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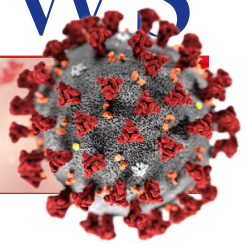

"INDETERMINATE" SARS-COV-2 PCR RESULTS: INTERPRETATION AND CLINICAL GUIDANCE

This document is a supplement to the recent PathCare clinical guidance document entitled "THE INTERPRETATION OF SARS-COV -2 RT-PCR CT VALUES IN CLINICAL PRACTICE"

Background

The polymerase chain reaction (PCR) test for the detection of SARS-CoV-2 in respiratory specimens is an essential tool in screening and diagnosing COVID-19[1]. Clinical virology laboratories often make use of a diverse repertoire of testing platforms with varying performance characteristics. The high analytical sensitivity of PCR assays often results in low-level detections. For very low-level detections, when the Ct value of a PCR test is above a certain threshold[2], and not all the analytical targets are detected, the result is inconclusive or "indeterminate" (IND). It has been estimated that up to 5% of SARS-CoV-2 PCR results may be inconclusive[3]. Up to 40% of these are subsequently shown to be "negative" with repeat testing[4]. This highlights the fact that patients with IND results should be managed as potentially infectious (Person Under Investigation [PUI]) until a definitive diagnosis can be made.

This document serves to provide practical guidance on the management of IND results.

What is an "indeterminate" SARS-CoV-2 PCR result?

An IND result means that a low-level signal was detected by the assay software. This could be due to a "false positive", a true positive with early infection, or a recent infection in the last 3 months where the patient is not considered to be infectious anymore. Thus this low-level signal does not definitively diagnose COVID-19, with the result reported as "indeterminate".

Why does an "indeterminate" category exist?

A substantial proportion of low-level detections are proven to be negative on repeat testing[4]. However the low-level detection could represent an acute infection where the viral load may still be increasing, hence the need for repeat testing for a definitive diagnosis.

What are the pros and cons of having an "indeterminate" result category?

IND results in symptomatic patients or during screening of asymptomatic contacts can result in clinical, diagnostic and infection control uncertainty.

However there is value in having an IND category in addition to either "positive" or "negative". There are multiple factors that may lead to IND results (Table 1). One of the commonest causes of low-level detections is the presence of remnant viral RNA from a previous infection. Without an IND category, these patients would be incorrectly classified as "positive", with potentially serious ramifications for the individual concerned. These ramifications include, but are not limited to:

- Inappropriate cohorting in a COVID-19 ward
- Unnecessary cancellation of elective procedures
- Unnecessary isolation and time off work
- Being unable to travel internationally
- Unnecessary quarantine for contacts

What are the factors associated with "indeterminate" results?

The various pre-analytical and analytical factors associated with IND results are presented in Table 1 (list not exhaustive).

Table1. Potential factors associated with "indeterminate" SARS-CoV-2 PCR results

PRE-ANALYTICAL		**ANALYTICAL**
Patient Factors	**Specimen Factors**	**Assay-specific factors**
• Asymptomatic /Mild dx • Timing of testing: Pre-symptomatic versus symptomatic • Prior Immunity	• Specimen type: Nasopharyngeal versus throat swabs • Dilution factor (volume of collection buffer) • Presence of PCR inhibitors (eg very mucoid specimens) • Adequacy of specimen collection • Random biological variation with respiratory swab specimens • Specimen integrity (specimen storage and transport conditions)	• Volume of sample tested • Gene targets • Primer and probe design • Nucleic acid extraction efficiency • Sample preparation method • Amplification efficacy

What to do after receiving an "indeterminate" result?

Refer to Table 2.

Table 2. How to manage "indeterminate" SARS-CV-2 PCR results

Action	Explanation
Treat the patient as a PUI until a definitive diagnosis is made	These patients may or may not have acute COVID-19. These patients should be considered potentially infectious until a definitive diagnosis is made. Urgent medical or surgical interventions should not be delayed pending repeat results. If an emergency procedure needs to be performed, the patient should be considered potentially infectious, with appropriate infection prevention and control measures instituted.
Repeat the PCR test if clinically indicated	Ideally re-swab after 1-2 days. If there are time constraints, immediate re-testing may be performed. If the repeat test is "positive" or IND again, regard as "positive".
Preferably repeat PCR on alternative platform	This will result in a delayed turn-around-time, but has the best chance of providing a definitive result. If time constraints are a factor, immediate re-testing may be performed. If the repeat test is "positive" or IND again, regard as "positive".
Re-swab from the oropharynx if possible	If the patient has a history of recent infection (last 3 months), then this low-level detection is most likely as a result of remnant RNA from a previous infection. Theoretically throat swabs would have less of a chance of detecting remnant RNA.
Repeat result negative	Considered to be COVID-19 negative and not infectious. The previous IND was likely due to remnant RNA from a previous infection.
Repeat result "indeterminate" or "positive"	Treat as "positive" and potentially infectious.

Conclusion

Inconclusive SARS-CoV-2 PCR results may cause confusion and delay infection prevention and control measures as well as patient management. However the risk of falsely classifying someone as COVID-19 positive may have serious ramifications for the individual concerned, thus compelling the need for an "indeterminate" category. Patients who test "indeterminate" should be regarded as a PUI until a definitive diagnosis is made.

References:

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