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Original Article

Microbiology Section

A Retrospective Observational Study to Assess the Quality Management System in a Molecular Diagnostic Laboratory of a COVID-19 Dedicated Hospital in Delhi, India

SONALI BHATTAR[1], SUKRITI SABHARWAL[2], SHIKHAR SAXENA[3]

ABSTRACT

Introduction: A molecular diagnostic laboratory is the cornerstone of Coronavirus Disease-2019 (COVID-19) disease diagnosis, as the patient's treatment and management protocol depend on molecular results. Therefore, the laboratory conducting these tests must adhere to quality management process to increase the accuracy and validity of the generated reports. Rajiv Gandhi Super Speciality Hospital established its molecular diagnostic set-up at the beginning of the pandemic. Hence, this study aims to generate quality management data to help improve weak points.

Aim: To assess the quality management system for COVID-19 diagnosis.

Materials and Methods: This retrospective observational study was conducted at Rajiv Gandhi Super Speciality Hospital in Delhi, India. A total of 14,561 samples were collected over six months, from February 2021 to July 2021. Data from all samples received during this period for COVID-19 Reverse-Transcriptase Polymerase Chain Reaction (RT-PCR) testing were included. Data were retrospectively collected from the electronic Laboratory Information Management System (LIMS). Quality variables were analysed over six months from July to December 2021 and classified into preanalytical, analytical, and postanalytical variables. Quality Indicators (QIs) were selected from a common model of QIs set by the International Federation of Clinical Chemistry and Laboratory Medicine. The results were presented in percentages, and descriptive statistics were analysed using Statistical Package for Social Sciences (SPSS) software.

Results: During the six-month study period, the molecular laboratory received 14,561 samples. Among the preanalytical variables, sample leakage was the most common cause of sample rejection (134 samples, 0.92%), followed by the non generation of Specimen Referral Form (SRF) identification (76 samples, 0.52%), and non compliance with triple packaging (44 samples, 0.3%). Other preanalytical aspects assessed included incomplete patient identification (17 samples, 0.11%), insufficient sample quantity (12 samples, 0.08%), missing forms/samples (7 samples, 0.04%), samples in the wrong vials/empty Viral Transport Media (VTM) tubes (5 samples, 0.03%), and incomplete LIMS entry (2 samples, 0.01%). Internal Quality Control (QC) was not obtained in 55 samples (0.37%), and two incidents of cross-contamination resulted in false-positive results. Among the postanalytical factors, 11 samples (0.07%) could not be dispatched within the stipulated time frame.

Conclusion: The assessment of the quality management system revealed some areas for improvement, emphasising the importance of adhering to QC processes for the smooth operation of diagnostic laboratories, especially those involved in critical reporting. The assessment of QIs helped monitor laboratory parameters effectively.

Keywords: Laboratory medicine, Quality indicators, Samples

INTRODUCTION

Rapid and accurate laboratory diagnosis of Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) infection is crucial for the management of COVID-19 patients and the control of the spread of the virus [1]. A well-established quality management system plays a very important role in patient management. A laboratory quality management system is a systematic, integrated use of activities to establish and control the work processes from preanalytical through postanalytical processes, manage resources, conduct evaluations, and make continual improvements to ensure consistent quality results [2]. This ensures accurate and timely test results. As the COVID-19 pandemic continued to create chaos, leading to a public health emergency, the Indian Council of Medical Research (ICMR) took charge of surveillance testing for COVID-19, which led to the expansion of testing capacity by using its existing laboratory network, developing standard protocols, and launching an online portal for reporting [3]. Although the COVID-19 emergency provided the laboratory with an opportunity to expand and utilise resources for diagnosing SARS-CoV-2 infection, the safety and quality of RT-PCR testing remain the priority for providing an accurate and interpretable results [4]. Unfortunately, it cannot be ruled out that this could be negatively affected by many preanalytical and analytical factors [5].

Rajiv Gandhi Super Speciality Hospital is a tertiary care super speciality hospital catering to a large metropolitan city population. It is a 600-bed hospital that was converted into a dedicated COVID-19 care centre during the COVID-19 pandemic catastrophe. The molecular laboratory under the Department of Clinical Microbiology was developed with a capacity to run 200-250 samples per day. It was planned and constructed at the beginning of the pandemic in May 2020 to meet the rising demand for testing needed to confirm and isolate COVID-19 cases.

In laboratory practice, the Total Testing Process (TTP) is classified into three essential phases: preanalytical, analytical, and postanalytical steps [6]. QIs are recognised as cornerstone tools for the quality of laboratory systems that can be measured to evaluate each step of the TTP [7]. The use of QIs in laboratory medicine enables the identification of error rates and reduces or prevents error risks regarding patient safety [7]. As COVID-19 testing had just started

everywhere during this time, it was decided to review the quality management system of the laboratory and share the learnings from implementing the same. The aim of the study was to assess the quality management system in COVID-19 diagnosis.

MATERIALS AND METHODS

This was a retrospective observational study conducted on a total of 14,561 samples collected over six months from 11th February 2021 to 11th July 2021. The quality variables were analysed over next six months from July 2021 to December 2021. Data were collected retrospectively from the electronic LIMS. This study was conducted at Rajiv Gandhi Super Speciality Hospital located in Delhi, India. As the study only observed data collected and no intervention in the routine sample collections and processing was done, ethics approval and consent were not sought. As it was only an observational study utilising the data of the laboratory itself and no interventions were carried out or deviations from the routine protocol of the laboratory, ethical clearance was not deemed necessary.

This study was a time-bound study, and only those samples available during the study duration were included. During the six months of the study period, 14,561 samples were received in the molecular laboratory.

Inclusion criteria: Nasopharyngeal swabs and oral swabs collected from outpatient and inpatient departments that were sent to the microbiology molecular lab for SARS-CoV RT-PCR diagnostics as part of routine patient management were included in the study.

Exclusion criteria: Samples other than nasopharyngeal and oral swabs were not included in the study.

The quality aspects monitored were classified into the following three categories based on the TTP. QIs used are based on the standard laboratory operating procedures followed for the test studies and are a modification of the common model of QIs set by the International Federation of Clinical Chemistry and Laboratory Medicine [7]. The QIs were categorised as follows:

- **Preanalytical variables:** Sample leaking, non generation of SRF identification number, non compliance with triple packaging, incomplete patient information, insufficient quantity of samples, missing forms/samples, samples in wrong vials/empty tubes, LIMS entry not done.
- **Analytical variables:** Inability to obtain internal QC, cross-contamination of sample batches, non compliance with PCR QC, reagent temperatures not maintained, instrument calibration, and external quality audits.
- **Postanalytical variables:** Increased Turnaround Time (TAT), and the number of duplicate reports.

The workflow of the COVID-19 molecular testing laboratory is as follows: The COVID-19 samples are received by the technical staff from 9:00 am to 4:00 pm. The laboratory staff recruited for sample receipt makes an entry in the sample receiving register and gives the sample a laboratory number. This is strictly done on a first-come, first-serve basis. The samples are also received on the LIMS. After screening the samples for any preanalytical errors as mentioned above, the analytical process begins. Those samples not adhering to the preanalytical quality aspects are rejected, and a repeat sample is requested. The analytical process begins with a routine check of all the equipment, and QC is run with each batch of samples. A negative control is used to check sample cross-contamination, and a positive control is used to assess the chemical integrity of the reagents, primers, and probes. The results are only read by the senior residents/consultants. Any deviation from the accepted range of QC value is documented, and a root cause analysis is performed. A logbook is maintained for the documentation of the results. All the reports are entered in the LIMS after appropriate validation by the senior residents and consultants. Apart from the above, biannually, samples are also sent to the assigned ICMR QC laboratory for External Quality Assessment (EQA). The observations of the above-mentioned parameters are presented in this study.

STATISTICAL ANALYSIS

Descriptive statistics were analysed using SPSS version 29.0 software. The results are expressed as percentages.

RESULTS

The quality aspects were classified into three categories: preanalytical, analytical, and postanalytical. In [Table/Fig-1], various preanalytical variables were identified and performance evaluated. Sample leaking was the most common cause of sample rejection, accounting for 134 (0.92%) cases, followed by non generation of SRF ID with 76 (0.52%) cases, and non compliance with triple packaging with 44 (0.3%) cases. Other preanalytical aspects are shown in [Table/Fig-1]

Variable	n (%)
Sample leaking	134 (0.92)
Non generation of SRF IDs	76 (0.52)
Non compliance with triple packaging	44 (0.3)
Incomplete patient information	17 (0.11)
Insufficient quantity of samples	12 (0.08)
Missing forms/samples	7 (0.04)
Samples in wrong vials/empty tubes	5 (0.03)
LIMS entry not done	2 (0.01)

[Table/Fig-1]: Analysis of the prevalence of preanalytical quality variables.

In [Table/Fig-2], various analytical quality variables of the laboratory were described. The main issue encountered was the inability to obtain internal QC, most likely related to incorrect sample collection techniques, accounting for 55 (0.37%) cases. Other variables included the absence of positive and negative controls in a run, which occurred three times, and cross-contamination among samples resulting in false-positive results, which happened twice, despite having a unidirectional workflow with staff movement prohibited from the extraction area to the clean reagent area. No errors were reported in external QC audits. The temperatures of reagent and sample storage refrigerators were recorded daily, with no outliers observed.

Variable	n (%)
Inability to obtain internal Quality Control (QC)	55 (0.37)
Positive and negative control not obtained	3 runs
Cross-contamination	2 incidence

[Table/Fig-2]: Analysis of the prevalence of analytical quality variables.

The postanalytical factors measuring the quality of the laboratory are illustrated in [Table/Fig-3]. Samples are manually delivered to the laboratory, and reports are attached to the hospital LIS. Given the critical nature of COVID-19 reports, a benchmark of 24-48 hours for TAT was set for these samples. A total of 11 (0.07%) samples could not be dispatched within the specified timeframe, and duplicate reports were generated for 82 (0.56%) samples.

Variable	n (%)
Increased Turnaround Time (TAT)	11 (0.07)
No. of duplicate reports	82 (0.56)

[Table/Fig-3]: Analysis of the prevalence of postanalytical quality variables.

DISCUSSION

Among the test methods available for SARS-CoV-2 diagnosis, real-time RT-PCR is considered the gold standard. Although molecular tests are highly accurate, there is still a chance of obtaining false results due to errors in preanalytical, analytical, and postanalytical

processes. Lippi G et al., have mentioned that the most important RT-PCR vulnerabilities include general preanalytical issues such as identification problems, inadequate procedures for collection, handling, transport, and storage of the swabs, collection of inappropriate or inadequate material (for quality or volume), presence of interfering substances, and manual errors. Specific aspects such as sample contamination and certain analytical problems that may lead to issues in diagnostic accuracy include testing outside the diagnostic window, active viral recombination, use of inadequately validated assays, insufficient harmonisation, instrument malfunctioning, or any other specific technical issues [5]. It is necessary to ensure that quality is not compromised due to the quantity of work. Therefore, an attempt was made to gauge the quality of the molecular laboratory over six months by evaluating its performance on various parameters.

1. **Preanalytical errors**

Preanalytical errors are an inevitable source of laboratory errors, and when it comes to the identification of SARS-CoV-2, these factors are particularly significant [8,9]. It has been demonstrated that most mistakes often occur before the sample is analysed [9]. The frequency of rejections was assessed due to sampling inadequacy, inappropriateness, and incorrect patient information resulting from incorrect sample collection practices and/or ignorance and non compliance by the technicians. Sample leaking was the most common anomaly observed during the assessment of preanalytical variables, followed by the non generation of SRF-IDs.

Sample leaking may be caused by faulty VTM tubes from the manufacturer, leaks during sample transportation, and inadequate triple packaging. In this set-up, cases of sample leaking were encountered as a few samples were received from other centres, leading to a lack of uniformity in the quality of VTM vials used as well as in triple packaging. Currently, preanalytical errors account for up to 70% of all mistakes made in laboratory diagnostics, most of which arise from problems in patient preparation, sample collection, transportation, and preparation for analysis and storage [10]. A study reports that preanalytical variables account for 32-75% of laboratory errors and encompass the time from when the test is ordered by the physician until the sample is ready for analysis [9]. In this study, maximum errors were observed in preanalytical variables. There is now incontrovertible evidence that the preanalytical phase is the major source of errors in laboratory testing when used for either diagnostic or research purposes [11,12]. The study by Naz S et al., highlights the need for stronger coordination between clinicians and personnel working outside the lab to improve test quality. Continuous communication with personnel in charge of the form requisition ensures that the form is filled correctly, and all details are entered [9]. The percentage of samples with incomplete patient information was 0.11%. The reason for the same in this study can be attributed to the increasing rush in the flu OPD during pandemic peaks and incomplete training of deputed staff posted at the sample collection area. Another study mentioned the same, expressing one of the critical challenges faced was the inadequate well-trained human capital in terms of sample collection and delivery, testing, and test result dispatching [13]. Preanalytical errors also lead to a prolonged TAT due to the need for fresh samples. As Kaufer AM et al., express, with limited resources and an overburdening workload, adhering to the recommended protocols may be difficult, but it should not be overlooked because breaking them can result in immediate cross-contamination, jeopardising the accuracy and quality of RT-PCR testing while increasing the risk of laboratory-acquired infections [14]. There is an urgent need to instill awareness about the complexities of a very basic activity that forms the mainstay of lab services, i.e., sample collection. While patient preparation and sample collection are widely recognised as frequent sources of errors, greater attention should be paid to sample transportation, especially when the diagnosis of a fastidious organism is anticipated. To reduce the frequency of preanalytical variables, several actions were initiated time and again, such as in-house training for technicians to familiarise them with the standard protocols of sample collection and transport. Administrative procedures were carried out wherever necessary, such as procuring good quality pieces of equipment and maintaining an adequate number of staff in the flu OPD, etc.

2. **Analytical errors**

The incidence of inability to obtain internal QC was observed in 55 samples. Molecular assays are susceptible to this type of error, which is directly associated with improper sampling techniques. As COVID-19 was a relatively new test when started in the laboratory, a hesitancy to collect samples or inadequate knowledge of collection techniques can lead to improper sampling, resulting in repeat sample collection and testing. Even though the sample may appear to have a satisfactory quantity, the required DNA content for molecular detection may not be present, leading to errors in internal QC. To minimise the occurrence of this issue, special attention was given to reinforcing sample collection and handling techniques to the staff posted in the flu OPD. In three instances, the lab was unable to obtain the positive control and negative control of the PCR run. It was also observed in two instances when the entire run got contaminated, which was probably due to an error in sample placement. These runs were repeated after a thorough root cause analysis was conducted. Khan MJR et al., have elaborated on tips that need to be kept in mind while performing the molecular process [1]. They agree that due to the complexity of the RT-PCR test procedure, it is vulnerable to cross-contamination [1]. Several measures were implemented in the laboratory to reduce contamination, including strictly prohibiting staff movement between the clean reagent room and extraction room during sample processing and not allowing any food/drink in these rooms. Contamination of surfaces, pipettes, and clothes by positive samples and PCR products can also lead to false-positive results; therefore, the molecular laboratory should be divided into different sections, including sample extraction, preparation of primers and reagents, and RT-PCR processing, to minimise cross-contamination [15]. Currently, there are three main extraction protocols: automatic extraction, magnetic method, and column-based. The automated method is known to be the safest and fastest with minimal staff intervention [16]. An automated nuclear extraction system was used in the laboratory, and the workflow is designed in such a way that movement is from a clean area to a dirty area to prevent the molecular laboratory environment from being affected by aerosols or particles containing a virus or viral genomes. Lippi G et al., emphasise that EQA schemes should be established as soon as possible for monitoring analytical quality and harmonising the assays [5]. Zero errors were reported in the external QC audits. External control audits were conducted by the EQA-scheme lab assigned by ICMR; for this hospital, it was the Maulana Azad Medical College in Delhi, India.

3. **Postanalytical errors**

There is a lack of studies that have reported postanalytical laboratory errors associated with the detection of COVID-19. Scrutiny of the postanalytical variables reveals that TAT could not be achieved for 11 (0.07%) samples. COVID-19 reporting is considered a critical value reporting, and maintaining TAT is of utmost importance. Reporting delays in critical results can lead to unfavourable outcomes in patients [17]. Positive reports of critical tests are considered important QIs for excellence in patient-centric care. The relative abundance of TAT reporting by the laboratory is an indicator of the conscious effort to appraise clinicians of reports indicating positive results. This facilitates decision-making that might prove to be lifesaving in certain cases. TAT is a measure of the number of tests that meet reporting deadline criteria. Delays

in the analytical phase and preanalytical phase may contribute to prolonged TAT. A percentage of 0.56% was reported for the number of duplicate reports given to patients or their attendants due to failure to receive the report or misplacement. The acceptable cut-off for the same, as quoted in the article by Chawla R et al., was 1.6% or 16/1000 [18]. However, a laboratory should strive to achieve 100% report delivery to clinicians or patients so that patients are not inconvenienced and treatment can be initiated at the earliest.

The audit of the various putative variables has revealed a few crevices in the laboratory system as the molecular laboratory was started from scratch. Therefore, a few glitches were faced initially, such as a lack of sufficient staff strength and increased lead time for training the staff; consequently, the QIs were slightly compromised in certain areas.

Limitation(s)

As it was a retrospective study covering a short period, a limited sample size was included. The study did not control for other factors, such as the experience of the laboratory staff or the type of equipment used. This makes it difficult to determine which factors are specifically responsible for the observed quality issues. Further studies are needed to draw firmer conclusions on the results presented in the present study.

CONCLUSION(S)

In conclusion, although the audit of the quality management system did reveal a few weaknesses that were later improved upon, the study also emphasises adherence to QC processes for the smooth operation of any diagnostic laboratory, especially those involved in critical reporting. The QIs assessed helped in monitoring the laboratory parameters effectively.

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PARTICULARS OF CONTRIBUTORS:

1. Associate Professor, Department of Microbiology, Institute of Human Behaviour and Allied Sciences, Delhi, India.
2. Senior Resident, Department of Clinical Microbiology, NCRIMS, Meerut, Uttar Pradesh, India.
3. Assistant Professor, Department of Clinical Microbiology, Rajiv Gandhi Super Speciality Hospital, Delhi, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Dr. Sukriti Sabharwal,
113 Ram Vihar, Delhi-110092, India.
E-mail: sabharwalsukriti@gmail.com

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