Interference in Results of SARS Cov-2 RT PCR Test by Rapid Method

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In humans, coronaviruses cause respiratory tract infections that can range from mild to lethal. SARS-Cov-2 Virus is responsible for Covid-19. The most reliable test for detection of Covid-19 virus is RT-PCR. There have been significant advancements in technology of detection since it was first discovered. Rapid RT-PCR technology is used in fast and reliable report within less time and less viral load. The amplification of artifacts may interfere in tests results with high Ct value.

Keywords: accula, amplification**,** COVID-19, CT value, gene checker, rapid RT-PCR, RT-PCR

Coronavirus disease (COVID-19) is an infectious disease caused by the SARS-CoV-2 virus.The first step in managing COVID-19 is the rapid and accurate detection of SARS-CoV-2 enabled by real-time reverse transcription–polymerase chain reaction (RT–PCR). RT–PCR detects SARS-CoV-2 nucleic acids present in nasopharyngeal fluids. In throat swabs and sputum, the viral shedding peaks at five to six days after symptom onset and ranges from 10^4 to 10^7 copies/ml. This reflects higher virus levels in the respiratory tract.[1] The viral RNA detection rate in nasal swabs of infected people has approached 100%. The positivity rates for blood, saliva and tears are 88, 78 and 16%, respectively.[2] Different methods used for Diagnosis of COVID-19 are RT-PCR, RT loop-mediated isothermal amplification (RT-LAMP), Detection of SARS-CoV-

Step		Temperature		Time		Cycles	
		Conventional method	Rapid method	Conventional method	Rapid method	Conventional method	Rapid method
1	Reverse Transcription	55°C	50°C	15 min	10 min	1	1
2	Pre-Denaturation	95°C	95°C	120 sec	30 sec	1	1
3	Denaturation	95°C	95°C	15 sec	5 sec	45	40
4	Annealing, Extension	58°C	-	35 sec	-		
5	Annealing	-	58°C	-	20 sec		
6	Extension	-	72°C	-	5 sec		
7	Instrument cooling	40°C	-	10 sec	-	1	-

Table 1: Steps in PCR procedure for detection of Covid 19

2 Antibodies, SARS-CoV-2 Antigens, etc. Comparing conventional RT PCR and rapid RT PCR; RNA Extraction time is about 40 minutes by automatic extraction machine and about 60 minutes by manual extraction method. In rapid method its less than 10 minutes in Genesystem swab Direct lysis kit method and less than 1 minute by Accula buffer method. PCR procedure includes following steps. It takes about 84 minutes to complete PCR Cycles by Conventional method whereas 45 minutes or even less by rapid method depending upon the technology used.

Materials and Methods

This is a retrospective study conducted in the department of pathology, Tribhuwan international airport division, B & B hospital from the time period 28th October 2021 to 15th December 2021. All the cases screened for international travelling purpose in whom RT-PCR test was done by rapid method (Genechecker and Accula) are included in the study. Detail history was taken including the COVID 19 vaccination status. Nasopharyngeal sample was collected by taking all safety precautions. Two genes were tested in genechecker (RNA-dependent RNA polymerase and Nucleocapsid gene) whereas accula tested nucleocapsid gene only. The result was delivered within one hour when performed with genechecker and within 30 minutes in accula. Therefore, accula technology was used in the cases who required the reports urgently. All other cases were subjected to gene checker technology. All the positive cases were subjected to retesting.

Results

A total of 2252 RT PCR tests were performed. Genechecker technology was used in 2087 cases and accula was used in 165 cases. Out of the total cases, 1799(80%) were males and 453(20%) were females. The maximum number of cases(2039) belonged to age group 20-50 years. Out of 2252 cases, 95% cases had no symptoms whereas 5% cases demonstrated mild symptoms. All the patients had received two full doses of COVID 19 vaccination.

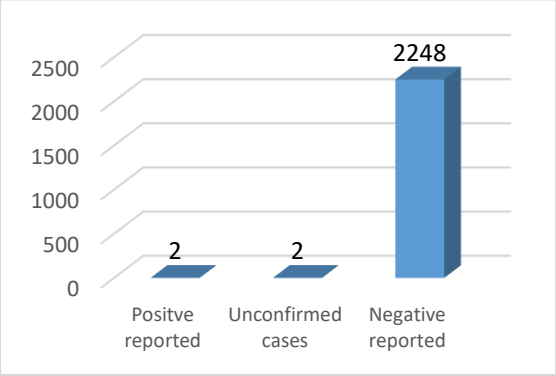

Figure 3: Total cases with respect to their results

SARS Cov-2 was not detected in 2244 cases whereas it was detected in 8 cases. Out of 8 cases, CT value was <30 in 2 cases (14 and 20) and had mild symptoms. The other 6 cases had CT value of >30 and were asymptomatic. Repeat sample was collected in 6 cases only as 2 patients refused **(Figure 1).** The repeat sample tested in two cases with CT value of <30 were positive and four patients with CT value of >30 showed negative results.

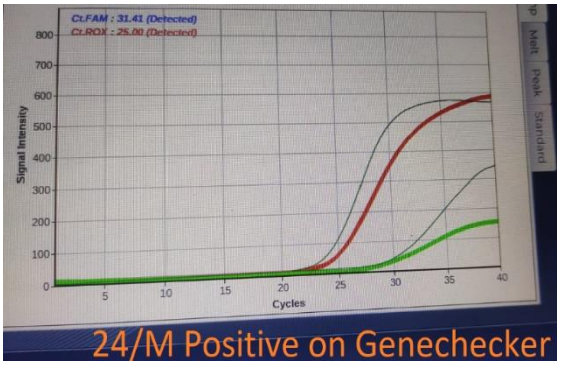

Figure 1: positive result in Genechecker

Figure 2: Shown Negative Result in Accula

Discussion

Real-time reverse transcription–polymerase chain reaction (RT–PCR) is the assay of choice and reliable test for detection of SARS-CoV-2 virus from the

nasopharyngeal, oropharyngeal, sputum samples.[5]The most preferred sample for test is nasopharyngeal swab. Sputum is also preferred regarding sample adequacy but it is more infectious. Any diagnostic test result should be interpreted in the context of the probability of disease, but also include proper internal controls to ensure a high level of clinical specificity when used as a tool for large-scale screening.[6] The internal control, positive control and negative control ensures the adequacy of sample and procedure is correct and validated. Those part were concerned while evaluating any results or reports.

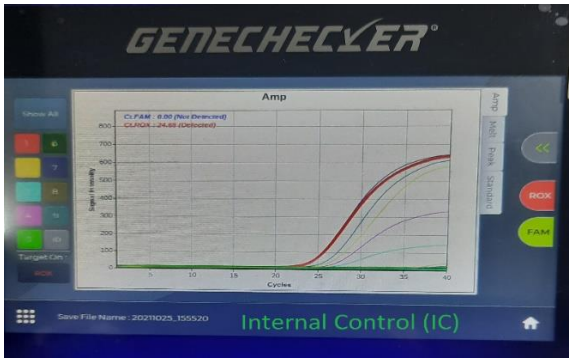

Figure 5: Internal Control

The majority of the cases in this study showed negative results for SARS-Cov2 which could be attributed to previous negative report 48 hours before, the vaccination status and asymptomatic cases (travelers only). The reason for positive result with Ct value > 30 may be due to artifacts that had either a higher or a lower melting temperature than the targeted

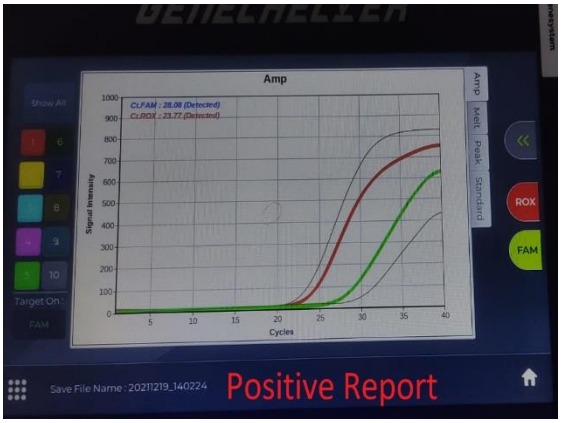

Figure 4: Positive result in Genechecker

product.[6] The amplification of artifacts is a recurring theme in qPCR literature but the conditions that lead to amplification of such off-target products remains rather elusive.[7]The main cause of the unspecific amplification in the target gene may be artifact that may be amplified in later stages of the cycle threshold. Due to preferential annealing of the primers and probe to nucleic acid template of positive samples, which occurs in earlier cycles of PCR (cycles 10–30, depending on the amount of viral genetic material). Although the detection of SARS-CoV-2 in positive samples seems not to be affected by unspecific signals, these signals are of great importance in the assessment of negative samples, leading to inconclusive results.[8]

Our study showed specificity of the test was 99.7% whereas sensitivity was 100% (False Negative test result was zero). Many studies have demonstrated

Figure 6: Positive result in Accula

difference in sensitivity whereas the specificity is found to be almost similar. A study conducted by Dinnes J et al found that summary sensitivity for the Xpert Xpress assay was 98.0% and sensitivity for ID NOW was 76.8%. The specificity for Xpert Xpress assay was 96.8% and specificity for ID NOW was 99.6%.[6]

Conclusion

Rapid RT PCR is useful as Point of Care (POC) instrument as well as for rapid diagnosis of Covid-19. There may be interference in the result by unspecific signals which may result in false positive results in late cycles. Therefore, it is recommended to repeat the test if the Ct value is > 30 in asymptomatic patients. Positive reports with Ct value less than 30 can be reported after correlating clinically.

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