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RESEARCH ARTICLE

FASTER AND COST-EFFECTIVE EXTRACTION FREE METHOD FOR SARS-COV2 DETECTION BY RT-PCR

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Abstract

Background: The World Health Organization (WHO) declared an outbreak of febrile respiratory illness caused by Severe Acute Respiratory Syndrome Corona Virus-2 (SARS-CoV-2) in Wuhan City, China in December 2019. Within the immediate outbreak of three months, the global community was challenged with a devastating pandemic that caused heavy morbidities and mortalities around the globe. The expeditious spread of the virus, challenged the diagnostic laboratories to rapidly develop the accurate molecular diagnostic methods. As SARS CoV-2 assays became available for testing on existing molecular platforms, laboratories devoted unprecedented energy and resources to evaluating the analytical performance of the new tests and in some cases developed their diagnostic assays under FDA-EUA guidance. **Aim**: This study aims to compare the diagnostic efficacy of the extraction-free method of COVID-19 PCR and the conventional RT-PCR with extraction. **Method**: To get an accurate view of how an omission of RNA extraction step would perform in a real-world setting, a comparative study was performed using TATA MD CHECK RT-PCR DIRECT (Direct PCR) and LabGun Genomics for Conventional RT-PCR method. **Result**: From this study, direct RT-PCR correctly identified 92.3% of samples (n = 50) identified positive for SARS-CoV-2 RNA by conventional RT-PCR featuring an RNA extraction. **Conclusion**: Direct methods may represent a reasonable alternative to meet higher testing demands with low turnaround time as reverse transcription PCR includes traditionally time-consuming RNA extraction and purification procedures.

Introduction:-

Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2), the seventh and most recent human beta-coronavirus, first appeared in Wuhan, China, in December 2019. This highly pathogenic virus rapidly triggered a global pandemic, resulting in millions of cases and deaths worldwide (Uhteg et al., 2020).

COVID-19 spreads primarily through respiratory droplets and aerosols released when infected individuals cough or sneeze and through extended contact with those infected. Symptoms of the disease varied widely, from mild

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respiratory issues to severe, life-threatening multi-organ failure. The virus's rapid transmission contributed to the outbreak's swift global spread (Uhteg et al., 2020). Within weeks of the virus's discovery, scientists had characterized the whole genome of SARS-CoV-2, enabling the development of multiple molecular diagnostic tests. The virus was first isolated from a patient's bronchoalveolar lavage (BAL) fluid in China using metagenomic whole-genome sequencing (Uhteg et al., 2020). On February 4th, 2020, the CDC's COVID-19 real-time PCR test was granted emergency use authorization (EUA).

Molecular identification methods are preferred for quick human diagnostic procedures, as virus isolation is often time-consuming. Reverse transcription-PCR (RT-PCR) became the gold standard for diagnosing SARS-CoV-2 infections. However, testing efforts were hindered by shortages of supplies and lengthy processing times (Cameron et al., 2021). One of the primary challenges in the early stages of the COVID-19 pandemic was the limited availability of RNA extraction reagents, which were both expensive and difficult to obtain due to global shortages and lockdowns, further complicating the testing process (Cameron et al., 2021).

The results from the antigen card test are determined in approximately 30-45 minutes, depending on the kit's make. However, positive results were accepted as reliable and accurate in antigen card tests, whereas when reported negative, the individual must undergo RT-PCR testing. When the individual is asymptomatic, antigen card tests are less likely to detect the virus than regular PCR tests. Therefore, a single negative antigen test cannot rule out infection. As per FDA recommendations, to be confident about the absence of COVID-19, it recommends two negative antigen tests for individuals with symptoms or three antigen tests for those without symptoms, performed 48 hours apart (WHO, 2023). The testing process becomes time-consuming and costly, and the individual must invest in many antigen test cards.

The nucleic acid detection approach introduced by Ochmann and colleagues avoids the need for molecular amplification, instead utilizing a direct physical fluorescence amplification mechanism (Ochmann et al., 2017). In a related development, Zhu and the team designed a device capable of quickly extracting and detecting the Zika virus (Zhu et al., 2020).

Aims and Objectives:-

Considering the other studies and of great interest for the use of extraction-free methods, which provide accurate results in a short time with less expenditure, this study aimed at determining SARS-CoV-2 RNA via direct RT-PCR assay without the step of RNA extraction and to compare the diagnostic accuracy of Direct RT-PCR with the conventional RT-PCR with extraction.

Materials and Methods:-

This study was conducted at the Viral Research and Diagnostic Laboratory (VRDL), Department of Microbiology, Coimbatore Medical College, Coimbatore. To get an accurate view of how an omission of the RNA extraction step would perform in a real-world setting, we tested a panel of 50 samples (including test controls). All sample collection procedures were performed according to standards established by WHO and CDC for COVID-19. For testing and comparative study, TATA MD CHECK RT-PCR DIRECT (Direct PCR) and LabGun Genomics for the Conventional RT-PCR method were used.

Instruments:

RNA Extraction Machine (KingFisher Flex, ThermoScientific), Thermal Cycler, Cooling Centrifuge, varying range micropipettes, heating plate, PCR consumables

Methodology:-

The routine RNA extraction step was performed as per the manufacturer’s instructions, using a MagMAX Viral Pathogen II Nucleic Acid isolation commercial kit (Applied Biosystems, ThermoFisher Scientific, USA). The Direct RT-PCR was performed as per the manufacturer’s instructions using the TATA MD CHECK RT-PCR XF diagnostic kit.

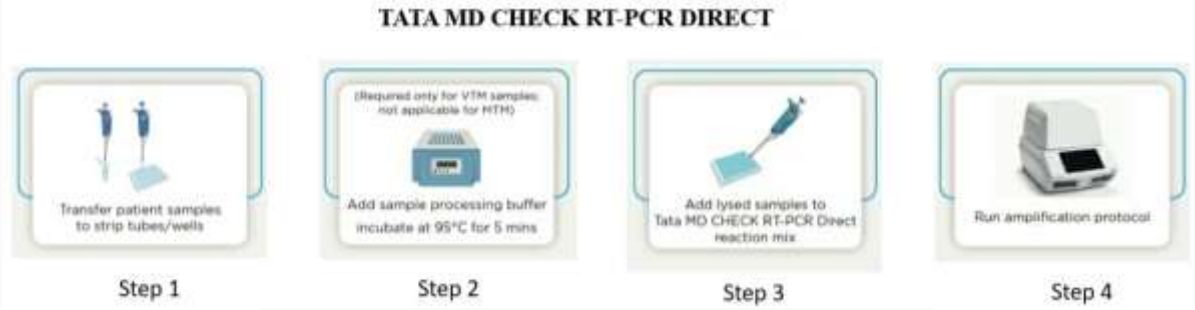

Figure 1:-Various steps involved in the Direct PCR technique.

Results:-

A total of 45 samples were tested with the plate map for 45 samples and 2 controls (positive and negative control) along with 3 known low and high-value controls to confirm the obtained result. The tests were performed in duplicates to confirm the obtained test result.

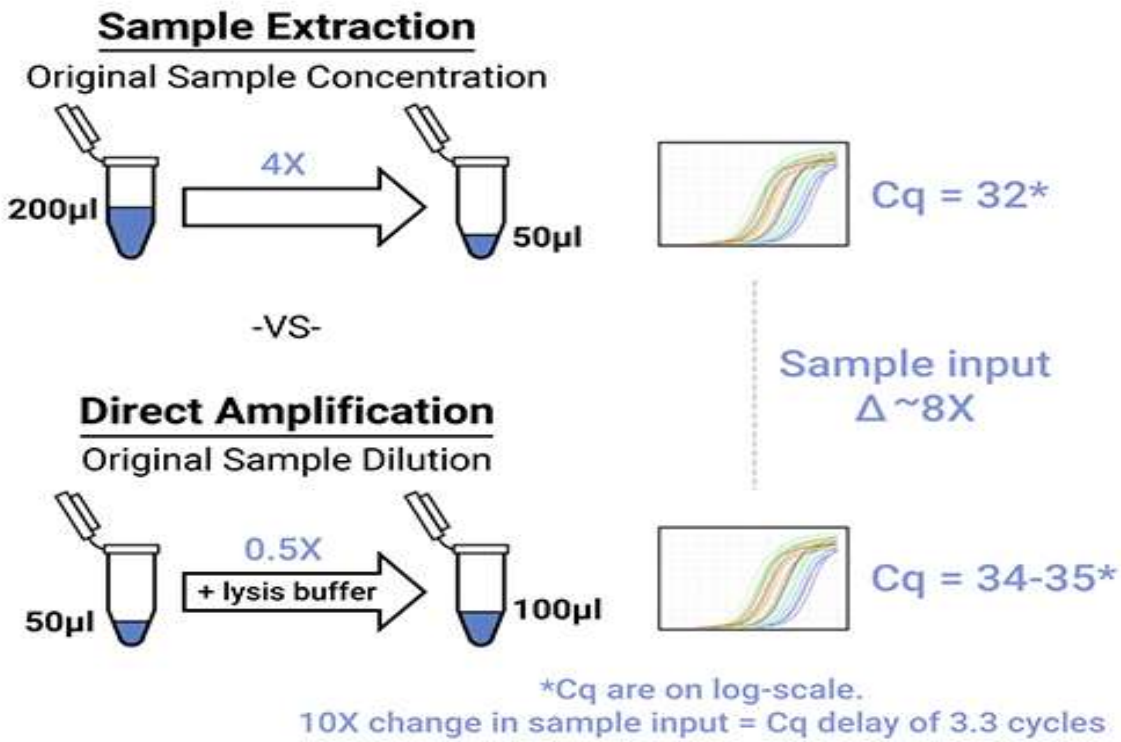

Figure 2:- Comparison of amplification results of the same sample with a sample extraction workflow and a direct amplification workflow.

Turnaround time for both methods:

Table 1:- Turnaround time (TAT) for both methods.

Method	Total Turnaround time/ sample
Direct PCR	1 hour
Conventional PCR	2-3 hours

Test results of the samples by both methods:

Table 2:- Results obtained by both methods.

Kit used	Positives	Negatives
Direct PCR	12	33
Conventional PCR	13	32

Number of samples processed (n) = 45

Comparison C_T values of the positive samples in both methods:

Table 3:- Comparison of Cycle threshold values of the positive samples obtained by both methods.

$C_{T\ values}$	Direct PCR	Conventional PCR
0-25.0	8	8
25.1-35.0	4	4
35.1 to 45.0	0	1
Total positives	12	13

The obtained Ct values were tabulated within a range and further, the cases were analyzed with the obtained test request form to know the conditions of the patients' samples as symptomatic or asymptomatic.

Table 4:- Data of symptomatic and asymptomatic positive cases and their Cycle threshold values from the Direct PCR method.

$C_{T\ values}$	Direct PCR	Symptomatic	Asymptomatic
0-25.0	8	7	1
25.1-35.0	4	2	2
35.1 to 45.0	0	-	-

Table 5:- Data of symptomatic and asymptomatic positive cases and their Cycle threshold values from conventional PCR method.

$C_{T\ values}$	Conventional PCR	Symptomatic	Asymptomatic
0-25.0	8	6	2
25.1-35.0	4	2	2
35.1 to 45.0	1	1	-

Discussion:-

The advancement and use of molecular diagnostic techniques have transformed how infectious diseases are diagnosed and monitored.

RT-PCR-based diagnostic methods are widely used to detect RNA in infectious agents and are regarded as the most sensitive and specific techniques for identifying them (Tang et al., 1997). For virological diagnosis, the Nucleic Acid Amplification Techniques (NAATs) are widely used as the detection of viral genome fragments are made possible. Several NAATs were developed to expand performance and specificity, such as real-time Polymerase Chain Reaction (real-time PCR), in which the amplified products are detected and quantified in each cycle of the PCR reaction (Costa, 2004). Whereas in the nested PCR technique, the product of the first amplification is used as a template for a second amplification, providing high sensitivity and specificity (Erlich, 1989). In multiplex PCR, more than one sequence is simultaneously amplified using different primer sets in the same reaction (Markoulatos et al., 2002). NAAT-based systems detect disease-causing agents directly from clinical samples without requiring culture, which are crucial for rapidly identifying unculturable or fastidious microorganisms. These methods have surpassed viral culture as the gold standard for viral diagnostics due to their broader applicability, higher sensitivity, faster results, and suitability for field use.

The COVID-19 pandemic has placed unprecedented pressure on hospital and commercial laboratories as they strive to meet the growing demand for SARS-CoV-2 testing (Bruce et al., 2020). A major impediment to large-scale SARS-CoV-2 testing is the ready availability of extraction kits for manual nucleic acid extraction and more extensive automated extraction processes. To address this issue, we tested the performance of the direct PCR technique by eliminating RNA extraction and directly using the VTM material for mixing. We report that this approach (direct RT-PCR) correctly identified 92.3% of samples (n = 50) identified positive for SARS-CoV-2 RNA by conventional RT-PCR featuring an RNA extraction.

The direct PCR method used in this study is simple, economical, and rapid. This technique reduces the result turnaround time. It can be performed in a simple lab setup with low investment. In terms of economy, this method saves machinery costs, consumables, and manpower to a greater extent. Direct PCR ensures safer testing with less contamination risk. The main challenge to overcome in direct PCR amplification is assay sensitivity. Before amplification, RNA extraction can serve as a concentrating step. For example, a 200 µL sample is concentrated to a 50µL eluent during the purification step. This results in a fourfold increased concentration of the RNA before going into the amplification reaction. In direct amplification there is no extraction step and hence lack of concentration which may at times lead to late cycle threshold for positive samples. Direct amplification workflows show high throughput and are also automation-friendly across multiple platforms. Our study had important limitations with the availability of testing kits.

Conclusion:-

Reverse transcription PCR typically involves lengthy RNA extraction and purification steps, often taking longer than the RT-PCR process. While slightly less sensitive than traditional RT-PCR assays, direct testing methods may offer a viable alternative for handling higher testing volumes with faster turnaround times.

Conflict Of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References:-

1. Bruce, E.A., et al. 2020. Direct RT-qPCR detection of SARS-CoV-2 RNA from patient nasopharyngeal swabs without an RNA extraction step. PLOS Biology. 18(10), e3000896.
2. Cameron, A., et al. 2021. Extraction-Free Methods for the Detection of SARS-CoV-2 by Reverse Transcription-PCR: a Comparison with the Cepheid Xpert Xpress SARS-CoV-2 Assay across Two Medical Centers. J. Clin. Microbiol. 59(2), e02643-20.
3. Costa, J. 2004. Reaccionencadena de la polimerasa (PCR) a tiempo real. Enferm. Infecc. Microbiol. Clin. 22(5), 299-305.
4. Erlich, H. A. 1989. Polymerase chain reaction. J. Clin. Immunol. 9, 437–447.
5. Markoulatos, P., et al., 2020. Multiplex polymerase chain reaction. Micromachines 11, 186.
6. Ochmann, S.E., et al. 2002. Optical nanoantenna for single molecule-based detection of zika virus nucleic acids practical approach. J. Clin. Lab Anal. 16, 47-51.
7. Tang, Y.W., et al., 1997. Molecular diagnostics of infectious diseases. Clin. Chem. 43, 2021–2038.
8. Uhteg, K., et al., 2020. Comparing the analytical performance of three SARS-CoV-2 molecular diagnostic assays. J. Clin. Virol. 127, 104384.
9. WHO, 2023 - https://www.cdc.gov/coronavirus/2019-ncov/symptoms-testing/testing.html#types-of-tests. (Accessed on August 30th, 2023)
10. Zhu, X., et al., 2020. A novel microfluidic device integrated with chitosan-modified capillaries for rapid ZIKV detection, Micromachines 11, 186.