

Interpretation of PCR results and infectivity

RAG 30/06/2020

1.1. DESCRIPTION OF THE PROBLEM

With the extended testing strategy (and increasing number of asymptomatic persons tested), in some cases, a positive PCR-result can lead to difficult interpretation and subsequent questions related to the necessity of quarantine measures, contact tracing and real-time epidemiological monitoring.

The absence of clear time point of infection (no known contact with an infectious case, nor date of symptom onset) together with prolonged detection of viral RNA (1-3) complicates decisions on whether this person could still be infective or whether the PCR positivity results from an older infection. Wrongly interpreting a positive PCR result as equivalent to infectiousness can have severe implications on all aspects of the life of patients and their household, ranging from access to education in children, postponement of surgery, use of health care resources or economic consequences.

Some examples:

1. The alert level in the South of Limburg is raised because of the detection of a higher number of cases. However, a significant proportion of the cases contributing to this alert have high Ct values and are likely older cases from when the epidemic hit the region hard. In Hasselt, 16 out of 17 recent positive PCR results were classified (after investigation) as probably old infections.
2. Some new screening initiatives in collectivities detected PCR positive cases with high Ct values resulting in lock down of a collectivity.

1.2. THE QUESTION

Is there a way to distinguish an 'older and non-infectious' PCR-positive infection from a 'recent infectious' one ?

1.3. BACKGROUND INFORMATION AND LITERATURE REVIEW

1.3.1. Correlation between infectivity and Ct values or viral load: evidence from literature

Several studies have investigated the correlation between Ct values or viral load and infectivity.

- A Chinese study using a hamster model found that that transmission of SARS-CoV-2 correlated well with detection of infectious SARS-CoV-2 from respiratory tract samples using in vitro Vero cell cultures, while not with detection of viral RNA (4).
- A French study including 155 patients (183 samples) observed a strong correlation between Ct value of RT-PCR with primers targeting the E gene (samples of upper and lower respiratory tract) and sample infectivity in a cell culture model. Patients with samples with Ct values ≥ 34 did not excrete infectious viral particles (5).
- A report from the Korean CDC (6) looked at 285 confirmed patients who had a 're-positive' test (60% after screening, 40% with symptoms) after discharge (up to 81 days after initial symptom onset). Ninety percent of these re-positives had Ct-values >30 , the remaining 10% had values between 25-30. In 180 of these re-positive cases, the researchers tried to culture virus, but they did not succeed in any. Of the 790 contacts of these re-positive patients, three became positive. These three also had a different exposure. Based on these findings, they decided no longer to isolate re-positive cases nor their contacts.
- A Canadian study looking at 90 RT-PCR SARS-CoV-2 positive samples (endotracheal and nasopharyngeal) found that cell culture growth was significantly reduced when RT-PCR Ct values

>24 (primers targeting the E gene). In a multivariable model they found that, for every 1 unit increase in Ct, the odds ratio for infectivity decreased by 32% (7).

- A German group concluded, based on the viral loads of nine hospitalized patients, that little risk of infectivity remained below a viral load of 100,000 viral RNA copies per ml sputum. They also estimated the RNA concentration for <5% isolation success to be 5.40 log₁₀ RNA copies per ml (95% confidence interval -4.11–6.51) (upper and lower respiratory tract). The primers used in this study targeted the E and RdPr genes (1).
- A Dutch, not yet peer reviewed study, investigated shedding of infectious virus in 129 hospitalized COVID-19 patients, and found that viral loads, quantified with RT-PCR targeting the E gene, above 7 log₁₀ RNA copies/mL were independently associated with isolation of infectious SARS-CoV-2 from the respiratory tract (odds ratio [OR]; CI 14,7 (3,57-58,1; p<0,001). The probability of isolating infectious virus was less than 5% when viral RNA load was <6,63 log₁₀ RNA copies/mL in respiratory samples (upper and lower respiratory tract) (8).
- A letter to the editor looking at nine asymptomatic cases detected during screening in Wuhan (positive for N gene and ORF1ab gene and IgG positive) found no transmission in the household before detection of these cases, despite having lived closely together during the lockdown before detection. At the moment of detection of these cases, the average Ct value in nine patients was 36.42 ± 2.06 (ORF1ab gene, range 31.27-38.89) and 34.87 ± 3.73 (N gene, range 27.30-39.47) (9).
- A study of the Belgian NRC has found that samples negative for the E gene and positive for the N gene with high Ct value are no longer infectious (unpublished data).

1.3.2. Uniformity of Ct values across different tests

- Although a cycle threshold (Ct) value correlates inversely with the amount of viral RNA in the sample, this **Ct value is not uniform across all used diagnostic tests**. Whereas low amounts of RNA will result in high Ct values, the exact value will depend not merely on the amount of RNA, but also on the sampling method, the RNA extraction method used and the primers used for amplification.
- In Belgium, **different tests and extraction techniques** are currently used. So although labs will be able to tell whether a sample is in a low range or a high range, exact Ct values are less informative, making it difficult to set an exact threshold.
- Moreover, currently the Belgium laboratories report **qualitative (positive/negative) results**, not all laboratories provide additional information on Ct value and in some cases, the machine that is used for the testing does not report these Ct values (this is the case for at least 11 laboratories).

1.3.3. Distinction between very recent and old infections

- Although studies suggest that viral loads (Ct values) (inversely) correlate with infectivity, a high Ct value/low viral load is also present at the very beginning of the infection, making it **impossible to distinguish very recent from old infections based on Ct value or viral load alone**.
- Additional information, such as the **presence of antibodies**, can be useful to make the distinction. The previously mentioned, not yet peer reviewed Dutch study found that, upon seroconversion, shedding of infectious virus dropped rapidly to undetectable levels (8). A recent systematic review concluded that the median time to antibody detection following symptom onset ranged from 5 to 17 days for IgM and 6 to 14 days for IgG and that IgG antibodies were detected in 90% of individuals 14 days and 100% 4 weeks post-infection (10). Little evidence is available about immune response after asymptomatic infection, however we can assume that if an antibody response is developed, this response will not be quicker than in symptomatic individuals.

1.4. ELEMENTS OF DISCUSSION

- Although studies suggest that infectivity of a person is correlated with the viral load or inversely correlated with Ct values, an international consensus on a cut off value has not yet been established:

- The cut offs mentioned in literature for **infectivity based on Ct values** are between >25 and >34, but as previously mentioned, this depends on certain test characteristics and on the type of sample used (upper versus lower respiratory tract) (1).
- The cut offs mentioned in literature for **infectivity based on viral load** are between <100000 and <6.63log₁₀ RNA copies/ml, but this should also be interpreted with caution since it also depends on the test characteristics and the type of sample used (upper versus lower respiratory tract) (1).
- Not all laboratories performing SARS-CoV-2 PCR can report Ct values.
- A correct interpretation of Ct value is influenced by several factors including pre-analytical conditions (timing and storage of the samples before analysis, specimen quality, type of sample) and analytical conditions (different targets, sample volumes varying according to the manufacturers). Therefore, comparisons of exact Ct values cannot be made between laboratories, and even not for the individual patient in different PCR runs.
- Since high Ct values/low viral loads also occur at the beginning of the infection, additional serological information could be useful for interpretation of results in asymptomatic persons. However, especially in the current epidemiological context with low virus circulation and low positivity rate of PCR testing (about 1%), it is not recommended to perform a serology test systematically with a PCR test, not even for asymptomatic people (overshooting). Performing a serology test for PCR+ results with high Ct value is logistically complicated (another visit to the GP) and would need an extra 1-2 days for final interpretation so it is not useful for e.g. pre-op screening (should be performed with the shortest delay possible). Also this would mean that the positive PCR result is not reported immediately (and contact tracing not triggered), while waiting for the serology result. However, if the serology result comes back negative, contact tracing would start with an extra delay, which must absolutely be avoided.
- Since the antibody response can appear soon (day 5 to 7), a positive PCR with positive serology does not automatically mean that the person is not contagious anymore. However, if Ct values are high, it seems extremely unlikely that the person is still contagious.
- For the moment, the number of persons with a positive PCR result is limited (< 100 a day), therefore the overall impact of placing people (with possible old infections) in isolation and performing contact tracing/testing is estimated to be limited. However, there will be an impact on the alert system for detecting clusters: regions with higher incidence during the epidemic peak are also more at risk to have false alerts now because of a higher chance of detecting old infections now through screening (see example of Hasselt). This is an extra workload for cluster investigation and may create false alarms.

1.5. CONCLUSIONS

Considering the limitations on use of Ct values and/or viral load for deciding on infectivity, it is at this stage not possible to define a unique algorithm for interpretation of a positive PCR result. If a laboratory has enough information (Ct value, E-gene-negative/N-gene-positive result with high Ct value, history of symptoms compatible with COVID-19, previous PCR positive result (e.g. through contact with the GP), serological results if available..), an interpretation can be reported to the physician, on a case by case base.

For all other situations, in the absence of any clinical information, a person with a positive PCR+ will be considered as infectious (precautionary measure).

If more scientific evidence or new PCR techniques (allowing to distinguish between active infection or not) become available the coming months, the current advice can be adapted.

Recommendations

- Because of the prolonged detection of viral RNA without correlation with infectivity, retesting of patients in a COVID-19 ward until they are PCR negative, is not considered useful (see release from isolation in procedure hospitalization).
- No screening/re-testing should be done if within 2 months of previous COVID-19 infection (PCR+ in symptomatic or asymptomatic person) (see previous RAG advice).

- Old non-contagious infections (based on decision laboratory and physician) should not be reported as a positive PCR result (no isolation, no contact tracing, no contribution to alert levels).
- Laboratories have been requested (25/06) to report the Ct values if available, and add a sentence to the result: ‘a positive PCR result is not equal to infectiousness, especially when Ct values are high’. Since Ct values are not comparable between different laboratories, it would be better to provide a semi quantitative information (low positive/positive/high positive) and, if available, information on the viral load if a common quantified standard is provided to all laboratories.
- This can be helpful for some physicians in decision making, case by case.
- Serology can assist in case-by-case decisions, but a systematic serology together with a PCR test is not recommended. Also, it should not delay contact tracing.
- The current guidelines on screening of asymptomatic persons in hospitals should be revised and adapted to the epidemiological context (ongoing).
- For communication to physicians, the most important focus is on the median time of infectivity.

The following persons participated to this RAG advice:

Alice Hannecart (Sciensano), Amber Litzroth (Sciensano); Benoît Kabamba (UCL); Charlotte Martin (CHU St Pierre); Chloé Wyndham-Thomas (Sciensano); Elizaveta Padalko (UZGent); Emmanuel André (KULeuven); Emmanuel Bottieau (ITG); Katrien Lagrou (UZLeuven); Laura Cornelissen (Sciensano); Marc Hainaut (CHU St Pierre); Michèle Gérard (CHU St Pierre); Olivier Denis (CHU Mont-Godinne); Patrick Waterbley (SPF Santé Publique); Petra Schelstraete (UZ Gent); Pierette Melin (Uliège); Romain MAHIEU (COCOM); Sigi Van Den Wijngaert, (LHUB); Stefan Teughels (Wachtposten Vlaanderen); Steven Callens (UZGent); Steven Van Gucht (Sciensano); Tinne Lernout (Sciensano); Wouter Dhaeze (AZG).

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