Indications for genomic sequencing and rapid molecular tests as POC tests

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CONTEXT AND QUESTIONS ADDRESSED

Indications for sequencing

The current indications for sequencing were set in January 2021 and updated in August 2021 in response to the evolution of the epidemic. They include baseline genomic surveillance (sequencing of a representative subset of all PCR-positive samples) and active surveillance (sequencing of additional priority samples, a selection of samples in unusual outbreaks and samples of travelers returning from a red zone).

In the context of a changing test strategy and lower number of PCR-tests performed, the question of maintaining the current indications of sequencing was raised. More specifically, the relevance of all indications of the active surveillance should be discussed, as well as the ideal amount of sequences needed for an optimal baseline surveillance (% of total positive tests or fixed number of samples).

Evaluation of the use of rapid molecular tests in primary health care and pharmacies

Rapid PCR tests offer quick and reliable result but are expensive and their availability is limited. Therefore, they are currently reserved for hospital settings where an urgent and accurate result is needed (see RAG advice from 19 April 2021). Indications were re-evaluated in December 2021 and maintained without changes.

As more tests become available and advertised, the question of the possibility to use rapid PCR tests in pharmacies and in primary health care is now asked.

RECOMMENDATIONS

Indications for whole genome sequencing

The proportion of positive samples included for the baseline surveillance will impact the sensitivity of the surveillance program to detect variants circulating in low proportion. Due to the cost of sequencing, a highly sensitive baseline surveillance program (ex: able to detect emerging variants before they reach 1% of the circulating strains) requires very important technical and financial investments, particularly when the virus is circulating at high levels. In order to decrease the overall cost of the surveillance program without altering its performance, we recommend the following:

- First, to limit the number of positive samples included in the baseline surveillance program to 400 - 500 samples/week for the country. The number of samples included can be lower during a wave, and therefore the proportion of randomly selected positive samples referred for the baseline surveillance will need to be adapted based on the epidemiological evolution.
- Second, to update the indications in the context of active surveillance:

- Infections in populations with enhanced risk for mutations (patients with long-time chronic infection, immunosuppressed patients, patients under specific COVID treatments such as monoclonal antibodies);
- Atypical PCR results (ex: S gene atypical or failed amplification when such result is not compatible with the dominant circulating variant);
- Samples from travelers returning from an area classified as VOC (list to be updated and communicated by Sciensano);
- A selection of samples from unusual outbreaks (see definition in Annex 1), in agreement with the regional health inspector

Reinfections and infections post-vaccination are no longer considered an indication for sequencing in the context of active surveillance.

Only samples with a clear indication should be included in the context of active surveillance, and this indication for testing should be systematically reported together with the associated metadata. If the indication is missing, the laboratory should not process the sample.

- The performance of the genomic surveillance program should be re-evaluated every 3 months. Alternatives to the current baseline surveillance sampling should be studied (ex: surveillance through the ILI/SARI surveillance networks, revision of the number of participating sequencing laboratories, …).

Use of rapid molecular tests in primary health care and pharmacies

- The indications for POCs are maintained. It remains important to allow easy access to testing “close to the patients” (at a GP or in a pharmacy), but rapid antigen tests are already available for this.

ELEMENTS OF DISCUSSION

Indications for whole genome sequencing

- Monitoring circulating variants remains essential. Although immediate public health actions following the detection of a new variant (ex: travel restriction, targeted test & trace) have not proven to allow a sustained containment of these variants, their early detection allows to initiate promptly a risk assessment which include clinical severity and level of immune evasion. Further, the ECDC and WHO still recommend to maintain high quality genomic surveillance at this stage of the pandemic. However, the surveillance represents an important cost.
- The two different arms of the Belgian genomic surveillance (active and baseline) play different and complementary roles. The purpose of the active surveillance is to rapidly detect new emerging variants more rapidly, by targeting populations with a higher risk of mutations. The main objective of the baseline surveillance is to follow-up trends for the circulating variants and to detect emerging variants when they reach a certain proportion, typically around 1%.
- For the active surveillance, sequencing of reinfections or breakthrough infections is not relevant anymore, since these are driven by the dominant circulating variants.
- For the baseline surveillance, it is more interesting to have a sufficient number of samples in a period of lower virus circulation, to follow-up trends and detect possible changes. However, during a wave of infections (generally caused by one dominant variant), a smaller number is sufficient. Setting a percentage of samples to be sequenced (e.g. 5%) will be less relevant if the test strategy

is changed (less tests performed). Therefore, it is easier to work with a fixed number, depending on the epidemiological situation and the number of participating laboratories. The proposal is to include a maximum of 400-500 samples a week in a period in between waves, and 200-300 samples a week maximum during a wave.

- Reducing the number of samples included in the baseline surveillance will increase the cost per sample or increase the time-to-result, as samples are typically analyzed by batches. To overcome this issue, reducing the number of sequencing laboratories may be required. Another alternative could be to replace the current sampling method by the IRI/SARI surveillance program, in the context of integrating SARS-CoV-2 surveillance in a broader surveillance of respiratory pathogens. But the latter alternative would first need to be assessed based (e.g. turn-around-time of results).
- A high proportion of samples in 2022 have no information on the indication for sequencing in the database available for Sciensano (data from health data), especially for the active surveillance (74% indication unknown for the last 3 months). The NRC reports that this proportion is lower in their database and in Gisaid, so it might be a problem of data transmission (to be investigated). Overall, samples without reported indication should not be sequenced.

Use of rapid molecular tests in primary health care and pharmacies

Arguments pro :

- Even if they can be less sensitive than PCR (e.g. the rapid isothermal NAATs assays), their performance is better than that of a rapid Ag test.
- They provide a rapid result at the PoC, which is becoming more and more important with the increasing availability of COVID-19 treatments that need to be initiated quickly after symptom onset, i.e. a maximum of 5 days and preferably within 3 days.

Arguments contra:

- The sensitivity of rapid Ag tests in patients with a recent onset of symptoms is considered sufficient to make a definite diagnosis. It can therefore be questioned if replacing it with a slightly better performing, but more expensive test is desired.
- Similar as for rapid Ag tests, they require additional time per patient, increasing the workload for the provider. While it is recommended that symptomatic patients be tested with a rapid Ag test, their use in general practice is still limited because of this higher workload, and at test centers they are not used for the same reason. It might be expected that the use of rapid molecular tests will face the same challenges.
- There is uncertainty about what the requirements are with regards to quality control of these tests. Tests using RT-PCR require a standardized quality control program, which should also apply to tests using other nucleic acid amplification techniques. Introducing these test at the PoC would imply setting up a QC system for general practitioners/pharmacies.
- Instead of increasing the use of (more expensive) PCR tests (as PoC or in laboratories), the focus should be on a broader use of RAT tests (for symptomatic persons with recent onset).

BACKGROUND INFORMATION

Indications for sequencing

Sequencing in Belgium

The current indications for sequencing are summarized in Annex 1, and the volumes of sequencing per indication, as reported in healthdata, are described in the table below. Baseline surveillance represents about half of the sequenced specimens (> 60% in the last 3 months). Other relatively common indications include post-vaccination or abnormal PCR results. We observe that sequencing for other indications is rare and that for most of the active surveillance samples, no information on the indication is available.

Indication	W1-W37 2021		W1-W37 2022		Last 3 months	
	N	%	N	%		
Baseline surveillance	26 491	44,9%	50 283	44,5%	13 021	60,6%
Post-vaccination	4 423	7,5%	3 185	2,8%	351	1,6%
Travelers	2 617	4,4%	1 097	1,0%	35	0.2%
Abnormal PCR result	2 543	4,3%	1 954	1,7%	570	2,7%
Outbreak	510	0,9%	391	0,4%	106	0,5%
Reinfection	39	0,1%	495	0,4%	0	0.0%
Immunocompromised	15	0,03%	72	0,06%	24	0,1%
Chronic infection	3	0,01%	3	0.0%	1	0,0%
Non-baseline	61	0,1%	22	0,02%	0	0,0%
Other	8 540	14,5%	3 535	3,1%	866	4,0%
Unknown	13 779	23,3%	51 285	45,4%	6 177	28,6%
Total	**59 036**		**113 032**		**21 484**	

International recommendations

The ECDC published a second update on the methods for detection and characterization of SARS-CoV-2 variants in August 2022. Priority for sequencing should be given to:

1. Sentinel samples from primary and secondary care sites;
2. SARS-CoV-2 positive specimens from special settings, e.g.
    a. outbreaks
    b. immunocompromised patients or patients with other underlying conditions associated with prolonged viral replication and shedding
    c. cases with an unusual clinical presentation or poor response to therapeutics including antiviral treatments.

Characterization and sequencing of samples from special settings may provide important signals that novel variants are emerging, with potentially changed characteristics.

ECDC has also published in May 021 a practical guidance to EU/EEA Member States on implementing genomic SARS-CoV-2 surveillance, including advice on the number of samples that need to be sequenced to achieve various objectives.

The indications for sequencing in our neighboring countries are summarized below.

	Baseline surveillance	Active surveillance
Netherlands	Yes 400-1200 samples/week	Sequencing possible in special settings : • unusual clinical presentation • immunocompromised patient • animal reservoir • unusual clusters • travelers if VOC
Germany	Yes 5 % of positive samples or 10 % if the number of new infections is < 70 000	Sequencing possible in special settings, at the initiative of public health institute : • unusual clinical presentation • animal reservoir • travelers if VOC • unusual clusters • unexpected transmission profile
France	Yes 3,4 % of positive tests in w36 2022	Targeted sequencing : • vaccine breakthrough • unusual clinical presentation • unusual clusters • immunocompromised patient

Use of rapid PCR tests in primary health care and in pharmacies

There are now several rapid molecular tests available on the market and CE approved that can be used at the point of care. A detailed list of available tests can be found on the website of FIND (https://www.finddx.org/covid-19/test-directory/; 113 tests listed as POC or nearly POC which detect SARS-CoV-2 RNA).

Some of these tests use RT-PCR and have a similar performance as lab-based RT-PCR (for example the cobas Liat test of Roche). They are however relatively expensive and there is limited availability of these tests on the market (4).

Other tests use isothermal amplification techniques (such as the Abbott ID NOW assay) and have a similar specificity as RT-PCR, but a lower sensitivity. Sensitivity is, however, higher than when using rapid Ag tests. The cost is lower than the lab-based PCR but higher than the rapid Ag test (for example the ID NOW reader cost 2,500 EUR, and 26 EUR per test). Their availability on the market is good (4,5).

In the recommendation made by the RAG in April 2021, there was agreement that rapid tests using RT-PCR (such as the cobas Liat) are best reserved for hospital settings, because of their high cost and limited availability. There was no consensus with regards to the usefulness of isothermal amplification at primary health care level.

The following persons participated to this advice:

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ANNEX 1: Current indications for whole genome sequencing

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:
    - Infections in fully vaccinated people (>7 days after full vaccination) with a severe clinical picture, requiring hospitalization, and in fully-vaccinated nursing home residents.
    - 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)[1].
    - 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 and a coverage of 90% was achieved
- Outbreaks in which the index case was confirmed to have an atypical variant

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

- 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.

[1] 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)