**INTERPRÉTATION D'UN RÉSULTAT RT-PCR AVEC SEULEMENT DES TRACES DE SARS-COV-2**

**RAG sous-groupe Testing – 31 mai 2021**

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

### Recommandations :

- **Dans les cas où seules des traces de SARS-CoV-2 sont trouvées (charge virale <10³ copies/mL pour le gène N et négative pour le gène S et l'ORF 1ab),** il est recommandé de distinguer les cas où le risque d'infection précoce est plus élevé de ceux où il est plus faible :
- **Les cas présentant un risque plus élevé d'infection précoce :**
  - Il s'agit de contacts à haut risque, de voyageurs revenant d'une zone rouge ou de personnes testées dans le cadre d'un cluster.
  - Dans la mesure du possible, on cherche à savoir si les traces sont un signe d'une infection précoce ou d'une ancienne infection/résultat faussement positif.
  - Le diagnostic de l'infection ancienne est posé selon les critères applicables (voir: [20201208_Advice RAG Interpretation and reporting of COVID PCR results.pdf](sciensano.be)) :
    - Absence de symptômes depuis au moins 10 jours, et apparition des symptômes précédents éventuels il y a au moins 4 semaines ;
    - Aucun contact à haut risque au cours des 3 dernières semaines ;
    - Test PCR positif antérieur au moins une semaine avant, ou sérologie positive connue.
  - Si les critères d'une infection ancienne sont remplis : déclarer comme infection ancienne, aucun isolement ou recherche des contacts n'est entrepris, les voyageurs sont autorisés de voyager.
  - Si les trois critères ne sont pas remplis ou si l'on ne dispose d'aucune information sur un précédent résultat positif de PCR ou sur une sérologie connue, une sérologie est demandée et un deuxième test PCR est effectué 36 heures après le premier.
  - Si la sérologie est positive et qu'il n'y a pas d'augmentation de la charge virale : déclarer comme une infection ancienne, pas d'isolement ni de recherche des contacts, voyages autorisés.
  - Si la sérologie est négative et qu'il y a une augmentation de la charge virale : déclarer comme un cas positif, l'isolement et la recherche des contacts sont mis en place, les voyages sont interdits.
Les personnes suivantes ont participé à cet avis :
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**CONTEXT**

The current advice with regards to PCR results in which only the N-gene is weakly positive ([RAG advice of 8 December 2020](#)) is that the labs of the ‘federal testing platform’ report samples with viral load RNA copies $<10^{3}$/mL for the N-gene but a negative result for S-gene and ORF1ab as a separate category ("traces of SARS-CoV-2") which should not trigger any contact tracing/isolation of the case, because the person is considered not (or no longer) contagious.

This recommendation is based on preliminary results that show that the N-gene remains positive for a longer time than other gene targets. Because 2 additional gene targets are simultaneously assessed, the risk is low that the negative results for both of these gene targets are due to technical issues. This applies only to the federal testing platform (which uses highly standardized processes and uses a three-gene assay) and should not be generalized to other platforms.

However, doubts were raised by some laboratories if all of these samples should systematically be considered as non-contagious, because some of them might indicate an early infection. Moreover, federal testing platforms no longer have a real ‘three-gene’ assay as the UK variant is dominant and is characterized by a drop-out of the S-gene. They rely on the curves of only the N-gene and the ORF1ab-gene. It was therefore proposed to allow different interpretations when the load of the N-gene is $<10^{3}$ RNA copies/mL and the ORF1ab and S-gene are negative:

- **Interpretation 1:**
  The patient is probably not or no longer contagious if there is also clinical and/or serological evidence of an old, cleared infection.

- **Interpretation 2:**
  Single target N genes are reported negative (if also single target N gene detected after reanalysis)

- **Interpretation 3:**
  Traces

- **Interpretation 4:**

Les cas présentant un risque moindre d’infection précoce :
- Il s’agit de tous les cas autres que ceux énumérés ci-dessus, par exemple les personnes asymptomatiques dépistées avant leur départ à l’étranger ou leur participation à un événement.
- Pas d’autres investigations, déclarer comme négatif, pas d’isolement ni de recherche des contacts, voyage autorisé.
Inconclusive; The patient should be retested.

- **Interpretation 5:**
  Negative

- **Interpretation 6:**
  This PCR result (N-gene positive, S-gene negative, ORFab negative) fits best with a post-COVID image, no longer infectious. To be correlated with clinic and serology.

The RAG testing was requested to provide advice.

**EVOlUTION OF ‘ONLY N-GENE POSITIVE’ SAMPLES OVER TIME**

The NRC has collected the available data from the platform laboratories on follow-up samples of initial samples that fulfilled the criteria of ‘traces of SARS-CoV-2’ for the period January-April 2021. Overall, for 40% of 4395 samples the result of a follow-up sample was available, and of these 12.8% tested positive.

**Table: Results of follow-up samples of ‘Only N-gene positive’ samples**

<table>
<thead>
<tr>
<th></th>
<th>January 2021</th>
<th>February 2021</th>
<th>March 2021</th>
<th>April 2021</th>
</tr>
</thead>
<tbody>
<tr>
<td># ‘Only N-gene positive’ samples (so S and ORF negative) (D0)</td>
<td>648</td>
<td>575</td>
<td>1688</td>
<td>1484</td>
</tr>
<tr>
<td># follow-up samples* after ‘only N-gene positive’ report (D2 – D7)</td>
<td>186</td>
<td>292</td>
<td>783</td>
<td>515</td>
</tr>
<tr>
<td># follow-up samples reported: ‘Positive’ (D2 – D7) (%)</td>
<td>29 (15,6%)</td>
<td>27 (9,3%)</td>
<td>108 (13,8%)</td>
<td>63 (12,2%)</td>
</tr>
<tr>
<td># follow-up samples reported: ‘Only N-gene positive’ (D2 – D7) (%)</td>
<td>12 (6,4%)</td>
<td>10 (3,4%)</td>
<td>26 (3,3%)</td>
<td>18 (3,5%)</td>
</tr>
<tr>
<td># follow-up samples reported: ‘Negative’ (D2 – D7) (%)</td>
<td>145 (78%)</td>
<td>255 (87,3%)</td>
<td>649 (82,9%)</td>
<td>434 (84,3%)</td>
</tr>
</tbody>
</table>

*follow-up samples*: amount of patients who were resampled within 2 – 7 days after they were ‘Only N-gene positive / traces of SARS-CoV-2’

**DISCUSSION**

- According to the data collected by NRC, an important percentage (about 10-15%) of the samples classified as ‘traces of SARS-CoV-2’ become positive later on and are thus early infections. Isolation and contact tracing is for these cases not systematically initiated, with a risk of further transmission. It has to be observed that only 40% of the samples a follow-up result was available. It is possible that these were cases in which there was a reason to retest (such as appearance of symptoms,...) and that there is a substantial bias. However, another reason for having no follow-up test is that it was done in another lab than the initial sample (especially in the context of ambulant patients that are tested in triage centers). It is thus unlikely that all not-retested samples would have tested negative.

- On the other hand, classifying all people with traces as ‘positive’ will put a lot of people unnecessarily in isolation and quarantine, and it can prohibit people from travelling. Even if not considered as a case,
people in which traces are detected are not classified as ‘negative’ either and can therefore not present a negative test certificate (and thus not travel). With the upcoming holidays, it is expected that this will put a lot of pressure on practitioners and laboratories from departing travelers.

- Moreover, it is unlikely that the tested person was infectious at the time of sampling, which is the key question to be answered by the testing. Not even negative test results should be taken as a guarantee for non-infectiousness in the future (>48h after sampling). This is the reason why quarantine is maintained despite a negative test result at the beginning of quarantine and or why a negative test result at day 7 of quarantine should still be followed by further heightened vigilance and low-threshold testing in case of symptoms. Results detecting “traces” or not different in this regard.

- The recommendation from a meeting with the platform laboratories was to classify cases with traces as ‘inconclusive’, to re-test and, based on the evolution of the viral load, to make a conclusive diagnosis (positive if increasing, negative if not). To accommodate the need of travelers who need the result rapidly, the federal platform recommends to retest within 48 hours, for example after 36 hours. There is evidence that by then, early infections will already present an increased viral load.

- In some cases (for example at ITM where the lab is in direct contact with the clinicians) it is possible to already make a conclusive diagnosis, based on the history. If there is clinical and/or serological evidence of an old, cleared infection, the departing traveler is classified as ‘negative’.

- One possible solution is thus to differentiate between old infections and suspected early infections, similar as is currently done for weak positive results. To have an early decision, serological testing (anti-Spik SARS-CoV-2 IgG) could be done and, if negative, a second PCR test at least 24 hours after the initial test. A limitation is that blood collection for serology is not possible at a test center and analysis not possible at a federal platform laboratory.

- Another possible solution could be to retest with a rapid Ag test after 1-2 days, and classify as negative if it is negative. That should pick up most early infections that by then have a higher viral load. Here also, there is the challenge that many laboratories do not perform rapid Ag tests.

- Travelers classified as an old infection, or as a negative result, should ideally have a certificate that clearly states them as ‘negative’. The certificate used in Switzerland could use as an example. This assumes that the destination country accepts such certificates.

- The EU Digital Green Certificate includes certificates for persons who have recovered from COVID-19. Once this certificate is implemented, this could resolve the issue of what certificate to provide to travelers with traces and believed no longer to be contagious.

**RECOMMENDATIONS**

- The decision to further explore cases in which only traces of SARS-CoV-2 are detected depends on the context:

- In a context in which the risk that the traces are the beginning of an early infection is relatively higher, such as among high-risk contacts, returning travelers or cluster investigations, it is advised to further explore if this is a false positive/ old, no longer infectious infection, or an early infection. To classify as an old infection, the same criteria as for weak positive (< $10^5$ copies/mL) results can be used:

  - Establish criteria for considering it an old infection, using
1. No symptoms for at least 10 days, start of previous symptoms at least 4 weeks ago; AND
2. No high-risk contact in the past 3 weeks; AND
3. Previous positive PCR test at least one week before, or known positive serology.
   - If criteria for old infection are fulfilled: classify as such, no isolation or contact tracing initiated, travelling allowed
   - If criteria for an old infection not fulfilled or, if no information available on previous positive PCR test or known positive serology: serology test and repeat PCR test after 36 hours
     - If serology positive and no increase in viral load: classify as old infection
     - If serology negative and increase in viral load: classify as positive, isolation or contact tracing initiated, travelling not allowed

- In a context in which the risk that the traces are a sign of an early infection is low, such as screening in asymptomatic people pre-traveling or pre-attending an event, no further exploration is necessary and the cases can be classified as negative. No isolation or contact tracing is initiated, and travelling is allowed.

**LITERATURE AND INTERNATIONAL GUIDELINES**

A literature review was done by the National Reference Laboratory in November 2020. This review focused on viral load limits that exclude infectivity. Only few articles were identified that differentiated between low viral load in early infections and low viral load in older infections.

Bonten et al. summarized the interpretation of weakly positive PCR results that can indicate (1) a very early infection in which the viral load will still increase; (2) a just-completed infection in which the virus concentration decreases again; or (3) a previously-completed infection in which residual RNA is detected (1). To interpret the result correctly, additional information from the patient is needed. If the infection is early, the patient will probably still become infectious and it is then recommended repeating the test. In other cases, the patient may be no longer infectious and there is no need for isolation and contact tracing.

Figure adapted from Sethuraman, et al. Interpreting diagnostic tests for SARS-CoV-2. JAMA.

No international or national guidelines were identified on the interpretation of traces of SARS-CoV-2 RNA.
An article by the Tropical and Humanitarian Medicine Division of the Geneva University Hospital describes the procedures applied for departing travelers (2). Asymptomatic travelers with a positive PCR result are called 12 to 24 hours after the result to verify the presence or absence of symptoms. If still asymptomatic, results are then interpreted according to three categories:

- **Acute (contagious) SARS-CoV-2 infection:** defined by a positive test (Ct value < 32) irrespective of an old RT-PCR-confirmed infection or the presence of acute symptoms.
- **Recent SARS-CoV-2 infection in the noncontagious phase (referred to in the text as post-Covid):** defined by a positive molecular test with a Ct value ≥ 32 in a person asymptomatic at the time of screening, and a past infection confirmed by RT-PCR or rapid antigenic testing (between 14 days and 3 months). Kinetics between Ct values should support the hypothesis of past infection.
- **SARS-CoV-2 infection at an unknown stage (potentially contagious):** defined by lack of information in persons who could not be reached by telephone or for whom the evidence does not allow a clear interpretation, regardless of the Ct value.

Only category 2 is allowed to travel, with a "negative result interpretation" certificate, certifying the absence of Covid-19 symptoms and contact with a confirmed case. During the period October-December 2020, 10 out of 210 positive testing travelers (4.8%) were classified as category 3 and 30% as category 2 (non-contagious post-COVID).

**REFERENCES**
