L'UTILISATION D'UN PROTOCOLE DE TEST PCR POUR DÉTECTER RAPIDEMENT LES VARIANTES PRÉOCCUPANTES DE SARS-COV-2

RAG sous-groupe testing –21 Juin 2021

Note : Les recommandations actuelles sont susceptibles d'être modifiées en fonction de nouvelles informations et/ou de l'évolution de l'épidémie.

Recommandations :

- Étendre le plus largement possible la capacité de réaliser un génotypage par PCR permettant une détection précoce des principales mutations. Dans un premier temps dans tous les laboratoires de la Plateforme Bis, puis dans tous les laboratoires effectuant des tests RT-PCR pour le SARS-CoV-2.
- Le protocole proposé par le NRC est un bon exemple et peut être utilisé par d'autres laboratoires. Cependant, d'autres protocoles qui produisent des résultats similaires à des coûts similaires sont également acceptables.
- Avant d'introduire le protocole dans les laboratoires, il convient de procéder à une validation pour s'assurer que le protocole comprend suffisamment de tests de génotypage et que les résultats sont correctement communiqués.
- La décision concernant les échantillons positifs à tester avec le protocole de génotypage (tous ou seulement dans certains situations ou aucun) est prise sur la base des principes suivants :
    - La présence ou l'absence d'un VOC nouvellement émergent pour lequel il est pertinent de prendre des mesures supplémentaires en temps utile (<3 jours après le test positif) pour contenir sa propagation.
    - La capacité locale de prendre des mesures supplémentaires pour les personnes chez qui un nouveau VOC émergent a été identifié, comme l'intensification du suivi de l'isolement/de la quarantaine (par exemple par téléphone ou via un agent de terrain).
    - S'il n'est pas possible/souhaitable de tester tous les échantillons positifs, donner la priorité aux échantillons provenant de :
        - voyageurs venant d'une zone où l'on sait que le VOC en question est en circulation
        - patients sévèrement malade, si cela est pertinent pour l'approche clinique ou pour prévenir la transmission nosocomiale
        - les infections post-vaccinales, y compris après une dose unique
        - réinfections
        - échantillons positifs en cas de clusters inhabituels

    - populations présentant un risque accru de mutation (patients atteints d'une infection chronique persistante ; patients immunodéprimés ; participants à des études cliniques COVID-19)
- En outre, poursuivre le séquençage du génome complet conformément aux recommandations convenues précédemment pour la sélection des échantillons à séquencer dans le cadre de la surveillance de base et de la surveillance active.

Les personnes suivantes ont participé à cet avis :

Emmanuel André (UZ/KU Leuven-NRC); Emmanuel Bottieau (ITG/IMT) ; Lize Cuypers (UZ/KU Leuven-NRC); Bénédicte Delaere (CHU-UCL Namur); Olivier Denis (CHU-UCL Namur); Achille Djiena (AVIQ); Herman Goossens (UAntwerpen); Marie Pierre Hayette (CHU-Liège); Sunita Janssenswillen (KU Leuven-NRC); Sofieke Klamer (Sciensano); Yves Lafort (Sciensano); Barbara Legiest (AZG); Tinne Lernout (Sciensano); Pieter Libin (UHasselt); Veerle Matheeussen (UZAntwerpen); Elizaveta Padalko (UZGent); Sophie Quoilin (Sciensano); Nancy Roosens (Sciensano); Patrick Smits (AZG); Olivier Vandenberg (LHUB-ULB); Ann Van den Bruel (KU Leuven); Steven Van Gucht (Sciensano); Pieter Vermeersch (UZ-Leuven).

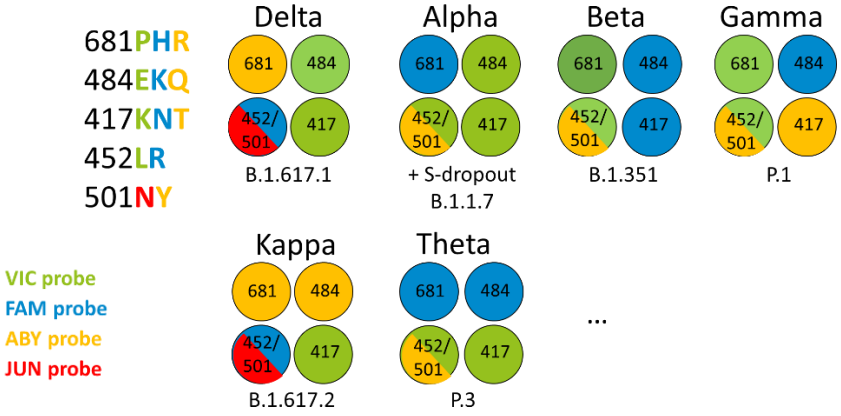
CONTEXT

In response to the increasing circulation of new SARS-CoV-2 variants of concern (VOCs), whole-genome sequencing of positive SARS-CoV-2 samples was reinforced in Belgium, and a RAG advice was formulated on indications for sequencing positive RT-PCR samples. The number of VOCs keeps increasing, with as latest addition the B.1.617.2 (Delta) variant that according to the genomic surveillance report of 15 June 2021 represented 6.1% of all variants in Belgium between 31 May and 13 June, 2021. The sequencing process, however, requires time and results are only available approximately one to two weeks after the sample was taken. This causes a delay in taking appropriate actions, such as intensified isolation and contact tracing, when a new VOC is detected. To address this problem, the National Reference Centre (NRC) developed a standardized protocol using a combination of PCR tests to rapidly (in one or a few days) detect mutations of concern at a limited additional cost (5.55 euro total extra cost per sample). UZ Antwerpen (UZA) also applies a similar approach using serial PCR tests to rapidly detect mutations of importance.

An advice was requested from the RAG Testing on the usefulness of such a testing protocol, the level of laboratory where it is useful and the indications for which it could be applied.

PROPOSED TESTING PROTOCOL

The testing protocol developed by the NRC uses a combination of custom TaqPath SNP Genotyping Assays (Thermo Fisher). Four PCR reactions are assessed per sample, detecting key mutations (681 P-H-R; 484 E-K-Q; 417 K-N-T; 452 L-R; 501 N-Y) that indicate a VOC (see figure below).



The protocol was applied to all positive RT-PCR samples with a Cq<30 during the period May 19 – June 13 and was able to document the evolution of the prevalence of the Delta variant, about one week prior to the whole genomic sequencing surveillance.

UZA uses a different approach of serial PCR testing, using primer/probes from TIB Molbiol + Taqman Fast Virus 1-Step Master mix (ThermoFisher). First, samples with S-gene drop-out are considered as alpha variant and excluded from further testing. Then, a test detecting N501Y, E484K and H655Y mutations is performed to identify beta and gamma variants, and finally a test

detecting L452R, P681R mutations to allow identification of the delta variant. Similarly to the experience by the NRC, this approach resulted in early detection of the Delta variant.

Other available assays include:

- the variant II assay, a reflex PCR with SARS-CoV-2 Variant I and II of Seegene that detects N501Y, 69-70del, E484K, K417T, K417N, L452R and W152C mutations, allowing identification of B.1.1.7 (with or without E484K), B1.351, P.1, B1.617 and B.1.429.
- the Sanger sequencing protocol, that provides results within 48 hours.

INTERNATIONAL RECOMMENDATIONS AND OTHER COUNTRIES

ECDC

ECDC issued a document on methods for the detection and identification of SARS-CoV-2 variants in March 2021 (1). The document proposes different diagnostic screening assays, mostly intended to detect the three VOCs in circulation at that time (alpha, beta and gamma). No screening assay to detect the delta variant was yet included, as it was not circulating at that time.

UK

In the UK, a genotyping PCR test is used, allowing a shorter turnaround time (12-24h after initial confirmation of COVID-19) for a probable variant result (2,3). The initial panel of targets used single nucleotide polymorphisms (SNPs): N501Y, E484K, K417N and K417T. On 11 May 2021, after rapid validation of targets to allow identification of Delta variant, P681R was introduced in the panel to replace N501Y. All positive RT-PCR results at Pillar 2 laboratories with sufficient quality to allow genotyping are processed.

France

In France, RT-PCR screening tests for mutations of interest ('tests RT-PCR de criblage') are systematically performed on all positive tests to allow for more reactive monitoring (4). In case of a positive diagnosis of a first RT-PCR test, a screening RT-PCR test is performed to detect the main mutations of interest E484K, E484Q and L452R because of their impact on transmissibility (L452R) or a possible escape from the immune response (L452R, E484K and E484Q). Data on these screening tests are analyzed by Santé Publique France to assess in real time their circulation in the territory. The available screening data associated with epidemiological indicators (incidence, positivity rate) and contact-tracing data (possible clusters) are useful to identify in a reactive way any abnormal situation that could lead to the suspicion of the emergence and diffusion of certain variants carrying mutations of interest in a given territory. In case of unusual situation identified, a sequencing reinforcement could be set up in the concerned geographical area.

Germany

The Robert Koch Institute recommends PCR-based genotyping assays for the timely detection of VOCs (5).

DISCUSSION

- Different opinions exist on the usefulness of systematically obtaining SNP genotyping PCR results for rapid identification of VOCs.
- On the one hand:
    - Early identification of VOCs allows for actions adapted to each VOC, such as a more strict control of isolation and contact tracing. This could potentially slow down the spread of a newly emerging VOC that possible is less susceptible to current vaccines and/or causes more severe disease.
    - The current rapid spread of the Delta variant is a good example, and a more strict control could slow its spread and prevent that it becomes dominant in a period in which the vaccination coverage is still low.
    - Precise monitoring of the spread of VOCs can at least inform clearly on the consequences of suboptimal isolation and contact tracing policies.
    - The supplementary financial cost of the test protocol (5.5 euro per tested sample) is limited.
    - This approach is already applied in some other countries, such as the UK and France
    - The use of rapid identification of the Alpha variant by S-gene dropout PCR was useful to monitor & inform policy makers.
- On the other hand:
    - The current guidelines with regards to isolation and contact tracing do not differentiate between VOCs, and a strict respect of the measures is needed for all variants.
    - Most VOCs are already endemic, and do not need different measures, and also the Delta variant is projected to become dominant soon (probably before the VOC PCR is widely available).
    - The rapid spread of the Alpha variant, where early identification by detecting S-gene dropout was possible, shows that it had little effect on the spread.
    - There is no sufficient capacity to systematically follow-up all people infected with a VOC.
    - Even if the additional cost is limited, it might still not justify the benefits.
    - The relevance of early identification of VOCs differs in function of the context. For example, VOCs might be more common in travelers returning/ arriving from countries where a VOC is known to circulate, in unusual cluster outbreaks, or in breakthrough infections.
- If SNP genotyping PCR testing is done, it should be available as widely as possible, including at peripheral (clinical) laboratories. The Platform Bis laboratories represent only 20-25% of all positive PCR tests and this is insufficient to have an impact.
- Before introducing a SNP genotyping PCR protocol, it needs to be validated to each laboratory's specific context.
- SNP genotyping PCR testing can only be done on RT-PCR positive test results with sufficient high viral load (threshold to be defined).

- The test protocol should not replace the current whole genomic surveillance system, but be complementary to it, because it still remains important to detect eventual new mutations not-detected by the protocol. Both the baseline routine surveillance and the active surveillance have to be maintained.
- A protocol using in-house RT-PCR tests offers the advantage that it can easily be adapted to newly emerging mutations. The current NRC protocol uses commercial RT-PCR tests, but is sufficiently flexible. NRC could relatively quickly alternate the combination of genetic markers according to emerging mutations.
- Several protocols can be applied, as long as they give similar results as the NRC protocol. The NRC protocol allows a rapid identification of more mutations than the UZA protocol. A disadvantage of the UZA protocol is that all samples with S-gene depletion are not included which might result in missing certain VOCs.

RECOMMENDATIONS

- To expand the capacity to perform SNP PCR genotyping, that allows early detection of key mutations, as widely as possible. In a first phase at all Platform Bis laboratories, in a later phase at all laboratories performing SARS-CoV-2 RT-PCR testing.
- The protocol proposed by NRC is a good example and could be used by other laboratories. However, other assays giving similar results at comparable cost are acceptable as well.
- Before introducing the protocol at laboratories, a validation needs to be done to ensure that the protocol contains sufficient genotyping assays and that the results are correctly reported.
- To continue the whole genome sequencing program, applying the indications for baseline and active surveillance previously agreed on[1].
- 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. Early identification of VOCs that are already endemic (such as de Alpha and Beta variants) is considered no longer useful. Early identification of the Gamma and Delta variant is currently still considered potentially useful.
    - 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. Precise guidance is needed on what additional measures are required.
    - If not possible/ useful to test all positive samples, give priority to samples from:

[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)

- Travelers returning/arriving from an area with known circulation of a newly emerging VOC. The indications for whole genomic sequencing in positive samples of travelers remain unchanged (maximum of 500 representative positive samples/week of travelers coming from a red zone).
- 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 infections in fully vaccinated people (>7 days after full vaccination) still need to be sequenced.
- All reinfections of which the first infection has been properly documented. Reinfections, or at least a selection, still need 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.

REFERENCES

1. Methods-for-the-detection-and-identification-of-SARS-CoV-2-variants.pdf [Internet]. [cited 2021 Jun 15]. Available from: https://www.ecdc.europa.eu/sites/default/files/documents/Methods-for-the-detection-and-identification-of-SARS-CoV-2-variants.pdf

2. C1297-VOC-and-Sequencing-FAQs-25-May.pdf [Internet]. [cited 2021 Jun 15]. Available from: https://www.england.nhs.uk/coronavirus/wp-content/uploads/sites/52/2021/05/C1297-VOC-and-Sequencing-FAQs-25-May.pdf

3. SARS-CoV-2 variants of concern and variants under .pdf [Internet]. [cited 2021 Jun 15]. Available from: https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/993198/Variants_of_Concern_VOC_Technical_Briefing.pdf

4. Circulation des variants : nouvelle stratégie de criblage par la recherche de mutations d'intérêt [Internet]. [cited 2021 Jun 16]. Available from: /les-actualites/2021/circulation-des-variants-nouvelle-strategie-de-criblage-par-la-recherche-de-mutations-d-interet

5. Hinweise zur Testung von Patienten auf Infektion mit dem neuartigen Coronavirus SARS-CoV-2 [Internet]. Available from: https://www.rki.de/DE/Content/InfAZ/N/Neuartiges_Coronavirus/Vorl_Testung_nCoV.html;jsessionid=1692ACA7D0ACF631627B6B9EB27EA35F.internet101?nn=13490888#doc13490982bodyText28