Internal Control Failure in SARS-CoV-2 PCR, Technical vs specimen Etiology

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Abstracts: SARS-CoV-2 (COVID-19) pandemic was global threat and preventing the re-pandemic considered the main target nowadays in many of healthcare commissions. Early and rapid diagnosis was achieved by PCR testing for COVID-19, against this concept was the rejection and or reptation of testing because of failure of processing mainly due to internal control failure (ICF). The objective of our study was emerged to find out the main cause of Internal Control Failure in SARS-CoV-2 PCR, and whether it caused mainly by Technical or Specimen Etiology.50 specimens of SARS-CoV-2 PCR were used with their original testing result were of Internal Control Failure then repeated to conclude whether it caused by Technical (the second reading gave a valid result) or Specimen Etiology (the second reading gave a ICF result). The result was highly significant. ($p < .05$). comparing the valid second result (technical causes) to ICF second result (specimen causes). We concluded that technical causes of ICF was the main cause of ICF result of PCR testing for COVID-19 with minimal specimen etiological cause that could recommend to interpret the result t of positive or negative from first reading with commenting on ICF with repetition especially in valid run and urgent specimen.

Keywords: SARS-CoV-2, PCR Testing, Internal Control Failure.

1. INTRODUCTION

Coronavirus disease 2019 (COVID-19) is a highly contagious viral illness caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). It has had a catastrophic effect on the world resulting in more than 6 million deaths worldwide. After the first cases of this predominantly respiratory viral illness were first reported in Wuhan, Hubei Province, China, in late December 2019, SARS-CoV-2 rapidly disseminated across the world in a short span of time. This compelled the World Health Organization (WHO) to declare it as a global pandemic on March 11, 2020.

Even though substantial progress in clinical research has led to a better understanding of SARS-CoV-2, many countries continue to have outbreaks of this viral illness that are attributed to the emergence of mutant variants of the virus.(1)

The standard diagnostic mode of testing is testing a nasopharyngeal swab for SARS-CoV-2 nucleic acid using a real-time PCR assay. Commercial PCR assays have been validated by the US Food and Drug Administration (FDA) with emergency use authorizations (EUAs) for the qualitative detection of nucleic acid from SARS-CoV-2 from specimens obtained from nasopharyngeal swabs as well as other sites such as oropharyngeal, anterior/mid-turbinate nasal swabs, nasopharyngeal aspirates, bronchoalveolar lavage (BAL) and saliva. The collection of BAL samples should only be performed in mechanically ventilated patients as lower respiratory tract samples seem to remain positive for a more extended period.

The sensitivity of PCR testing is dependent on multiple factors that include the adequacy of the specimen, technical specimen collection, time from exposure, and specimen source.[2] However, the specificity of most commercial FDA-approved SARS-CoV-2 PCR assays is nearly 100%, provided that there is no cross-contamination during specimen processing.

SARS-CoV-2 antigen tests are less sensitive but have a faster turnaround time compared to molecular PCR testing.[3] Comprehensive testing for other respiratory viral pathogens should be considered for appropriate patients as well.

PCR-based pathogen detection requires the use of appropriate controls. These aid in result interpretation by identifying adverse factors such as contamination, inhibition of the amplification reaction, or problems during nucleic acid extraction. For example, ruling out the possibility that your reaction has been contaminated leading to a false positive result requires the use of adequate negative controls. Alternatively, ensuring that your test would have detected the pathogen had it been present in the sample (i.e., reducing false negative results) requires the use of appropriate positive controls.

Negative controls are well established in detection workflows. Including multiple negative controls in an assay to rule out contamination is the basis for a valid positive result. The importance of controlling for false negative results in highly sensitive techniques such as PCR is recognized to a lesser extent. Several factors can generate a false negative result, such as errors in sample extraction or thermocycler malfunction. Assay failure due to PCR or RT-PCR inhibition is the most common cause. The most practical approach to control for the presence of inhibitors is to include an Internal Positive Control, or Internal Control (IC). This IC is simultaneously extracted and amplified (or only amplified) in the same tube with the pathogen target, and should always be combined with an external positive control to prove the functionality of the reaction mix for amplification of the pathogen target. This combination rules out inhibition, among other malfunctions, and confirms that a negative result is truly negative.

False negative results due to ICF is one of the common causes of false negative beside other causes like Presence of amplification inhibitors, organisms in quantity below the detection level of the assay and inappropriate collection, transportation, and improper handling or processing, variability in virus shedding, sample collection too early and low analytic sensitivity of the kit can be various reasons for a false negative sample. This could lead to delay of result, False negative result due to delay in processing can have major implications on the spread of infection and thus can pose great challenge to control of the current pandemic. (4)

2. MATERIALS AND METHODS

A 60 specimens were selected with their primary reading was ICF (Internal Control Failure) result, the specimens then repeated twice with double pathologist reading to conclude result as positive or negative or repeated ICF. We considered second sample reading with NO ICF represented a technical error for the primary sample, on the other hand, second sample reading with repeated ICF represented a sampling cause error for the primary sample Statistical analyses were performed by standard methods, and a “p” value of less than 0.05 is considered statistically significant.

3. RESULTS

60 indeterminate specimens of SARS-CoV-2 PCR Value were used with their primary reading was ICF (Internal Control Failure) result their mean were 32, median 35 and average were 35 and STD were 1.9. Upon reputation of all specimens with double check reading the mean were 17, median 33 and average were 32.8 and STD were 7.8 with only 18 specimens concluded as negative and 42 specimens concluded as positive. The p value was 0.004915 the result was highly significant. (< .05). comparing the result first indeterminate specimens and Double checked specimens. Table 1 showed all results.

Table 1. Results of repeating the ICF specimens of SARS-CoV-2 PCR Value

	First ICF specimens	Double checked specimens
Number of specimen	60	60
Number of positive	04 (if we neglect the Internal Control)	04

Number of negative	54 (if we neglect the Internal Control)	54
Number of ICF	60	2
Specimen Etiology Causes of ICF	2	
Technical Etiology Causes of ICF	58	
Value of z	-7.6	
P value	< .00001	
Significance	Highly significance	

4. DISCUSSION

In general, it is attempted to reduce the repeated specimens during PCR testing of COVID-19 patients. In daily practice, PCR ICF-values are common cause for repeating test and false negative results, the main two causes for ICF result were technical error that lead to repeating the specimen with negative or positive result of same original result and specimen etiology that causing repeated ICF. However, it is not yet known how interpreted SARS-CoV-2 result of ICF and is repletion make a significant change of the original reading if we neglect the Internal Control reading in the primary specimen.

The importance to define whether ICF results have to be repeated or issued as its if we neglect the Internal Control reading with commenting of that was emerged and to conclude the importance of reproducibility if those specimens. Our results showed a high significance difference between the technical error that lead to repeating the specimen with negative or positive result of same original result and specimen etiology that causing repeated ICF as p Value was < .00001 Also there was no difference between sample reading after repetition if we neglect the Internal control reading of original sample testing.

The result of this study resolve the debate of the relevance of ICF cause of repetition and fond no significant need for this repetition as results were same if we neglect the Internal control reading of original sample testing as well as, wee proof that most of the cauyses of ICF were due to technical error etiology that lead to internal control acceptance upon repeation. From this point our result enforced the suggestion if NO significance of ICT value causes for repetition.

5. Conclusion

In conclusion, technical causes of ICF was the main cause of ICF result of PCR testing for COVID-19 with minimal specimen etiological cause that could recommend to interpret the result t of positive or negative from first reading with commenting on ICF with repetition especially in valid run and urgent specimen.

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