Guidance on the management of weak positive (high Ct value) PCR results in the setting of testing individuals for SARS-CoV-2

V1.5 07.07.2021

Version	Date	Changes from previous version
1.5	07/07/2021	Change in terminology and definitions on vaccine protection
1.4	22/06/2021	Updated to reflect NPHET guidance on immunity extending to 9 months post infection People with significant vaccine protection should generally not be tested is asymptomatic
1.3	14/04/2021	Updated introduction on evidence of RNA persistence and culture of virus High Ct value and low viral load are essentially interchangeable Greater emphasis on the role of the laboratory director in determining cut off values and interpreting results Replacement of term "testing" throughout when "sample" or "sampling" is more accurate Updated to reflect NPHET recommendation to extend period of presumptive immunity to six months Reference to virus variants and their relevance to acquired immunity and asymptomatic testing Statement that laboratories should provide Ct values on request in the context of expert interpretation Simplification to high Ct value/low viral load with removal of very high Ct value Reference to management of people with high Ct value results in hospital or residential care setting Reference to the absolute change in Ct value in the context of a fall in Ct value between samples Resequencing to place unintended testing after intended testing
1.2	22/12/2020	Expanded scope to encompass symptomatic people Revision of the title to reflect wider scope Indication that this approach may not be readily applicable in all settings
1.1	08/10/2020	Added information about previous positive cases Isolation/transmission-based precaution duration changed from 14 days to 10 days in community settings
1.0	19/08/2020	Initial guidance

Note: If you have any queries on this guidance please contact the AMRIC team at hcai.amrteam@hse.ie

Table of Contents

Background

SARS-CoV-2 RNA remains detectable in upper respiratory tract samples from some patients for extended periods. The reported median interval to first sample reported as "not detected" is in the range of 16 to 24 days with one sample reported as detected up to day 92. Additional details are available at the following links:

https://www.eurosurveillance.org/content/10.2807/1560-7917.ES.2020.25.30.2001292

https://casereports.bmj.com/content/bmjcr/14/3/e241087.full.pdf

The experience of laboratories in Ireland is consistent with this. There are accounts of detectable RNA in repeated samples on people at, and beyond 12 weeks, and at least one instance of a result reported as detected at 19 weeks. However, studies of recovery of culturable virus indicate that virus is generally not cultured after 9 to 10 days but may be recovered up to day 12 in people with moderately severe disease, and has been reported up to day 20 in those with severe disease.

The immune response, including duration of immunity to SARS-CoV-2 infection, is not fully understood. The frequency with which reinfection with SARS-CoV-2 can occur and the time frame of recurrence is also subject to ongoing research. Based on updated review of available evidence and advice provided by the Health Information and Quality Authority (HIQA), the National Public Health Emergency Team has taken the view that the period of presumptive immunity should now be considered as nine months following natural primary infection. There are reports of a second infection, confirmed as a distinct infection by differences in sequence between the virus initially detected and the virus subsequently detected. Some second infections may occur in less than nine months after the first infection so that immunity cannot be considered total at any time. This limitation is particularly pertinent in the context of emerging variants that may evade an immune response made to variants that circulated earlier in the pandemic and to people who have compromised immune function.

Testing, in particular testing of asymptomatic people, can result in the identification of people with positive tests for SARS-CoV-2 RNA which can be difficult to interpret. Specifically, interpretation is difficult when a person, often a healthcare worker (HCW) or a patient scheduled for a procedure or admission to a hospital, with no symptoms, tests positive for viral RNA at a low level. Testing of asymptomatic people should only be performed within the parameters of a clearly defined public health policy regarding the testing of asymptomatic individuals or on the basis of advice from a Public Health specialist or IPC

practitioner. The Consultant Microbiologist who is director of the laboratory performing the analysis has a critical role to play in guiding the interpretation of results as they have detailed knowledge of the platforms in use and their performance characteristics.

Scope

This guidance is intended to support practitioners in avoiding testing for SARS-CoV-2 where this is unlikely to be useful, and to interpret certain difficult to interpret results.

It may not practical and is not essential that the complexity of interpretation outlined here is applied in all settings. The detailed approach to interpretation outlined here may be particularly applicable in the context of an acute hospital setting where a multidisciplinary team is available and has capacity to critically interpret individual results and ensure appropriate communication and follow up.

When reporting confirmed results as SARS-CoV-2 RNA detected in settings where case by case evaluation is not practical it is appropriate, whenever possible, to include a comment differentiating a detected result qualified as a high Ct value/low viral load from a detected result without qualification.

Testing for SARS-CoV-2 antigens is not within the scope of this paper.

When it is not practical to implement the process outlined here and no differentiation between a positive result (unqualified) and a positive result qualified as high Ct value /low viral load is accessible it is necessary to proceed on the basis that a positive test is evidence that a person is infectious.

Terms

Cycle threshold

Ct (cycle threshold) values represent the number of cycles of amplification elapsed before the test system signals detection of the target. In general terms, the higher the Ct value the lower the quantity of virus target (viral load) present in the sample. Precise definition of what constitutes a high Ct value is difficult because a Ct value is not comparable to the quantitative output from a calibrated assay. The Ct value for a given sample will be different in different laboratories depending on the test platform and other factors (see Carroll and McNamara at https://www.eurosurveillance.org/content/10.2807/1560-7917.ES.2021.26.6.2002079).

In general terms for this report a Ct value of 30 or greater is taken as indicative of a high Ct value but it is appropriate for the director of each laboratory to make their own determination as to what constitutes a high Ct value based on their experience with the platform they are using.

Some laboratories may estimate viral load and /or prefer to use the terminology of level of virus detected in the sample in preference to reference to high Ct values. Both high Ct value and low viral load convey essentially the same information for clinical purposes and either terminology may be used.

A SARS-CoV-2 detected test result

For the purposes of this paper a "detected" result means that the test result meets appropriate criteria to be reported as detected. In general terms, in **an assay detecting multiple targets**, detection of a single target at a high Ct value/low viral load should be:

1. reported as either equivocal or not detected based on the reporting policy of the laboratory and their experience with the platform and assay in use or;
2. retested on the same or a second platform before reporting.

In **an assay detecting a single target**, detection of that target at a high Ct value/low viral load should result in retesting on the same or a second platform before reporting. Samples with high Ct values that are not reproducible on re-testing should generally not be reported as SARS-CoV-2 RNA detected. Reporting as SARS-CoV-2 RNA not confirmed or as equivocal may be more appropriate in the context of the laboratories experience. If reported as not confirmed, no further action is required and the result should not be

notified to public health. If the laboratory reports the result as equivocal, it is generally appropriate to request a repeat sample. If reported as equivocal the result should not be notified to public health.

Vaccine protection.

Individuals are considered to have vaccine protection if they are vaccinated as follows:

1. 15 days after the second dose of AstraZeneca (Vaxzevria);
2. 7 days after the second Pfizer-BioNTech dose (Comirnaty);
3. 14 days after the second Moderna dose (Spikevax);
4. 14 days after Janssen (one dose vaccination course);

If other vaccines become available the requirement for vaccination will be as advised by HSE.ie.

Guidance

In general, someone who has vaccine protection **or** a person who has had a positive test for SARS-CoV-2 in the previous nine months should not be tested for SARS-CoV-2 unless they develop symptoms suggestive of COVID-19. This statement encompasses people who are identified as close contacts of COVID-19 cases but who are noted to have tested positive in the previous nine months. Exceptions may apply based on risk assessment (for example if there is a specific concern about exposure to a particular variant that is expected to evade the immune response to previously circulating variants, if a person is immunocompromised or in certain high risk settings).

The application of this guidance should take account of the epidemiological situation (time and place) in which the sample is taken. In general terms, a high Ct value/low viral load result in an asymptomatic person is more likely to represent residual RNA detection of no public health or infection prevention and control (IPC) significance in a situation in which the incidence of infection in the population is low and falling. Such a result is more likely to represent an early pre-symptomatic RNA detection that is of public health and IPC significance in a situation in which the incidence of infection is high and increasing. **Such a result is more likely to represent an early pre-symptomatic positive in the context of a multiple tests reported as not-detected in the preceding days, as for example in serial testing of hospitalised patients.**

Interpretation of results is dependent on the availability of Ct values/viral load. Laboratories may not report Ct values routinely **but should provide the result in the context of expert interpretation on**

request. If Ct values/viral loads are not available for any reason (for example some platforms do not display Ct values) the default is to assume a positive result represents a significant result and that the person is infectious. It may be appropriate however to retest the sample on a platform that does display Ct values.

Confirmed detection of SARS-CoV-2 RNA at high Ct value in a person tested on the basis that they had no symptoms or other clinical features at the time of sampling may represent:

1. Pre-symptomatic infection in a person who subsequently will develop symptoms or other clinical features. [Likely to be infectious];
2. Symptomatic infection in a person who has symptoms or other clinical features not noted prior to or at the time of sampling. [Likely to be infectious];
3. True asymptomatic infection. [Likely to be infectious];
4. A person with residual RNA detectable more than 10 to 14 days after onset of infection. [Unlikely to be infectious];
5. A true false positive.

Guidance on the management of weak positive (high Ct value) PCR results in asymptomatic individuals who have not had a diagnosis of COVID-19 in the previous nine months

Asymptomatic people who have never had a diagnosis of COVID-19 and those who have had diagnosis more than nine months previously managed in the same way as follows:

1. When a positive result with a high Ct value/low viral load is obtained on a person understood at the time of sampling to be asymptomatic, it is important to establish if they had relevant symptoms either in the recent past, or if they have developed symptoms since the sample was taken. If not already know to be a COVID-19 Contact it is important to establish this;
2. If they have developed relevant symptoms since the sample was taken they should generally be regarded as a recent onset infectious case;

3. If they report relevant symptoms with a date of onset in the 10 days prior to sample collection, they should generally be regarded as a recent onset infectious cases;
4. If they report relevant symptoms with a date of onset of more than 10 days prior to the test ***OR*** if they report no symptoms at any time, the following approach is appropriate:

   If not in hospital or residential care setting

   a. If the Ct value is high they should be advised to self-isolate, but notification and initiation of contact tracing may await the outcome of further evaluation or change in the clinical condition. If there is no process for further evaluation in place the test is assumed to indicate that the person is infectious and contact tracing should commence without delay.

   If in hospital or residential care setting

   b. They should be provisionally managed as an infectious case (with transmission based precautions and contact tracing within the facility) pending further evaluation.
5. Further evaluation should include a repeat sample. This sample is generally taken on the second day after the initial sample (for example, if the initial sample was taken on Monday the repeat sample should be taken on Wednesday) but in the setting of a hospital or residential care setting it may be appropriate to repeat the sample after 1 day. Ideally the second sample should be tested on the same platform as the initial sample to facilitate comparison.
   a. If the Ct value remains high/viral load remains low on the repeat sample, the person may generally be considered as a remotely acquired infection and non-infectious at the time of testing. If self-isolation/transmission based precautions and contact tracing were initiated they can be stood down and the person need no longer self-isolate. Greater caution in this interpretation is appropriate if the repeat sample interval is shorter.
   b. If the Ct value has fallen below the high range /viral load has increased on the repeat sample, the person should be generally regarded as a recent onset infectious case. However it is appropriate also to consider the absolute change in Ct value/viral load (for example change in Ct value from 30 to 29 is unlikely to represent a meaningful change).
   c. In the event of a fall in Ct value (for example Ct value changes from 39 to 31)/increase in the viral load it is generally appropriate to take a further sample 1 to 2 days later even if the repeat value is in the high Ct value/low viral load range.
6. The person should also be asked to contact their doctor (or a dedicated phone number in the service where they work in the context of a healthcare worker) immediately if they develop new relevant symptoms at any time during the period of further evaluation. If the person develops

relevant symptoms at any time during the period of further evaluation they should be treated as a recent onset infectious case.

Unintended sampling and testing of a person with vaccine protection or within nine months of a previous diagnosis of infection

In the event that an asymptomatic person who has vaccine protection or who is within nine months of a previous diagnosis of COVID-19 is sampled and tested unintentionally and the Ct value is high/low viral load, it can generally be assumed to represent residual viral nucleic acid unless they are symptomatic. Under these circumstances asymptomatic people do not need to restrict their movements or take any specific measures other than those that apply to everyone. Exceptions apply based on risk assessment.

If the person has symptoms or other clinical features consistent with a diagnosis of COVID19 and has a positive test with a high Ct value/low viral load the default is to assume that the result is clinically significant and that they are infectious. Variation from this assumption should be based on a written assessment by a senior medical practitioner with relevant expertise taking account of all available information including testing for other infections as appropriate.

If the sample has a low Ct value/high viral load, even if the person is asymptomatic, the default is to regard the person as a new infectious case. However, the interpretation depends very much on the time interval since the previous diagnosis and Ct value/viral load at time of diagnosis. An individual assessment made by an appropriate specialist may indicate that the result can be considered to be consistent with residual viral nucleic acid in the context of the overall clinical situation. For example an asymptomatic person with a Ct value of 29 on a sample taken inadvertently 14 days after diagnosis with a Ct value of 15 may not need to be considered as infectious and would generally not be considered a new case of infection.

If a sample from their original test is available and reinfection is considered likely, sequencing the viral nucleic acid in both samples should be performed is possible, although it is unlikely that this result will be available within a time frame that supports the management of the individual person.

Appendix 1 – Notes on the Utility & Limitations of PCR

1. PCR is primarily a method for amplifying DNA and (by extension) RNA;
2. PCR as a diagnostic methodology is exquisitely sensitive, capable under conditions of optimal sample quality of detecting fewer than 10 copies of viral RNA in a clinical sample;
3. However, PCR does not distinguish between viable virus and non-infectious RNA;
4. In individuals infected with SARS-CoV-2, PCR can often detect viral RNA for many days and weeks after the resolution of the clinical features and after the person is no longer infectious;
5. PCR-based assays can yield non-specific (or 'false positive') results near the limit of detection of the assay: this does not mean that the test is bad;
6. A very good PCR assay with a specificity of 99.5% can still generate 5 'RNA detected' results in a cohort of 1000 individuals without the infection;
7. Although there may be variation between platforms and amplification efficiency in general standard PCR assays run for 40 cycles: in the case of a commercial, CE marked PCR assay, the assay manufacturer determines for how many cycles the assay should run;
8. Under optimal PCR conditions the amount of genetic material present doubles with each cycle, and increases by a factor of 10 every 3.3 cycles;
9. PCR results can be reported with cycle threshold (Ct) values: in general terms the lower the Ct value, the more viral RNA that is present in the clinical specimen. Note: The same sample tested on different assays/platforms can give different Ct values reflecting differences in the targets detected and the chemistry of the test used. When considering trends in Ct values it is preferable to test samples with the same assay/platform each time;
10. There are very few reports of viable SARS-CoV-2 virus being retrieved in culture from clinical specimens with a Ct value of >34;
11. Some PCR assays will try to detect more than one target (piece of viral RNA) in a clinical specimen;
12. If the assay sees all targets (usually 2 or 3) as present, then RNA is detected; if no targets are present, then RNA is not detected; if only some of the targets are present, then the test should be repeated and if the result is reproducible the result may be reported as indeterminate:
    a. Individuals whose samples yield indeterminate results should be recalled for repeat sampling if this is appropriate in the clinical context;
    b. If the second sample also yields an indeterminate result, the individual should generally be considered as confirmed SARS CoV2 infection and the result notified to public health.

ENDS