RAG INTERPRETATION AND REPORTING OF SARS-COV-2 PCR RESULTS

Meeting 08/12/2020

Aanbevelingen:

1. Labo's moeten PCR-resultaten standaard semi-kwantitatief rapporteren, gebruikmakend van de indeling in 4 niveaus (zwak positief tot zeer sterk positief) zoals die door het NRC werden voorgesteld.
2. De labo's van het federaal platform kunnen bovendien een resultaat rapporteren als "sporen van SARS-CoV-2" indien enkel het N-gen zwak positief is en indien de twee andere genen (ORF1a en S) negatief zijn. Een dergelijk resultaat staat qua maatregelen gelijk aan een negatief resultaat.
3. In de categorieën positief en zwak positief kunnen de criteria die eerder vooropgesteld werden om een infectie als 'oude infectie' te beschouwen, verder behouden worden. Enkel in deze gevallen zijn geen contact tracing en isolatie nodig
4. Indien het testresultaat bekomen wordt in het kader van screening van asymptomatische personen (d.w.z. minstens 1 week geen symptomen van SARS-CoV-2, minstens 3 weken geen hoog-risico contact), maar een voorgaand positief PCR- of serologieresultaat zijn niet beschikbaar, kunnen volgende elementen in rekening worden gebracht om een positief of zwak-positief testresultaat te klasseren als "oude infectie":
    a. positieve serologie afgenomen binnen 48h na PCR-staal
    b. PCR-staal afgenomen minstens 2d na initiële PCR-staal met dezelfde of lagere virale lading.
    c. anamnese sterk suggestief voor doorgemaakte SARS-CoV-2 voorbije 8 weken (bv. plotse anosmie, respiratoire symptomen na hoog-risico contact bevestigd geval…).

    In afwachting van bevestiging/uitsluiten van een oude infectie wordt de persoon in dat geval in individuele druppel-contact-isolatie geplaatst (geen cohorte of COVID-afdeling).

Background

In June and July, RAG advices were given on SARS-CoV-2 PCR values and contagiousness. The threshold was set at 10^5 viral RNA copies/ml for a "low viral load", based on scientific literature.
Labs were also requested to report in a semi-quantitative way to patients/requesting physicians. It is particularly important when test results are obtained by screening of asymptomatic patients (e.g. pre-hospital admission or in residents of long-term care facilities) to be able to distinguish a recent, contagious infection from an 'old infection' for which no further measures are required. For the Sciensano database, old infections should not trigger contact tracing and should not be counted towards new case numbers.

Based on more literature and own lab research, the NRC respiratory pathogens (UZ Leuven) now prepared a guideline regarding the interpretation and reporting of SARS-CoV-2 PCR-results.
Ct values for the same viral load vary widely between assays and between laboratories with the same assay. Cutoffs for Ct values that predict negative viral culture are therefore very variable between studies (ranging from >24 to >35).

Because of these known issues with Ct-values, the proposal is to use absolute quantification (viral RNA copies). In order for labs to be able to report this, they will need to analyze standardized samples of the

NRC and make their own corresponding calibration curves Ct-values/viral RNA copies. Steps have been taken to initialize this project and it is estimated that this should be technically ready (from the lab-side) by January 2020.
This initiative will know two phases:

- Phase 1: inactived and quantified viral cell culture (very strong positive) will be distributed to all labs, together with guidelines to prepare dilutions and set up a calibration curve.
- Phase 2: commercial quantified panel of 4-5 samples will be distributed to all labs

Timeline: phase 1 December (before Christmas) – phase 2 February

The proposal for reporting and the associated evaluation of contagiousness is as follows:

Semi-quantitative reporting of RT-PCR values in four categories:

- very strongly positive: ≥ 10^7 RNA copies/mL
  'the patient is contagious'
- strongly positive: ≥ 10^5 - <10^7 RNA copies/mL
  'the patient is probably contagious'
- positive: ≥ 10^3 - <10^5 RNA copies/mL
  'the patient is potentially contagious, unless there is clinical and/or serological evidence of an old, cleared infection'
- weakly positive: < 10^3 RNA copies/mL
  'the patient is probably not or no longer contagious if there is also clinical and/or serological evidence of an old, cleared infection'.

Operational issues on reporting to the central database and compliance of the national platform will be clarified by the IFC Test&Trace. The RAG is asked for advice on implications on previously established guidelines.

Summary of the discussions

1. **Consequences of "weakly positive":**
   - in the suggested interpretation it is stated that the patient is not contagious if there is evidence of an old, cleared infection
   - however, the NRC proposed for reporting to the central database, that all of these results would be discarded

   The RAG advices that decisions on contact tracing / isolation of cases with a positive result, should always be informed by additional clinical information. "Weakly positive" results should not be automatically discarded in the contact tracing database.

   However, if the infection is deemed to be an old infection (see criteria below), these cases are no longer contagious so isolation and further contact tracing are not necessary.

2. **Distinction between "strongly positive" and "very strongly positive".**
   - Should different measures be taken for "strongly positive" and "very strongly positive" cases?
   - If not, is it useful to report them as separate categories or would reporting of the viral copies be more useful?

Although the measures that need to follow the identification of a “strongly positive” person will not be different from the measures for “very strongly positive” persons, the addition of an extra category does provide extra information that might be helpful for clinicians and epidemiologists. Therefore, **the RAG accepts the proposed distinction.**

3. **Samples with only N-gene weakly positive**
   The NRC further proposes that the labs of the ‘federal testing platform’ will report samples with viral load RNA copies $<10^3$/mL for the N-gene but a negative result for S-gene and ORFab
   - will be reported as inconclusive.
   - should not trigger any contact tracing / isolation of the case

   The RAG agrees with the proposal to report these results as a separate category for which no additional measures are required and which should not be counted towards new case numbers. 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. However, 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.
   The RAG further advices that the word “inconclusive” already has a meaning and should not be used for reporting this result. **The wording “traces of SARS-CoV-2” is proposed** as an alternative.
   Communication as proposed to federal testing platform partners by the NRC is as follows:
   Wanneer aan de hand van de Applied Biosystems TaqPath COVID-19 CE-IVD RT-PCR kit met de ORF1a, S en N genen als virale doelwitten, in de context van de federale testplatformen, enkel een zwak positief SARS-CoV-2 resultaat (<10^3 copies/ml) bekomen wordt voor het N-gen en een negatief testresultaat voor zowel het ORF1a als het S gen, stellen wij de volgende rapportering voor:
   ‘Sporen van SARS-CoV-2 werden gedetecteerd. De patiënt is niet (meer) besmettelijk.’
   De maatregelen die gekoppeld worden aan dit resultaat staan gelijk aan deze van een negatief testresultaat.

4. **Serological and/or clinical evidence of a cleared infection**
   - What is considered evidence of an old, cleared infection?
   - Should the previous criteria proposed by the RAG be maintained or can repeat testing and/or serology e.g. 1-2 days after the initial weakly positive result also be taken into account?

   Those previously established criteria were:

   1. de persoon vertoonde geen COVID-symptomen gedurende minstens 1 week. Indien er tevoren ernstige symptomen waren, moet de start daarvan minstens 4 weken geleden zijn;
   2. de persoon had geen contact met een bevestigd positief geval in de afgelopen 3 weken (2 weken incubatie + 1 week infectieuze periode);
   3. er was een eerdere positieve PCR-test, minstens 1 week voor de staalname, of een gekende positieve serologie

   The first criterium should be updated to reflect the recently revised guidance on discontinuing isolation of confirmed cases. **The RAG judges that if all three criteria are met, the result**

can be safely classified as an 'old infection', and the patient is no longer contagious so isolation and further contact tracing are not necessary. However, these criteria are quite strict. There might be additional situations where one of the criteria is not met, but contact tracing / isolation are not necessary. This can have important implications for the persons involved (index and contacts).

Most problems arise if the result was obtained after screening of an asymptomatic person (e.g. pre-travel request, nursing home staff/resident, pre-hospital admission, new resident of a collectivity). Criteria 1 and 2 should always be met. **If criteria 1 and 2 are fulfilled, but criterium 3 is not, the person should be isolated individually (not cohorted) whilst confirmation of an old/recent infection is being sought.** Further confirmation can be obtained by one or a combination of the following:

- **requesting additional serology (within 48h after initial sampling)**
  although the interpretation of serology is not straightforward and also depends on the target, negative serology should be interpreted as a recent infection.
- **repeat PCR testing at least 2 days after the initial sampling**
  although viral loads are also dependent on sampling technique and other factors like swab type, if the result remains weakly positive or turns negative after an interval of 48h, this should be interpreted as an old infection. The repeat test should be sent to the same laboratory to rule out other factors that might cause a difference in reporting.
- **detailed clinical anamnesis**
  If the clinical history is strongly suggestive of a past SARS-CoV-2 infection (e.g. respiratory symptoms after contact with a confirmed COVID-case, sudden onset anosmia…) in the past 8 weeks but no PCR result is available, this points towards an old infection

In case of a strong clinical suspicion, a weakly positive result should be considered as positive and the test should be repeated if necessary.

The following experts contributed to this advice:

Emmanuel André (UZ Leuven, NRC respiratory pathogens), Emmanuel Bottieau (ITG, UZA), Steven Callens (UGent), Laura Cornelissen (Sciensano, Epidemiology), Lize Cuypers (UZ Leuven, NRC respiratory pathogens), Olivier Denis (ULB), Frédéric. Frippiat (AVIQ), Herman Goossens (UAntwerpen), Frédérique Jacobs (ULB, Hôpital Erasme), Sofieke Klamer (Sciensano, Epidemiology), Valeska Laisnez (Sciensano, Epidemiology), Bénédicte Lissoir (Grand Hôpital de Charleroi), Romain Mahieu (COCOM), Pierrette Melin (ULG), Elizaveta Padalko (UGent), Paul Pardon (FOD Volksgezondheid, PHE), Catherine Sion (Grand Hôpital de Charleroi), Diane Stroobant (Grand Hôpital de Charleroi), Koen Vanden Driessche (UZA), Steven Van Gucht (Sciensano, Virology)