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

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Background
It is increasingly apparent that SARS-CoV-2 RNA remains detectable in upper respiratory tract samples from some patients for many weeks. The published literature on this is limited but there is experience described on teleconference and emails from multiple laboratories of repeated tests on people at and beyond 12 weeks and at least one instance of a positive result at 19 weeks.

The immune response including duration of immunity to SARS-CoV-2 infection is not well understood. The frequency with which reinfection with SARS-CoV-2 can occur and the time frame of recurrence is unclear at this point. However clinical recurrence in patients after a symptom free period associated with detection of SARS-CoV-2 in nasopharyngeal samples has been reported. The symptom free interval was reported as from 4 to 27 days. It is not clear the extent to which clinical recurrence represents reinfection or relapse of symptoms related to primary infection. Of note one patient became symptomatic 26 days after initial recovery and had a low CT value and positive viral culture during the second symptomatic episode (Batisse D et al. June 2020. Journal of Infection. Clinical recurrences of COVID-19 symptoms after recovery: viral relapse, reinfection or inflammatory rebound?).

Current Challenges
In general, someone who has ever had a previous positive test should not be retested unless they develop symptoms.

Testing of asymptomatic people either because they work in healthcare or because they are scheduled for a procedure is resulting in the identification of people with positive tests for SARS-CoV-2 RNA which are difficult to interpret in a person who has no prior report of symptoms or other clinical features.

In addition, the recent implementation of mass testing programmes targeting healthcare workers (HCW) has led to the detection of RNA (at a low level) in a number of asymptomatic HCWs who had a previously documented SARS-CoV-2 infection between March and June. The interpretation of results being generated as part of the above programmes is challenging for public health policy, so guidance is required.

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 result is more likely to
represent and early pre-symptomatic detection of public health and infection prevention and control significance in a situation in which the incidence is high and increasing and it is more likely to represent late detection of no public health or infection prevention and control significance in a situation in which the incidence is low and falling.

**Detection of SARS-CoV-2 RNA in person who has no prior report of symptoms or other clinical features may represent**

1. Pre-symptomatic infection in a person who subsequently will develop symptoms or other clinical features
2. Symptomatic infection in a person who has symptoms or other clinical features not noted prior to or at the time of testing
3. True asymptomatic infection
4. A person who has recovered from infection and has residual RNA detectable

In the event that a person with previously confirmed SARS-CoV-2 infection is retested and has a positive test result in the low or mid-range Ct value or a significant decrease in the Ct Value (i.e. a rise in the viral load) the result should be reported in the context of any previously available results and the clinical features as per Batisse *et al.* above. Cases assessed as reinfection should be reported to public health.

**Guidance Principles for the management of weak positive (high Ct value) PCR results in asymptomatic individuals**

The evidence bases on which to address this complex issue is limited however from a pragmatic clinical point of view there is a need to define an approach to managing this as follows:

1. Testing of asymptomatic people should generally be avoided unless there is a clear clinical indication or a specific requirement based on a nationally mandated policy.
2. Retesting of asymptomatic individuals with previous laboratory confirmed COVID-19 should be avoided indefinitely unless there is a clinical indication for testing based on clinical features of illness that raise suspicion of COVID-19. This statement applies to people who are identified as Contacts of COVID-19 cases but have previously confirmed infection.
3. In the event of a positive result in a sample from a person that appears, based on clinical details provided, to be asymptomatic the Ct value of the result should be considered in the context of the laboratories experience with the specific platform: if the Ct value is “low” or “mid-range” in the context of the platform used the result should generally be assumed to reflect current infection of infection prevention and control and public health significance; if the Ct value is “high” in the context of the platform used the result requires further evaluation as follows:

Proposal

Step 1: Confirm the result

1.1. For all specimens positive with a high Ct value (generally more than 30 but this will be assay/platform dependent) the original sample should first be retested. Retesting of the sample should ideally be performed on a second assay platform, but can be on the same platform if an alternative is not available

1.2. If the initial result is reproduced on retesting, then it should be considered a genuine (true positive) result: proceed to step 2

1.3. If the initial result is not reproduced on retesting, then the sample report can generally be reported to the effect that SARS-CoV-2 RNA was not confirmed or SARS-CoV-2 RNA was not detected. If there are specific clinical circumstances a different interpretation may be appropriate. If reported as not confirmed or not detected no further action is required and the result should not be notified to public health.

Step 2: Result confirmed; check the history

2.1 If the individual being tested has a prior history of confirmed SARS-CoV-2 infection, compare the Ct value from the time of their infection with the present Ct value.

2.1.1 If there has been no change, or a significant increase in the Ct value (i.e. a decline in the viral load), and the individual remains asymptomatic, then this is likely to reflect residual non-infectious RNA and no further action is required. The result does not need to be notified to public health.
2.1.2 If it becomes apparent that testing has been performed because the person is symptomatic it is recommended to also perform additional investigations including a respiratory virus panel PCR and to seek virology/microbiology input on the interpretation of all results, including SARS-CoV-2 Ct values, to determine if this is a new infection or detection of a small amount of persistent, non-viable virus material. If an alternative diagnosis does not explain the clinical presentation, cases assessed as reinfection should be reported to public health.

2.1.3 If it is not possible to access the previous results, or if the individual has no previous record of infection, proceed to step 3.

Step 3: If there is no previous record of laboratory confirmed infection or it is not possible to access previous results

3.1 In the absence of a clear history, it is not possible – on the basis of a reproducible positive ‘RNA detected at a low level’ PCR result – to ascertain whether the viral load is on the way up (early infection) or on the way down or at a relatively stable residual low level. The following steps should be taken:

3.1.1 The result should be notified to public health as the first laboratory detection of a COVID-19 case.

3.1.2 Follow up of contacts should be arranged as per Public Health Risk assessment & testing of contacts as appropriate.

3.1.3 If the person has no symptoms or other clinical features at the time of testing, on a precautionary basis they should be advised to self-isolate in line with public health advice (or if in a healthcare/ residential care facility should be isolated with appropriate transmission-based precautions) and be retested on day 7.

3.1.4 If no features of COVID-19 develop within the 7 days and the repeat test still has a high Ct value or SARS CoV2 RNA is “not detected” the possibility of early pre-symptomatic infection with rising RNA level can be discounted. If there is no other indication for isolation/transmission-based precautions these can be discontinued at that time.

3.1.5 If the person subsequently develops symptoms they should be re-tested at the time of symptoms onset as a fall in Ct value (to low or medium range Ct value) may help to confirm that this is an acute infection. In that case the duration of isolation/transmission-
based precautions should be as per public health/IPC guidance that is 10 days\(^1\) from date of onset of symptoms (at least 5 days fever free).

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**Appendix 1**

**Notes on the Utility & limitations of PCR**

1. 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
2. PCR does not distinguish between viable virus and non-infectious RNA
3. In individuals infected with SARS-CoV-2, PCR can often detect viral RNA for many days and weeks after the resolution of the clinical syndrome
4. 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
5. 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
6. Although there may be variation between platforms and amplification efficiency in general standard PCR assays run for 40 cycles: under optimal conditions the amount of genetic material present doubles with each cycle, and increases by a factor of 10 every 3.3 cycles
7. PCR results can be reported as 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.
8. In the diagnostic setting, a Ct value of 30 in a very efficient PCR assay represents a viral load of around 1000 (3 log) copies: a Ct value > 34 represents a viral load of fewer than 100 copies; a Ct value of >37 represents a viral load of fewer than 10 copies
9. There are very few reports of viable SARS-CoV-2 virus being retrieved in culture from clinical specimens with a Ct value of >34

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\(^1\) 10 days in community settings. In residential care facilities and hospitalised cases isolation/transmission-based precautions should be in place for 14 days (with at least 5 days fever free).
10. Some PCR assays will try to detect more than one target (piece of viral RNA) in a clinical specimen

11. 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.

Approved by the Pandemic Incident Control Team August 14th 2020

ENDS