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SARS-CoV-2 Polymerase Chain Reaction (PCR): Limitations and Interpretation Guidelines

SARS-CoV-2 is the virus that has been identified as the cause of the disease, COVID-19. It is most important to understand that we are dealing with a new virus that is causing a pandemic of unprecedented severity. In the course of a very short period of time, the diagnostic laboratory community has had to validate a large number of assays, mainly based at this stage on reverse transcriptase PCR technology, as the primary diagnostic test for acute SARS-CoV-2 infection. The course of this diagnostic journey has been plagued with shortages of laboratory reagents, diagnostic kits and consumables on an unprecedented scale. These shortages are the result of limitations in production due to the effects of the pandemic and drastically reduced distribution of material due to grounding of aircraft and limitations in overland travel associated with lockdown activities all over the world, all coupled with dramatic increases in global demand. This affects every aspect of the laboratory service from the pre-analytical stage presenting at the most basic level with shortages in collection materials such as swabs, the analytical stage in the laboratory, and the post-analytical stage in analysing the results obtained on very new assays with a very new pathogen.

Pre-analytical factors:

The journey to producing a successful laboratory result starts in the pre-analytical stage. This begins with testing the appropriate patients and realising that the risk of falsely negative results is higher when asymptomatic patients are screened for COVID. While viral shedding is detectable in the 2-3 days or so prior to symptoms appearing, asymptomatic screening will include those who are tested prior to viral shedding being detectable, and a negative result obtained under these circumstances does not exclude infection.[1] Detection of viral RNA is also dependent on the timing of the specimen collection after the onset of symptoms – viral shedding tends to decrease after day 7 following the onset of symptoms, and PCR testing from day 7 post onset of symptoms can result in a negative result. The clinical sensitivity of PCR tests are highest if the specimen was collected within the first three days of symptom onset. In addition, viral shedding can be intermittent, and a patient may simply have had a specimen collected at the time when virus is not being shed resulting in a false negative result, despite being symptomatic.

An additional factor to consider is the type of specimen collected, and adequate sampling of clinical material in this process. Nasopharyngeal swabs are generally preferred, but

oropharyngeal, mid-turbinate, and anterior nares swabs are also acceptable alternatives.[2] Lower respiratory tract samples such as sputum, tracheal aspirates and bronchoalveolar lavage specimens should also be collected for patients with lower respiratory tract pathology. The use of stool specimens, particular for those with atypical presentations is currently being investigated.

There is currently a global shortage of the appropriate collection swabs for nasopharyngeal sampling, and oropharyngeal swabs are being used as an acceptable alternative where nasopharyngeal swabs are not available. A poorly collected nasopharyngeal or oropharyngeal specimen may also result in a false negative PCR result.

Analytical factors:

The PCR assays in use are all very new, use different gene targets, come in different formats from manual to automated batch based tests. New PCR tests are being developed and validated that will allow testing in peripheral laboratories, closer to the patient, however availability in SA, limited supply and cost may limit their use. Experience with all PCR assays is limited, and while they are validated in each laboratory implementing them according to their internal processes that comply with ISO15189 for SANAS accredited labs, we can at this stage work only on analytical sensitivity and have limited experience with clinical sensitivity. Currently, while there are some estimates available, the true clinical sensitivity of the available PCR assays is unknown and is not 100% with various factors including those mentioned, as possible causes of false negative results.[3] The assays in use each look for multiple gene targets, but each of the targets has varying analytical sensitivities. In addition, particularly as SARS-CoV-2 is a RNA virus, we can expect mutational variability to arise which might also affect the sensitivity of the targets chosen for any particular PCR assay.

Post analytical factors:

After completion of the PCR assays, each result needs to be scrutinised. Some of the gene targets are less specific for the SARS-CoV-2 such as the E gene target which may be Sarbecovirus (the subgenus) specific rather than SARS-CoV-2 specific, and some targets are more specific for SARS-CoV-2. The pathologist needs to determine whether the result should be reported as positive or not, or whether further testing on different assays might be required. This is one of the reasons that we recommend that the laboratory performing these assays is SANAS accredited to ISO15189 and that there is an experienced pathologist interpreting the results prior to releasing them for clinical use. At this stage, as the assays are new, they are not yet on

the laboratory's list of SANAS accredited tests, however, SANAS has indicated that they will be performing remote assessments, due to the lockdown, to extend the scope to include COVID-19 PCR tests. Until then, it is recommended that the laboratory performing COVID-19 testing should be a SANAS accredited laboratory in order to ensure that the same stringent quality standards, as per any other test performed in an accredited lab, is followed.

With all these factors to consider, it is clear that PCR testing, while a very valuable tool, has limitations with regards to the acute diagnosis of COVID-19. With time, we will hopefully find ways of supplementing the diagnostic solutions for laboratory testing for this virus. These will likely include properly evaluated serology assays, which used algorithmically with PCR testing, may improve the clinical sensitivity and specificity of our current testing procedures.

Should a situation arise where a patient is tested more than once and one result is positive and the other negative, it is of crucial importance to respond clinically to the positive rather than the negative result.

Key points:

- A positive PCR results confirms a diagnosis of a SARS-CoV-2 infection.
- A negative result does not exclude an infection with SARS-CoV-2. It is crucial to consider the patient's clinical presentation, radiological findings and epidemiological exposures and perform repeat testing on alternative specimen types as indicated.
- There are valid reasons for obtaining discrepant laboratory results and this does not imply that a laboratory error was made. Clinicians should always act on a positive PCR result if obtained.

References:

1. Temporal dynamics in viral shedding and transmissibility of COVID19: XI He et al; Nature Medicine https://doi.org/10.1038/s41591-020-0869-5
2. Diagnostic Testing for Severe Acute Respiratory Syndrome–Related Coronavirus-2: Cheng MP et al; Ann Intern Med. doi:10.7326/M20-1301
3. Report from the American Society of Microbiology COVID international summit 23 March 2020: Value of diagnostic testing for SARS-CoV-2/COVID19; Patel R et al; https://doi.org/10.1128/mBio.00722-20.