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REAL TIME RT PCR TESTING FOR SARS- COV 2: KEY PERFORMANCE QUALITY INDICATORS

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ABSTRACT **Introduction:** A quality management system should be in place in order to ensure accurate and reliable results from laboratories. However in developing countries like India with resource limited settings, many laboratories which were not performing molecular tests and also are not accredited to international standards ,may only be implementing few parameters of quality control. Introducing a molecular test like RT PCR during the covid pandemic where accurate and timely results are essential step to control the outbreak may therefore come with a high risk of errors. In the absence of quality assurance (QA), use of inaccurate test results can lead to the wrong treatment and management decisions and lapses in surveillance of disease epidemics. **Aim:** To identify the basic key performance quality indicators in a RT PCR testing laboratory for covid-19. **Method :** Key performance quality indicators were identified and retrospective analysis was done for all nasopharyngeal swabs received for SARS- CoV 2 PCR testing over a period of seven months from November 2020 to May 2021. **Conclusion:** Collection and analysis of data at each step of the testing cascade serves as quality indicator for correct performance of the whole testing process. It is important to analyze and report them on a regular basis so as to minimize laboratory error and generate accurate results.

KEYWORDS :

INTRODUCTION

The novel SARS-CoV-2 virus is a new public health threat to the world. Three different coronavirus outbreaks: SARS-CoV-1 in 2003, MERS-CoV in 2012, and SARS-CoV-2 pandemic in 2019 have occurred in the last twenty years and diagnostic laboratories have played a key role in the rapid diagnosis of these new microorganisms using the molecular diagnostic techniques[1]. During the SARS CoV-2 pandemic aggressive mass testing is the first step to prevent transmission. In order to prevent nosocomial and community transmission, rapid identification of suspected cases is important [2,3]. Molecular based diagnosis through real time reverse transcription PCR real time RT-PCR) method using specific primers and probes is the gold standard to identify and differentiate SARS-CoV-2 from other beta-coronaviruses such as SARS and MERS.. Real time RT-PCR is a modification of PCR which detects target RNAs in clinical samples specially for diagnosing RNA viruses in molecular diagnostics laboratories. Laboratory errors in three different steps including pre-analytical such as sampling method, sampling location, sampling time, sample size, sample transfer and storage errors, factors during the test (analytical), such as nucleic acid extraction, cDNA synthesis, and PCR process and lastly post-analytical errors such as interpretation and analysis of results and test report can affect the PCR results. Since the clinical laboratories have a major role in the diagnosis and control of covid 19 pandemice , it is quite crucial to minimize laboratory errors so that there is no delay in reporting plus there is no wastage of time, reagents and manpower [4] . For accurate and reliable results implementing a quality management system (QMS) is important. Laboratories should be accredited to international standards. However, in developing countries like India with limited resources , during this covid pandemic, introducing a molecular based test like PCR at all healthcare levels was a herculean task. And during an outbreak situation when there is already so much of panic and a new test is introduced with the need to give fast results, there are always chances of high errors. The limited laboratory staff working in high throughput settings have to face high workload with the increasing number of cases during the ongoing covid pandemic [5]. So it is very essential that laboratories identify the key performance quality indicators in order to minimize the risk of laboratory errors , thus preventing the lapses that might occur in the disease surveillance during the pandemic because of wrong reporting. In the present study we have summarized the main quality indicators of RT PCR testing for SARS-CoV 2 in a covid PCR testing laboratory of a tertiary care hospital.

MATERIAL AND METHOD

This study was done in covid PCR testing laboratory of a tertiary care hospital after taking approval from institutional ethical committee. Key performance quality indicators were identified at each step of the testing cascade, especially during the analytical phase, that can serve as indicator for correct performance of the whole testing process. The data was collected and analyzed retrospectively for all nasopharyngeal swabs received for SARS- CoV 2 PCR testing over a period of seven months from November 2020 to May 2021. After sampling each swab was placed inside VTM (viral transport medium). Virus RNA extraction was performed by nucleic acid extraction kit (PATH KITS MDS™ Viral RNA extraction kit) and analyzed by real time reverse transcriptase-polymerase chain reaction using the Q-line molecular ER nCov-19 RTPCR kit (POCT services private limited) with specific primer and probe targeting E gene and Rdrp genes.

Key performance quality indicator is the number of times a sample could not be processed properly at each step of the testing cascade. This process might be at sample collection level, transport level or while processing or interpretation level and can be used to assess laboratory performance. It is an improvable measure through which process performance can be quantified.

RESULT

Over a period of seven months, 13725 samples were received in the PCR laboratory. Out of which 79 (0.57%) samples were rejected. A total number of 13646 nasopharyngeal swab samples were tested successfully for SARS-CoV 2 by real time RT PCR, out of which 1230 (9.01%) samples were positive. All key quality indicators identified are shown in table 1. Less than 4% samples have been reported as invalid or inconclusive. Only 1.06% samples were reported after the TAT. The number of samples that were retested due to contamination during RNA extraction were 139(1.01%). The positive and negative control failed in less than 5% of PCR runs. Overall, all key performance quality indicators showed that laboratory errors occurred in less than 5% of the samples.

DISCUSSION

As soon as SAR-CoV 2 was declared a pandemic, the WHO (World Health Organization) on 30 January 2020 notified all states worldwide to set up rapid diagnostic testing for SARS-Cov-2 in order to implement mass screening of populations so that carriers can be identified and isolated fast to prevent the spread of the virus [6]. India too scaled up its covid testing capacity under the guidance of ICMR (Indian Council of Medical Research) with the introduction of the rapid antigen detection test in addition to the molecular tests, which remain the mainstay of diagnosis. Regular advisories are issued by the ICMR regarding the testing strategies for SARS CoV-2 as per the changing pandemic situation based on the scope, need and capacity of

tests performed each day across the country. The number of laboratories in the country rose from 14 in February 2020 to more than 1596 with the surge in cases. All covid testing laboratories are ICMR approved in order to ensure quality [7]. Although WHO and CDC have approved Real time RT PCR test as the gold standard to detect SARS CoV 2 genome in clinical samples [4], the results of the test can be affected by laboratory errors during the testing cascade. The reliability of results depend on several factors during the pre analytic, analytic and post analytic phase of the test [6,8]. Identification of key performance quality indicators at each phase , majority being in the analytical phase, and analyzing them regularly definitely increases the confidence of the laboratory in reporting reliable results. All the key quality performance indicators for PCR testing of SARS-CoV-2 and laboratory concerns related to them have been summarized in table 2.

Key Quality Indicators During The Pre Analytic Phase -

The pre-analytical phase includes collection, transportation and storage of samples. Laboratory errors during this phase leading to poor performance may be due to many reasons like - collecting the sample hastily and superficially without plunging deep enough (decreased sensitivity); Poor cold chain maintenance during transport conditions; Presence of interfering substances; Patient identification failure; Failure to take antiviral treatment into account. The Centers for Disease Control and Prevention in the United States (CDC) have published precise recommendations for the collection of samples from the respiratory tract (type of sample, equipment to be used, how to go about it) and their handling (storage and transportation)[9]. Since the laboratory staff is not present at the sample collection area it is difficult to observe and point out errors in the pre analytical phase in real time. However certain parameters like the sample rejection rate when analyzed can indicate about the quality of sample collection and transport. In our laboratory, 79 (0.57%) out of 13725 samples received in the PCR laboratory over seven months were rejected. The reason for rejecting the samples were mainly due to incomplete patient details, inadequate sample and cold chain not maintained properly during transportation. All samples are checked inside a Class II BSC by the lab staff wearing PPE. In order to reduce exposure risk to the HCW collecting and transporting the samples to the laboratory and to the laboratory staff handling the sample at the sample receiving area, it is important to follow safe infection control practices and develop a robust transportation protocol for samples. Based on the WHO guidelines [10], all specimens (swabs, body fluids, blood, faeces) must be considered potentially infectious, and appropriate personal protective equipment (PPE) must be donned while obtaining samples from the suspected patient or manipulating such samples. PPE includes a disposable gown, gloves, cap, shoe cover, protective eyewear and an N95 respirator mask. A rejection rate of 0.57% in our laboratory is the result of a successful training programme. All our staff was trained for PPE donning and doffing, nasopharyngeal swab sample collection and transport. Sample rejection rate will probably be less if trained personnel handle the specimens while collection and transport. It is recommend that specimens should be tightly capped, completely labelled and transported safely to the laboratory sealed in biohazard zip-lock bags kept inside a leak-proof cryobox / thermocol box (if cryobox not available) .Samples should be transferred to the laboratory as soon as possible (CDC recommendation) and stored between 2 °C and 8 °C for up to 72h after collection. If they are to be kept longer than that, samples should be stored at -20 °C or -70 °C according to CDC and WHO Recommendations [9]

Key Quality Indicators During The Analytic Phase

Analysis of samples from suspected SAR-CoV- 2 patients are processed in our laboratory within a class 2 biological safety cabinet (BSC) by laboratory staff wearing full PPE . Total 13646 nasopharyngeal swab samples were tested successfully for SARS-CoV 2 by real time RT PCR with 9.01% samples reported as positive and less than 4% samples reported as invalid or inconclusive. All samples were processed and interpretated according to the kit protocol. It is essential that a validated and approved kits are used . The annotated genome of SARS-Cov-2 was published in January 2020 on the site of the US National Center for Biotechnology Information, or NCBI [11], since then many test kit suppliers have been working to develop new real time RT-PCR (Reverse Transcription Polymerase Chain Reaction) kits. As soon as the covid pandemic was declared by the WHO there was an urgent need for actions and resources and so the reliability of test kits and the actual state of health of patients tested were seriously in question. In India all commercial kits for PCR testing of SARS CoV-2 are validated and approved by the ICMR. The choice of PCR kit by a laboratory is based on several criteria like kit performance (which impacts the reliability of the result, particularly when it comes to sensitivity); Ease of use, Quality and storage conditions of the reagents, costs , whether extra equipment needs to be purchased or any specific consumables are required [6]. Laboratories do not have enough time to compare data in order to assess the kits during this covid pandemic when testing load is so much. All kits used by our laboratory are ICMR validated and approved.

Various factors contribute to false negative or invalid results for the detection of SARS-Cov-2. Most important factor is the choice of sample collection site. There is high probability of detecting the virus in the sample with high viral load thereby increasing the diagnostic sensitivity. The WHO recommends taking samples from the upper and the lower respiratory tract, especially if a sample from the upper respiratory tract appears to be negative when there is a strong suspicion of infection in order to improve the reliability of the diagnosis [12,13,14]. However, logistical problems, biosafety issues for the health professionals who take the samples, time and cost might affect the choice of anatomical site. Nasopharyngeal sample collection is nevertheless the most common choice for mass testing, despite not being the region where the most significant viral load is typically found [6]. Other factors that may cause false negative results are human error, low target or poor amplification and detection protocols or inhibition caused by interfering substances in a patient's sample.[15] The number of samples that were retested due to contamination during RNA extraction were 139(1.01%). There are two crucial challenges for a PCR ,firstly, this molecular diagnostic test is technically demanding and requires more expertise from the user than most of the older conventional tests and secondly, the extreme sensitivity of PCR gives rise to contamination issues [15]. The first diagnostic results that were obtained by the new PCR technology were a disaster because of the high rate of false-positive tests created by contamination [16, 17]. False-positive result with PCR may be due to human errors (e.g. mixing of specimens or mislabeling), or non-specific amplification/detection and contamination. It is difficult to rule out human error but it can be minimized by regular training and supervision of personnel, all measures that can be ascribed to good laboratory practice procedures.[9] All our laboratory staff has been trained in performing PCR and they are under constant supervision. Contamination is a major issue. Every time when a PCR is done, a new sample, reagent, disposables or instruments can get contaminated in the subsequent round of amplifications. To overcome this serious problem it should be ensured that PCR test is performed in three different rooms (pre-pcr, pcr and post-pcr) maintaining a unidirectional workflow [10,19]. The lab staff should change their PPE when entering a new zone. Exchange of material and instruments is not allowed between the rooms. Each room should have its own dedicated set of Biosafety cabinet, instruments and pipettes. Contaminating material can also be transferred by pipettes as the amplicons might invade the pipette. To prevent this, filtered tips are used that act as a physical barrier between pipette tip and instrument. Also it is important that the laboratory be cleaned with a surface disinfectant daily before and after starting the test in order to prevent contamination and lab personnel safety also. The WHO recommends disinfectants with proven activity against enveloped viruses, such as sodium hypochlorite 0.1%, a minimum of 62%–71% ethanol, 0.5% hydrogen peroxide ammonium or phenolic compounds [17].

The positive and negative control failed in less than 5% of PCR runs. Controls are an absolute must in every PCR run. Especially the negative control which serves as contamination control. If it is positive then entire run needs to be repeated. Similarly positive control is essential to tackle false negative results due to technical errors. If positive control is negative then the entire run is invalid. False negative result may also occur due to inhibitions because of biological material present in the clinical specimen like hemoglobin, heparin, certain hormones, detergents and solvents coming from the sample preparation (sodium dodecyl sulfate, ethanol etc.) and/or particulate matter [20,21].To rule out inhibition, amplification in each individual PCR test needs to be monitored by means of an internal control (IC) [8]. An IC is a piece of DNA that is added by the user to anamplification assay and has to show up positive in every case, where amplification has taken place.[22] If an IC shows up negative in a particular test, the possible reasons are Inhibition, technical error , reagents used, issue with thermal cycler or inappropriate sample preparation.

Ideally each run should include the following controls: NC- Extraction negative control (Indicates whether contamination was introduced from the extraction phase), PC-Extraction positive control(Provides an indication of the quality of the extracted template), NTC -No template control(Indicates whether contamination was introduced in from the PCR phase and quality of PCR reagents) , Positive template

control(s): synthetic SARS-CoV-2 RNA/DNA (either gene fragment or whole genome) that usually comes with the kit used(Indicates limit of detection and robustness of the assay). Water/universal transport media/viral transport media can be used as NTC and a known negative and positive patient sample can be used as NC and PC respectively for extraction control .

Key Quality Indicators During The Post Analytic Phase –

The real-time RT-PCR method kit used to detect SARS Cov- 2 is multiplexed, with co-amplification of two targets –E gene and RdRp gene. For a test to be considered positive, an amplification curve for each molecular target should be observed. Interpretation of Positive/negative/ inconclusive/invalid result is based on the presence of an exponential amplification curve for each target with a Ct value (Cycle threshold) below a given threshold (according to kit literature) depending on the total number of cycles programmed for the test [20,21,23]. It is important to determine the correct baseline and threshold for right interpretation of result. None of the samples were misinterpretated in our laboratory. Only 1.06% samples were reported after the TAT (within laboratory TAT) i/e 12hrs. Total TAT is defined as the time between specimen collection and result reporting to the clinician while Within laboratory TAT is defined as time from sample receipt at the laboratory to result reporting . Only those samples were reported after the TAT which were retested due to contamination or were invalid due to technical error. Timely communication between both clinician and laboratory staff is important. An alert call of all positive cases should be made immediately post analyses to the clinician in order to rapidly initiate the treatment and infection control protocols to prevent the transmission of infection.

CONCLUSION

There are several challenges that a covid testing PCR laboratory can face. By identifying the key quality indicators (QI) at each phase of the testing process right from sample receipt in the lab to final reporting these challenges can be dealt with successfully. It is recommended that the following QIs should be analyzed and reported on a regular basis, at least three monthly: 1) Total Number of successful sample results 2) Total Number of positive, negative , inconclusive and invalid test results 3) Total number of samples retested due to contamination 4) Sample rejection rate 5) Total Number of failed positive and negative controls 5) Total samples misinterpretated 6) Total samples reported after the TAT.

Table 1: Key Performance Quality Indicators for SARS-CoV 2 PCR Test in a Tertiary Care Hospital

Quality indicator	Number (%)	
Pre analytical phase	Total number of samples rejected - Due to incomplete patient details - Due to improper transport - Inadequate sample	79(0.57) 14(0.10) 45(0.32) 20(0.14)
Analytical phase	Total number of successful test results - Total samples tested - Total number samples positive - Total number of samples negative	13646 1230(9.01) 11538(84.5)
	Total number of samples inconclusive	382(2.7)
	Total number of samples invalid - Number of samples invalid in first run but negative in repeat run - Number of samples invalid in first run but positive in repeat run	496(3.6) 486(3.5) 10(0.07)
	Total number of samples retested due to contamination during extraction	139(1.01)
	Number of time NTC (no template control) failed	17(7.3)
	Number of times NC(negative control) failed	10(4.3)
	Number of times PC (positive control) failed	5 (2.17)
Post analytical phase	Number of times result was misinterpretated	0(0)
	Total number of samples reported after TAT	145(1.06)

Table 2: Summary of Key Quality Performance Indicators for PCR Testing of SARS-CoV 2 and Related Concern with Action Needed.

Phase of testing cascade	Quality indicator	Concern	Preventive and Corrective Action needed
Pre analytical	-Total samples with incomplete patient details -Total samples with improper transport - Total samples with Inadequate sample volume -Total samples not collected properly	- Report mismatch due to patient misidentification - Leakage of samples leading to exposure risk to the HCW - False positive/negative reports - Invalid results due to poor quality samples	- Sample with incomplete details should be rejected - Use of proper PPE while handling the samples - Samples should be opened inside class II BSC - Ensure sample was received maintained in a proper cold chain inside a cryobox/thermocol box with ice packs. -Train the staff in sample collection area regarding proper sample collection from the correct anatomical site
Analytical	- Total number of successful test results -Total number of invalid/inconclusive results -Total number of times controls failed in a run	-False positive/negative results due to human error, technical problems, contamination during extraction, low concentration of target in the sample , presence of PCR inhibitors - Invalid results due to pipetting errors -Poor quality of reagents -Incorrect extraction protocol or PCR amplification procedure followed.	- Each step of PCR should be performed in different rooms maintaining unidirectional workflow. -Equipments, PPE and consumables should be kept and stored separately for clean area(PCR amplification and analysis room)and dirty area (extraction room). -Trained laboratory staff should perform the test to minimize technical errors - All pipettes should be calibrated, tips with filters should be used. - Surface decontamination with 70% alcohol or 0.1% sodium hypochlorite should be done daily before and after the test. -In c/o contamination , prepare all reagents freshly. Discard all already prepared reagents during the previous run. Clean the room and all instruments with approved disinfectant. -Use of controls is must-Positive control for amplification and negative control for contamination. -Use of IC (internal control) for PCR inhibitors -Read kit insert properly and follow protocol according to the manufacturer's instructions.

Post analytical	-Total number of samples reported after TAT -Number of times result was misinterpretated	- Delayed patient diagnosis and management -Reports getting misplaced or lost during transit from laboratory to the ward/ICU etc. -Misinterpretation due to incorrect determination of baseline and cycle threshold. -Untrained personnel reporting the results	-Alert calls to be made for positive reports. Immediate communication between lab and clinician should be established to initiate timely treatment and infection control protocol. -kit insert to be read properly for correct interpretation of results. - Trained personnel to report the results. -Proper report dispatch system should be in place.

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