

## INTERPRETATIE VAN EEN RT-PCR RESULTAAT MET ENKEL SPOREN VAN SARS-COV-2

RAG subgroep Testing – 31 mei 2021

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

### Aanbevelingen:

- Bij gevallen waarbij enkel sporen van SARS-CoV-2 gevonden worden (virale lading  $<10^3$  kopies/mL voor het N-gen en negatief voor het S-gen en ORF1ab) wordt aanbevolen om een onderscheid te maken tussen gevallen waar er een hoger, en gevallen waar er een lager risico bestaat op vroege infectie:
- Gevallen met een hoger risico op vroege infectie:
  - Dit zijn hoog-risico contacten, personen die terug komen uit een rode zone, of personen getest in de context van een cluster onderzoek.
  - In de mate van het mogelijke wordt verder onderzocht of de sporen een teken van een vroegere infectie of een oude infectie/ vals positief resultaat zijn.
  - De diagnose oude infectie wordt gesteld volgens de geldende criteria (zie: [20201208 Advice RAG Interpretation and reporting of COVID PCR results.pdf \(sciensano.be\)](https://www.sciensano.be/20201208-Advice-RAG-Interpretation-and-reporting-of-COVID-PCR-results.pdf)):
    - Geen symptomen sinds tenminste 10 dagen, en begin van eventuele vroegere symptomen tenminste 4 weken geleden;
    - Geen hoog-risico contact in de voorbije 3 weken;
    - Eerdere positieve PCR-test ten minste een week voordien, of bekende positieve serologie.
  - Indien voldaan aan de criteria van een oude infectie: rapporteren als oude infectie, geen isolatie of contact tracing gestart, reizen toegestaan.
  - Indien niet voldaan aan alle drie de criteria of er is geen informatie beschikbaar over een eerder positief PCR-resultaat of bekende serologie, wordt een serologie aangevraagd en een tweede PCR-test uitgevoerd 36 uur na de eerste.
  - Indien serologie positief en geen toename van virale lading: rapporteren als oude infectie, geen isolatie of contact tracing gestart, reizen toegestaan.
  - Indien serologie negatief en een verhoging van de virale lading: rapporteren als positief geval, isolatie en contact tracing wordt gestart, reizen is niet toegestaan.
- Gevallen met een lager risico op vroege infectie:
  - Dit zijn alle andere gevallen dan bovenvermelde, zoals bijvoorbeeld asymptomatisch personen gescreend voor het vertrek naar het buitenland of voor deelname aan een evenement.
  - Geen verder onderzoek en rapporteren als negatief, geen isolatie of contact tracing gestart, reizen toegestaan.

## De volgende personen hebben deelgenomen aan dit advies:

Emmanuel André (KU Leuven); Emmanuel Bottieau (ITG/IMT); Lize Cuypers (NRC); Achille Djiena (AVIQ); Olivier Denis (CHU-UCL Namur); Herman Goossens (UAntwerpen); Marie Pierre Hayette (CHU-Liège); Reile Janssen (NRC); Yves Lafort (Sciensano); Barbara Legiest (ZG); Tinne Lernout (Sciensano); Elizaveta Padalko (UZGent); Olivier Vandenberg (LHUB-ULB); Ann Van den Bruel (KU Leuven); Pieter Vermeersch (KU Leuven).

## 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 <math>10^3</math>/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 <math>10^3</math> 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:  
Inconclusive ; The patient should be retested.
- Interpretation 5:  
Negative
- Interpretation 6:  
This PCR result (N-gene positive, S-gene negative, ORF1ab 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**

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

\* **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 of 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-S(pike) 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 \(< 10<sup>5</sup> copies/mL\) 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.

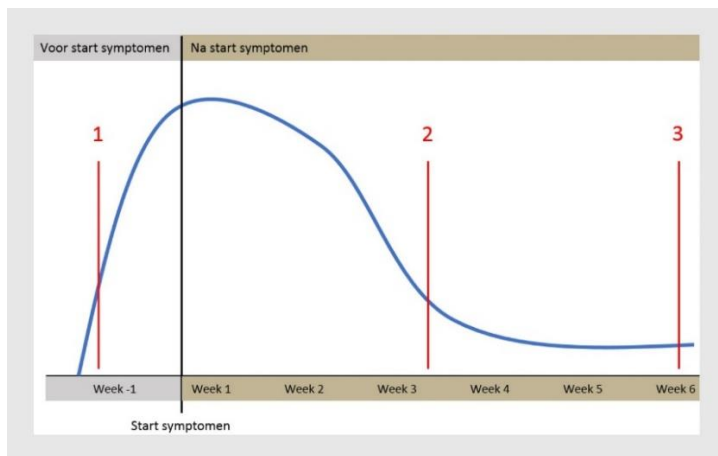


Figure adapted from Sethuraman, et al. Interpreting diagnostic tests for SARS-CoV-2. JAMA.

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  $\geq 32$  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

1. Covid-19: een fout-positieve PCR-testuitslag | Nederlands Tijdschrift voor Geneeskunde [Internet]. [cited 2021 May 21]. Available from: <https://www.ntvg.nl/artikelen/covid-19-een-fout-positieve-pcr-testuitslag/volledig>
2. Revue Médicale Suisse - la revue médicale francophone de référence pour la formation continue des médecins. [Internet]. Revmed.ch. [cited 2021 May 25]. Available from: <https://www.revmed.ch/revue-medicale-suisse/2021/revue-medicale-suisse-737/depistage-de-l-infection-a-sars-cov-2-chez-les-voyageurs.-quelle-approche-pour-evaluer-le-risque-de-transmission>