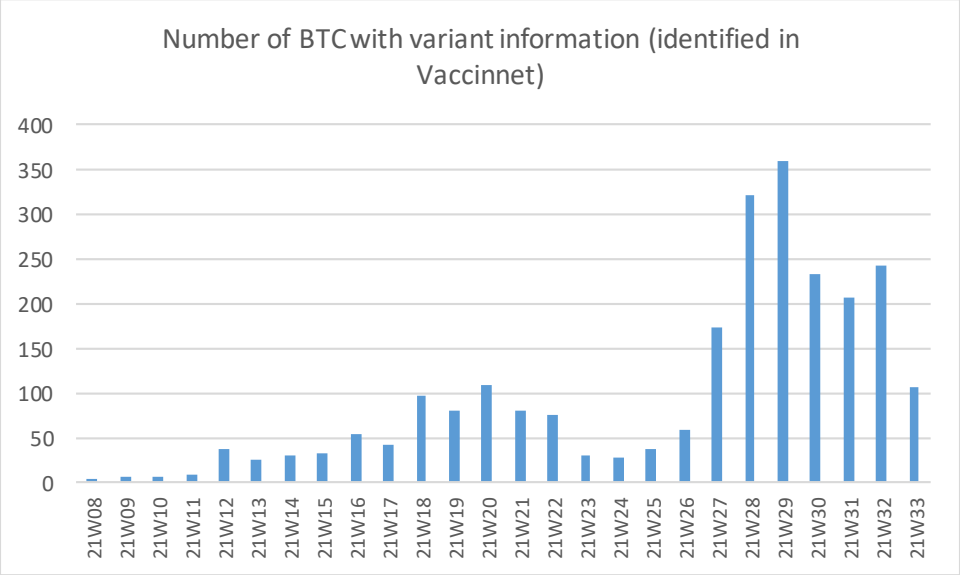
Related to the above, the current recommendation to test all suspected cases in fully vaccinated people with an RT-PCR test, and not with an Ag test (rapid or automated), has been questioned. Several studies have shown that viral load in breakthrough cases with the Delta variant is similar to that in unvaccinated people and the sensitivity of an Ag test might therefore be similar. A main argument to systematically test all fully vaccinated people with an RT-PCR test, was that it allows to sequence the positive samples. If positive samples in fully vaccinated people were no longer to be systematically sequenced, this argument is no longer valid. The question specifically applies to people with an onset of symptoms <=5 days.

[1] See : 20210315_Advice RAG_Selection for samples for sequencing - update_NL.pdf (sciensano.be) or 20210315_Advice RAG_Selection for samples for sequencing - update_FR.pdf (sciensano.be)

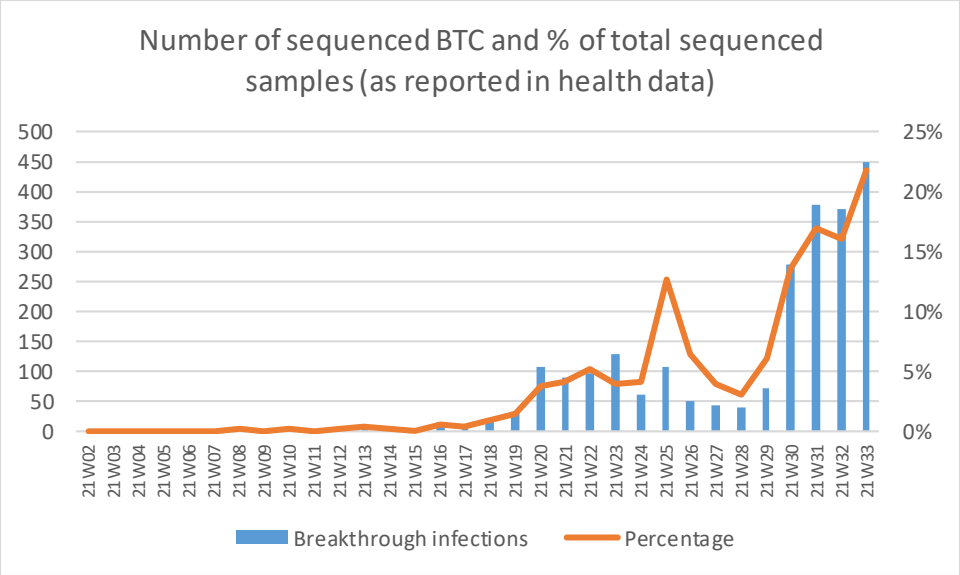
Another topic related to sequencing, concerns optimized sequencing of hospitalized patient samples to analyze clinical outcomes related to COVID-19 variants. The LINK-VACC project, initially set up to monitor vaccine effectiveness in Belgium by linking the vaccine registry (VACCINNET+) with other already existing databases, allows researchers to merge SARS-CoV-2 variant information with detailed hospital records. However, this repurposing of existing database faces specific methodological challenges that need to be addressed before it can efficiently answer clinical questions related to variants of concern. Namely, the infrastructure is in place but requires uniform adoption by all stakeholders that record and register hospital and variant data. In this way the limitations related to selection bias (selective sample of intensive care patients?), low sample size and near real-time collection of data can be improved. The question

NUMBER OF BREAKTHROUGH INFECTIONS

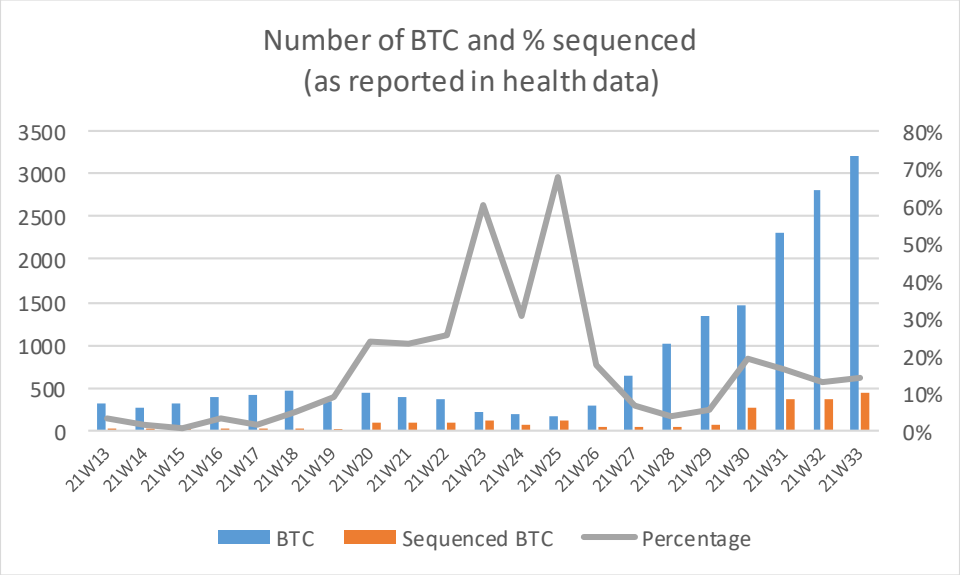
The graph below presents the number of breakthrough cases (BTC) identified in the Vaccinet database with information on the SARS-CoV-2 variant causing the infection (and thus assumed to have been sequenced). A breakthrough case is defined as an infection in a person who has been fully vaccinated for at least 14 days. Identification of the variant was mostly through whole genome sequencing (82%), although sometimes by PCR (18%). The highest number was reached in the week of 19-25 July with 359 reported breakthrough infections. In later weeks, the number was between 200-250 infections/week, but the data are possibly not yet complete.



The graph below presents the number of samples reported in health data as having been sent for sequencing because of being a breakthrough infection (defined as an infection in a person fully vaccinated for at least 7 days). This number is slightly higher, with a peak of 450 samples in the week of 16-22 August, representing 22% of all sequenced samples and 35% of the samples sequenced in the active surveillance.



The following graph shows the total number of BTC reported in health data, and the number and percentage reported as having been sent for sequencing. We note that in recent months only a small proportion of BTC are being reported as having been sent for sequencing (around 15-20%), and that the actual number of BTC is much larger (more than 3000 in the week of 16-22 August, representing 25% of all cases).



The table below summarizes the number of whole genome sequencing performed by indication, as reported in health data, for the total period since the beginning of the sequencing program and for the last 4 weeks (26/7-22/8). Baseline surveillance represents about half of the sequenced specimens. Post-vaccination is the next most common indication, and also travelers represented a substantial proportion during the summer holidays (10%). We observe that sequencing for other indications is rare and that of an important proportion no information on the indication is available (about ¼).

Indication	Total period		Last 4 weeks	
	N	%	N	%
Baseline surveillance	23,440	50%	4036	47%
Post-vaccination	2367	5%	1475	17%
Travelers	1407	3%	900	10%
Abnormal PCR result	2516	5%	162	2%
Outbreak	481	1%	12	0.1%
Reinfection	34	0.1%	4	0.05%
Exhaustive hospital	15	0.03%	0	0%
Immunocompromised	15	0.03%	0	0%
Chronic infection	3	0.01%	0	0%
Other	6750	14%	1164	13%
Unknown	10,164	22%	904	10%
Total	**47,229**		**8,657**	

DISCUSSION

Indications for whole genome sequencing

- It was agreed that it is no longer feasible, nor necessary, to sequence all samples of COVID-19 infections in fully vaccinated people.
- The most relevant criteria for the selection of which infections to sequence in fully vaccinated people (in addition to those already sampled for the baseline surveillance) is the clinical status of the infected person. It was agreed that only patients with a severe clinical picture (requiring hospitalization) need to be systematically sequenced.
- A need for a more extensive sequencing of positive specimens in nursing home residents was expressed. Breakthrough infections are more common and severe among this population and not all residents with a severe clinical picture are being hospitalized. It was agreed to recommend systematic sequencing of infections in fully vaccinated nursing home residents.
- Breakthrough infections are a result of a combination of factors. The individual inflammatory response is an important factor that is insufficiently documented and, in addition to sequencing, there is a need to establish a system to better characterize the immune response in infections in certain vulnerable populations, such as nursing home residents.
- It was agreed that the current information on which variant is causing infections in hospitalized patients is insufficient to establish relationships between variants and severity of disease. The number of sequenced specimens in the baseline surveillance is insufficient, in particular now that less people present a severe disease following vaccination. In addition, the available specimens are not a representative sample of all hospitalized patient, with, for example, an overrepresentation of ICU patients.
- It was agreed that systematically sequencing of all hospitalized patients in a selected number of hospitals could resolve this problem. The hospitals currently participating in the Clinical Hospital Survey appear to be the most logical choice.

- The aim is to build up a systematic registry of hospitalized COVID-19 patients with variant data. Patients who were hospitalized for a reason other than COVID-19, but who subsequently suffered the consequences of a COVID-19 infection (nosocomial infection or late presentation due to an extended incubation period), should also be systematically sequenced.
- The reporting system should clearly indicate that the sample was sequenced because of being of a hospitalized patient ("exhaustive hospital surveillance").
- Selected hospitals performing systematic sequencing would need to register all their hospitalized patients in the Sciensano COVID-19 Clinical Hospital Survey. In this way, sequencing results can be linked to the clinical impact on the hospitalized patients (demographics, severe disease course, mortality).
- With regards to the other current indications for sequencing, no major changes are currently necessary. Reinfections represent only a small proportion of samples sent for sequencing, and the recommendation to sequence all of them can be maintained. Also the number sequenced in a context of unusual outbreaks is relatively low, although that there appears to be serious under-reporting. It was agreed that the criteria to consider an outbreak unusual need to be updated. It is no longer necessary to consider all outbreaks in collectivities in which vaccination has been completed as unusual, regardless of the coverage that was achieved. Only outbreaks in collectivities with a high vaccination coverage should be considered unusual. There is also no need to sequence specimens in outbreaks in which the index case was confirmed to have an immune escape VOC, because the definition of what is an immune VOC is disputable (almost all infections are currently by a variant considered as an immune escape VOC). It was agreed to change the criteria to outbreaks in which the index case was confirmed to have an atypical variant. The recommendation to ideally sequence all positive specimens in travellers returning/ arriving from a red zone can be maintained, because the number of positive tests in travellers is expected to reduce after the holiday period.
- Errors in the reporting of the sequencing indication appear to be frequent (a lot of 'unknowns' and 'others', active surveillance reported as baseline,…). Only one indication can be reported and sometimes a patient fulfills the criteria for more than one indication (for example post-vaccination and cluster outbreak).

Testing of vaccinated people with an Ag test

- Although that it has still insufficiently been proven that infections in fully vaccinated people by the Delta variant have a viral load similar to the one in unvaccinated people, it was agreed that it is acceptable to test fully vaccinated people with symptoms <= 5 days with a rapid Ag test.

RECOMMENDATIONS

- To no longer systematically sequence all positive RT-PCR tests in infections in fully vaccinated people. Only infections in fully vaccinated people (>7 days after full vaccination) with a severe clinical picture, requiring hospitalization, should be systematically sequenced.

This includes both patients requiring hospitalization in the ICU and patients not requiring hospitalization in the IUC.

- To systematically sequence all positive RT-PCR tests in infections in fully vaccinated nursing home residents.
- To revise the criteria to consider a cluster outbreak as unusual:
    - 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 and a coverage of 90% was achieved
    - Outbreaks in which the index case was confirmed to have an atypical variant
- To establish a surveillance system in a selected number of hospitals in which all positive specimens of patients hospitalized for COVID-19 are systematically sequenced.
- To improve the reporting of the indication for which sequencing was done, and reduce the number of missing information and reporting errors. For patients with more than one indication, either the reporting of multiple indications should be possible, or a priority listing should be established of which indication gets priority.
- To approve the use of rapid Ag tests in fully vaccinated patients with COVID-19 symptoms <=5 days.

INTERNATIONAL RECOMMENDATIONS

The latest update from **ECDC** on sequencing of SARS-CoV-2 dates from March 3 (Guidance for representative and targeted genomic SARS-CoV-2 monitoring). With regards to targeted sampling and sequencing from special settings or populations, ECDC recommends to sequence the following:

- Vaccine breakthrough infections and reinfections: comprehensive sampling to detect and characterize variants causing infection in the presence of SARS-CoV-2 antibodies;
- Outbreaks and clusters: a representative sample, with a minimum of five specimens per event to investigate virus transmission dynamics; detect novel genetic variants; assess the relatedness of viral strains within epidemiological clusters, and support contact tracing and other public health interventions;
- Confirmed cases with travel history in areas where VOCs or VOIs are endemic: to detect potential introductions of variants and slow down their spread, ECDC recommends comprehensive sequencing sampling of all SARS-CoV-2 positive cases with travel history in areas/countries where new VOCs or VOIs are circulating;
- Unusual events: a representative sample, with a minimum of five specimens from super-spreading events or settings with unusually high transmission; for cases with unusual clinical presentations, ECDC recommends comprehensive sampling to support investigations of virus transmission dynamics and detection of novel genetic variants.

WHO published new guidance on August 9, 2021 (Guidance for surveillance of SARS-CoV-2 variants). With regards to targeted sampling, it recommends to focus on specific subsets of cases associated with public health risks: diagnostic failures, vaccinated cases, reinfections,

immunocompromised cases, and on outbreaks, alerts or other unusual events. They list three potential triggers for targeted sequencing: (1) specimen-level characteristics (e.g. genomic sequencing based on results from screening assays such as PCR-based single nucleotide polymorphism (SNP) detection assays); (2) individual-level characteristics (e.g. clinical characteristics; immunocompromised patients and selective sequencing of vaccine breakthrough) and (3) environmental characteristics (e.g. evidence of variant sequences from wastewater surveillance.

Specimens that deserve prioritization based on individual-level characteristics include:

- cases of SARS-CoV-2 infection in people who have been fully vaccinated;
- cases of SARS-CoV-2 infection in people who have been previously infected;
- cases where there is unexpected discordance between diagnostic tests, such as in clusters of individuals testing positive by rapid antigen test but negative by RT-PCR (or vice versa); characteristic and recurrent drop-out in a single gene target in a multi-target PCR assay; or where sample compartment test results are discrepant (e.g. upper versus lower respiratory tract);
- patient groups with underlying conditions that increase the likelihood of prolonged viral replication and shedding, such as immunocompromised patients (19–21);
- case clusters with unusual clinical presentations (e.g. unusually severe disease, unusual symptoms);
- case clusters suggestive of zoonotic transmission (e.g. among people working with animals susceptible to SARS-CoV-2 infection);
- cases with unexpectedly poor response to therapeutics.

In its guidance on COVID-19 Vaccine Breakthrough Case Investigation and Reporting, **CDC** states that respiratory specimens of breakthrough cases should be collected for genomic sequencing to the fullest extent possible. It recommends local and state health departments to hold any residual respiratory specimens from the positive SARS-CoV-2 test and either sequence them at a local laboratory or send it to CDC.

As of May 1, 2021, CDC transitioned from publicly reporting the passive surveillance of all vaccine breakthrough cases to focus on hospitalized or fatal vaccine breakthrough cases due to any cause. The purpose of the shift was to maximize the quality of the data collected on cases of greatest clinical and public health importance. Nevertheless, some health departments continue to report all vaccine breakthrough cases to the national database and continue to submit specimens to CDC for sequencing.

The Robert Koch institute has developed guidance for primary diagnostic laboratories to select SARS-CoV-2 positive samples for sequencing in **Germany**. It does not define precise indications, but states that the following clinical-epidemiological indicators or indications of exposure to novel or worrying variants of SARS-CoV-2 justify further analysis by whole genome sequencing:

- unexpected disease severity or unexpected clinical course
- vaccination breakthroughs (cases of illness in fully vaccinated)
- suspected zoonotic infection
- suspected reinfection, according to the case definition of the RKI

The RIVM of **The Netherlands** does neither provides a precise list of indications but stipulates that in addition to the baseline surveillance, sequencing should be considered in certain unusual cases, such as cases with an unusual clinical picture/illness, suspicions of reinfection or

(re)infection after vaccination with an abnormal disease pattern, infections in immune-compromised persons, and infections in which a possible animal reservoir plays a role.

Sequencing is also important in people with a travel history to a country where a particular variant is circulating and/or positive contacts of a case infected with a particular variant.

In clusters and outbreaks with an unusual course (for example: rapid spread, newsetting, different clinical picture, difficult to control), sequencing can provide additional insight that can be used for control. It is, however only of added value if a thorough epidemiological investigation can be done. In case of many positive samples, sequencing only part of the samples can be considered.

LITERATURE BACKGROUND

Viral load in breakthrough infection

An overview of the scientific evidence with regards to the effectiveness of the current vaccines against the Delta variant is available in the latest advice on testing, isolation and quarantine[2]. In summary, vaccine effectiveness of full vaccination against hospitalisation appears to be similar as against the Alpha variant, and against symptomatic disease slightly lower. Evidence on vaccine effectiveness against infection with the Delta variant is still scarce. However, one study found a substantial lesser protective effect, even after complete vaccination. The lesser protection of vaccination against infections by the Delta variant is also shown by several studies investigating post-vaccination breakthrough infections.

Of interest is that some of these studies found that the viral load in breakthrough infections by the Delta variant was high.

A study in Houston, Texas, among 1118 Delta variant and 3802 other variant infections found that Delta variants caused a significantly higher rate of vaccine breakthrough cases (17.4% compared to 5.8% for all other variants, p<0.0001). Individuals with vaccine breakthrough cases caused by Delta variants had a low Ct value that was not significantly different than the Ct value observed in unvaccinated patients with COVID-19 caused by Delta variants (median of 20.67 in both, p=0.9231) (1). Also in an outbreak investigation in Barnstable County, Massachusetts, Ct values among Delta infections were similar among specimens from patients who were fully vaccinated and those who were not (2), and also a study in Wisconsin came to the same conclusions (3). A study in Singapore observed the same among hospitalized patients (4), but found that viral loads decreased faster in vaccinated individuals. In its update on what is known about the Delta variant, CDC concludes, based on the above studies, that fully vaccinated people with Delta variant breakthrough infections can spread the virus to others, but that vaccinated people appear to be infectious for a shorter period (5).

A study in The Netherlands analyzed 161 vaccine breakthrough infections in health care workers, of which 91% were by the Delta variant (6). The samples were compared to samples from infections that occurred in the same cohort of HCWs prior to the onset of vaccination (caused by pre-VOC variants). The mean Ct-value upon diagnosis was similar between the two groups: 24.6

[2] See : 20210812_Advice_RAG_Update measures August_NL.pdf (sciensano.be) or 20210812_Advice_RAG_Update measures August_FR.pdf (sciensano.be)

(15.3 - 33.9) for vaccinated HCWs and 24.2 (14.53 - 33.8) for unvaccinated HCWs (p=0.53). However, the culture was positive in 68.6% of vaccinated HCWs versus 84.9% of unvaccinated HCWs (p = 0.005).

A study using data from India also found lower Ct values in Delta breakthrough cases in health care workers (mean Ct 16.5), fully vaccinated with the Covishield vaccine, compared to non-Delta breakthrough cases (mean Ct 19) and a larger cluster size with Delta breakthrough (7).

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ANNEX 1: CURRENT INDICATIONS FOR WHOLE GENOME SEQUENCING AND VOC PCR

Indications for whole genome sequencing

The strategy comprises of a baseline genomic surveillance and active surveillance.

Baseline genomic surveillance comprises of routine surveillance in a nationally representative sample among all RT-PCR positive tests with a sufficient high viral load (>=10^4 RNA copies/mL). The exact % of positive samples to be sequenced is continuously evaluated by NRC and Sciensano, but should comprise between 5% and 10% of all RT-PCR positive tests.

Active surveillance consists of sequencing of additional priority samples; a selection of samples in unusual outbreaks; and samples of travelers returning from a red zone.

- The *additional priority samples* include:
    - All infections in fully vaccinated people (>7 days after full vaccination). Infections in partially vaccinated people (>7 days after the first of two doses) are not systematically sequenced. A subset can be screened after screening with a VOC PCR detecting atypical PCR results.
    - 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
    - All reinfections of which the first infection has been properly documented. If the number of reinfections is high, screening with a VOC PCR can be considered.
    - Infections with specific atypical PCR results. These are 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, possibly indicating new genetic modifications. A separate advice on the use of VOC PCR was developed for this purpose (see summary below)[3].
    - Other, ad-hoc, indications decided case-by-case by the health inspector.

- 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, using the following criteria:

- 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

A representative sample of all positive cases (that in principle should not exceed 20%) is sequenced.

[3] See : 20210621_Advice_RAG_Use of a genotyping PCR protocol_NL.pdf (sciensano.be) or 20210621_Advice_RAG_Use of a genotyping PCR protocol_FR.pdf (sciensano.be)

- A selection of travellers *from a red zone*

Ideally all positive samples in travellers returning/ arriving from a red zone are sequenced. If the number becomes too high, a maximum of 500 representative positive samples/week of travellers are to be sequenced.

Indications for VOC PCR

To allow a more rapid detection of key mutations, it was recommended to expand the capacity to perform SNP PCR genotyping ('VOC PCR') as widely as possible; in a first phase to all Platform Bis laboratories, in a later phase to all laboratories performing SARS-CoV-2 RT-PCR testing

The decision which positive samples to test with the genotyping protocol (all or only in certain circumstances or none) will use the following principles:

- The presence of newly emerging VOCs for which early identification is relevant to take timely additional measures to contain its spread. To be relevant, results should be available in less than 3 days and communicated to the responsible physician.
- The capacity to take timely additional measures, with regards to reinforcing follow-up of isolation of people identified with a newly emerging VOC and quarantine of their high-risk contacts.
- If not possible/ useful to test all positive samples, give priority to samples from:
    - Travelers returning/arriving from an area with known circulation of a newly emerging VOC.
    - Severely ill (hospitalized) COVID-19 patients, if relevant to guide clinical treatment (different clinical approach required for the VOC) or to prevent a nosocomial cluster outbreak.
    - All post-vaccination infections, including after a first dose of a vaccine for which two or more doses are required.
    - All reinfections of which the first infection has been properly documented. A selection still needs to be sequenced.
    - All positive samples in unusual outbreaks (according to the criteria established in the advice on indications for sequencing). A selection still needs to be sequenced.
    - 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). Sequencing still needs to be done as well.