Fast PCR test for the diagnosis of SARS COV-2: Necessity, Success and Challenges

By

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A thesis submitted to the School of Pharmacy in partial fulfillment of the requirements for the degree of Bachelor of Pharmacy (Hons)

School of Pharmacy
Brac University
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Declaration

It is hereby declared that

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2. The thesis does not contain material previously published or written by a third party, except where this is appropriately cited through full and accurate referencing.
3. The thesis does not contain material which has been accepted, or submitted, for any other degree or diploma at a university or other institution.
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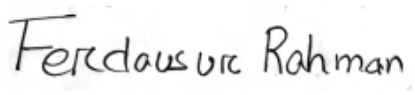

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Approval

The thesis/project titled "Fast PCR test for the diagnosis of SARS COV-2: Necessity, Success and Challenges" submitted by Ferdausur Rahman (17346014) of Summer, 2017 has been accepted as satisfactory in partial fulfillment of the requirement for the degree of Bachelor of Pharmacy.

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Ethics Statement

This study does not include any human or animal trials.

Abstract

The SARS-CoV-2 pandemic has kept the whole world on a halt. Even after two years of emergence, we could not contain the infection due to its highly transmittable nature. Early and fast detection of the causative SARS-CoV-2 virus is necessary to take proper steps to isolate the patient and/or medications on time. RT-PCR is the most conventional diagnostic method to detect SARS-CoV-2 that requires appropriate samples as well as trained resources for smooth operation. Most importantly, a longer turnaround time remains a major challenge on the way to mass testing for SARS-CoV-2. The newly developed fast PCR techniques eliminate several limitations of the previous RT-PCR, capable of detecting SARS-CoV-2 from the patient sample within 30 minutes. This study focuses on two fast PCR instruments Cobas Liat and MIC qPCR to assess the success, necessity, and challenges of the fast PCR methods for intervening in the pandemic.

Keywords: SARS CoV-2, RT-PCR, Fast PCR, Cobas Liat, Magnetic Induction Cycler.

Dedication

Dedicated to all my teachers who were and are with me. I am grateful to have you as my teacher.

Acknowledgment

All praise to the Almighty Allah for the courage, patience, and strength to complete the project. I am grateful to my supervisor, Dr. Md. Abul Kalam Azad, Assistant Professor, School of Pharmacy, Brac University for his constant support, patience, guidance, and suggestions without which I would not have been able to complete this project. I am also grateful to Professor Dr. Eva Rahman Kabir, Dean, School of Pharmacy, Brac University, and all the faculty members of the School of Pharmacy, Brac University for their teaching, support, encouragement, and precious advice. I am also grateful to my family and friends for their support and love.

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List of Acronyms

2019-nCoV	2019 novel coronavirus
ARDS	Acute Respiratory Distress Syndrome
COVID-19	Coronavirus Disease-19
DNA	Deoxy Ribonucleic Acid
LAMP	Loop-Mediated Isothermal Amplification
MERS-CoV	Middle Eastern Respiratory Syndrome Coronavirus
Mic	Magnetic Induction cycler
MRI	Magnetic Resonance Imaging
mRNA	Messenger RNA
NGS	Next-Generation Sequencing
NSP	Non-structural Protein
PCR	Polymerase Chain Reaction
qPCR	Quantitative Polymerase Chain Reaction
RNA	Ribonucleic Acid
RT-PCR	Reverse Transcriptase Polymerase Chain Reaction
SARS-CoV	Severe Acute Respiratory Syndrome Coronavirus
SARS-CoV-2	Severe Acute Respiratory Syndrome Coronavirus-2
WHO	World Health Organization

Chapter 1

Introduction

1.1 Severe Acute Respiratory Syndrome (SARS-CoV-2)

The highly infectious coronavirus incidence originating in Wuhan, China in December 2019 was named Coronavirus Disease-19 (COVID-19). The pathogen was named SARS-CoV-2 comparing its genomic similarity with previously occurring coronaviruses. It produces common flu-like symptoms in a majority of the patients but a fraction of patients may also witness severe outcomes, acute respiratory distress syndrome (ARDS), multiple organ failure, even death (Adil et al., 2021; Chiara et al., 2021; Ciotti et al., 2019; Cui et al., 2021; Umakanthan et al., 2020; V'kovski, Kratzel, Steiner, Stalder, & Thiel, 2021; F. Wu et al., 2020; Xiong et al., 2020). The World Health Organization (WHO) has declared COVID-19 as a global pandemic in March 2020 considering its abrupt global spread and disease severity (Cucinotta & Vanelli, 2020). According to the "Worldometer" the total confirmed cases crossed 438 million by the year 2022 with a death rate of 1.37% while, Bangladesh is ranked 41th based on the total confirmed COVID-19 cases (Worldometer, 2022). The Asian Development Bank has estimated a global economic loss between USD 6.1 to 9.1 trillion during the COVID-19 pandemic (Mottaleb, Mainuddin, & Sonobe, 2020). While the COVID- 19 pandemic has terrorized the mass population even in the advanced parts of the globe, experts implicate a much more challenging situation in developing countries like Bangladesh. Lack of resources, high population density, the lower income of the majority greatly limits the ability to implement preventive measures, social distancing, personal hygiene, etc, (Anwar, Nasrullah, & Hosen, 2020; Biswas, Huq, Afiaz, & Khan, 2020; Mofijur et al., 2021). Immunotherapy and vaccination are slowly but surely improving the current scenario. As studies and reports

establish a positive link to vaccination and effectiveness and protection from infection, more people are responding to the government's vaccination initiative every day. Yet, the efforts to detect SARS-CoV-2 for monitoring and diagnostic tests are still inadequate. The most commonly used real-time reverse transcription-polymerase chain reaction (real-time RT-PCR) is still an expensive and time-consuming process. It limits the daily sample testing ability of the diagnostic centers resulting in a fraction of suspected patients remaining untested and failing to attain proper medication and hospitalization (Haidere et al., 2021).

1.2 Polymerase Chain Reaction (PCR)

Polymerase Chain Reaction (PCR) detects even minute quantity DNA present in the sample by amplifying it for further experimental procedures. Repeated cycles of controlled heating and cooling of the sample DNA loaded into PCR tubes along with a master mix containing suitable primer, polymerase enzyme and oligonucleotide bases exponentially increase the product yield on each cycle (*Figure 1*) (Bhatia & Dahiya, 2015; Green & Sambrook, 2018). Selecting the desired genetic fragment through binding to a complementary sequence before amplification enables qualitative detection of the specific sequence in the sample. Although this method provides a clear indication of the presence or absence of the sequence, it does not directly produce a quantitative result. Real-time PCR or qPCR integrates radioactive or fluorescent markers designed to specifically bind to target sequence and correlates fluorescence intensity over PCR cycles quantitatively (Kralik & Ricchi, 2017; Maddocks & Jenkins, 2017). As genetic materials amplify exponentially in each cycle, the curve also shows exponential growth (Heid, Stevens, Livak, & Williams, 1996; Kubista et al., 2006; Schmittgen & Livak, 2008; Wong & Medrano, 2018). Incorporation of reverse transcriptase enzyme introduces to another variation of PCR, reverse transcription PCR (RT-PCR) that enables amplifying RNA, which translates into functional proteins, especially important in amplifying genetic materials of

organisms that only constitute RNA (*Figure: 4*) (Bachman, 2013; DC, 2014; Farrell, 2010; Jalali, Zaborowska, & Jalali, 2017). High sensitivity, reproducibility, and multidimensional scope make RT-PCR one of the most conventional diagnostic pathogen detection techniques of today (Genersch, 2005; Hirsilä et al., 2001; K, P, P, & L, 2017; SL et al., 2004). Real-time RT-PCR is regarded as the gold standard for detecting SARS-CoV-2 amid the current COVID-19 pandemic; as a result, clinicians across the globe employ it as a confirmatory test for SARS-CoV-2. However, RT-PCR may take several hours to days to perform and provide a result (Gao et al., 2020; Lamers et al., 2020; Soremekun, Omolabi, & Soliman, 2020). Other methods for SARS-CoV-2 detection include whole-genome sequencing, antigen testing, etc. These methods are applied alone or in combination with RT-PCR for screening purposes and, they need to meet specific requirements like, expert technician, a certain period from infection, volume and availability of sample, etc. limits their use (Eftekhari et al., 2021; Kevadiya et al., 2021; R. Liu, Fu, Deng, Li, & Liu, 2020). A few relatively new and faster PCR-based systems has been developed as an emergency measure that shown to detect SARS-CoV-2 within 20 to 50 minutes (Mahmoud et al., 2021). They include Cobas Liat system of Roche Molecular Systems, Inc. and Mic qPCR cycler of BioMolecular Systems, which provides a result within 30 minutes and also does not require skilled technician, difficult calibration (BioMolecular Systems, 2022a; FDA, 2020). Although the methods lack sensitivity and accuracy compared to conventional RT-PCR, it might act as an immediate screening test in the hospital setting to determine treatment, reduce hospital stay and treatment cost as well as aid in faster traveling and transition (Mahmoud et al., 2021).

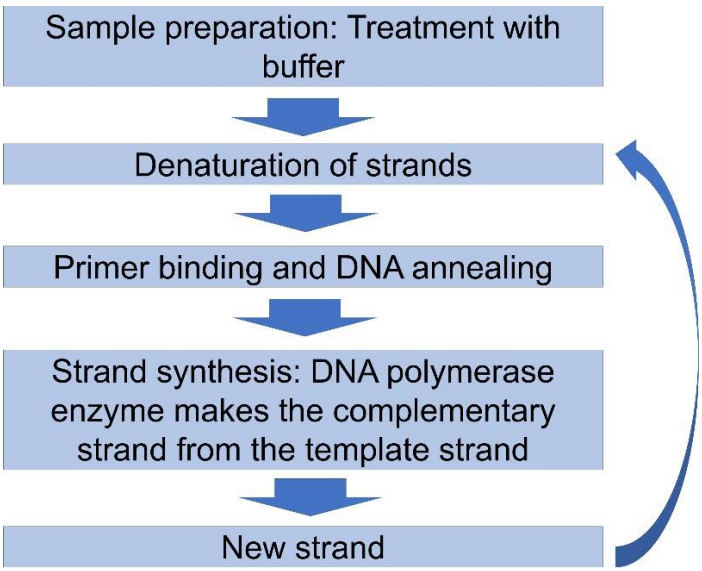

Figure 1: PCR amplification steps. The sample is first prepared by lysing the cells to obtain genetic materials and DNA is separated from all the other substances. DNA is denatured to make the single-stranded template. Primer binds on the DNA to initiate complementary strand synthesis by DNA polymerase. After completion of the strand synthesis, it will increase the genetic material by two-fold. The cycle is run several times to sufficiently amplify the genetic material (Bachman, 2013; Stephen A. Bustin & Nolan, 2020; Green & Sambrook, 2018).

1.3 Aim of the Study

The study aims to assess the current understanding of the SARS-CoV-2 detection using faster PCR techniques, their application, success, and challenges.

Chapter 2

Methodology

The study implied a keyword-based literature search to extract the current knowledge and discuss the applications of the Roche Cobas Liat and BioMolecular system's Mic qPCR system. Two prominent literature search engines, PubMed and Google Schooler were utilized to select the relevant literature. SARS-CoV-2, COVID-19, coronavirus, rapid, PCR, diagnostic, detection, application was used to procure results on SARS-CoV-2 diagnostic detection. Liat, Roche, Cobas for Cobas Liat system and Mic, Mic-qPCR, Magnetic Induction Cycler, BioMolecular System for Mic qPCR were inquired separately. Additional filtering on only Full text, publication year threshold 2020 to 2021, and English language were applied. Finally, the extracted literature were read and manually screened and only the articles that specifically focus on the application and implementation of the Cobas Liat and Mic qPCR for rapid detection of SARS-CoV-2 were selected for the study.

Chapter 3

SARS-CoV-2 and Diagnosis of SARS-CoV-2

3.1 SARS-CoV-2 and its structure

SARS-CoV-2, the causative agent for the current COVID-19 pandemic is the third human infecting coronavirus instance followed by SARS-CoV and MERS-CoV. The metagenomic analysis of the infected patient sample confirmed it as a new strain that had genomic similarity with previous Coronavirus incidences (Lu et al., 2020; V'kovski et al., 2021). SARS-CoV-2 however, showed a greater genomic similarity, 96% to a bat coronavirus species and less than 80% with the previously occurring SARS-CoV and MERS-CoV. Scientists predict the virus might either witness a zoonotic shift from bat or some other species through genetic recombination and bat acted as a secondary reservoir. The virus is a single-stranded positive sense RNA virus, that shares structural similarity with SARS and MERS-CoV (Blanco-Melo et al., 2020; Gordon et al., 2020; Haimei, 2020; Khorsand, Savadi, & Naghibzadeh, 2020). The SARS-CoV-2 genome is approximately 29,903 base pairs long and constitutes 4 structural proteins, 16 non-structural proteins (NSP). The structural proteins include nucleocapsid (N protein) on the core, membrane protein (M protein) on the membrane of the virus, envelop protein (E protein) covers the membrane and spike protein (S protein) is embedded on the membrane *(Figure: 2)*. The non-structural proteins are named nsp1~16. The S protein attaches to the host organism for cellular entry *(Figure: 3)*, N protein encapsulates the viral genetic material and prepares for replication in the host while, NSPs regulate host machinery for immune evasion and viral replication (Banerjee et al., 2020; Srivastava, Daulatabad, Srivastava, & Janga, 2020; V'kovski et al., 2021; Wang et al., 2020).

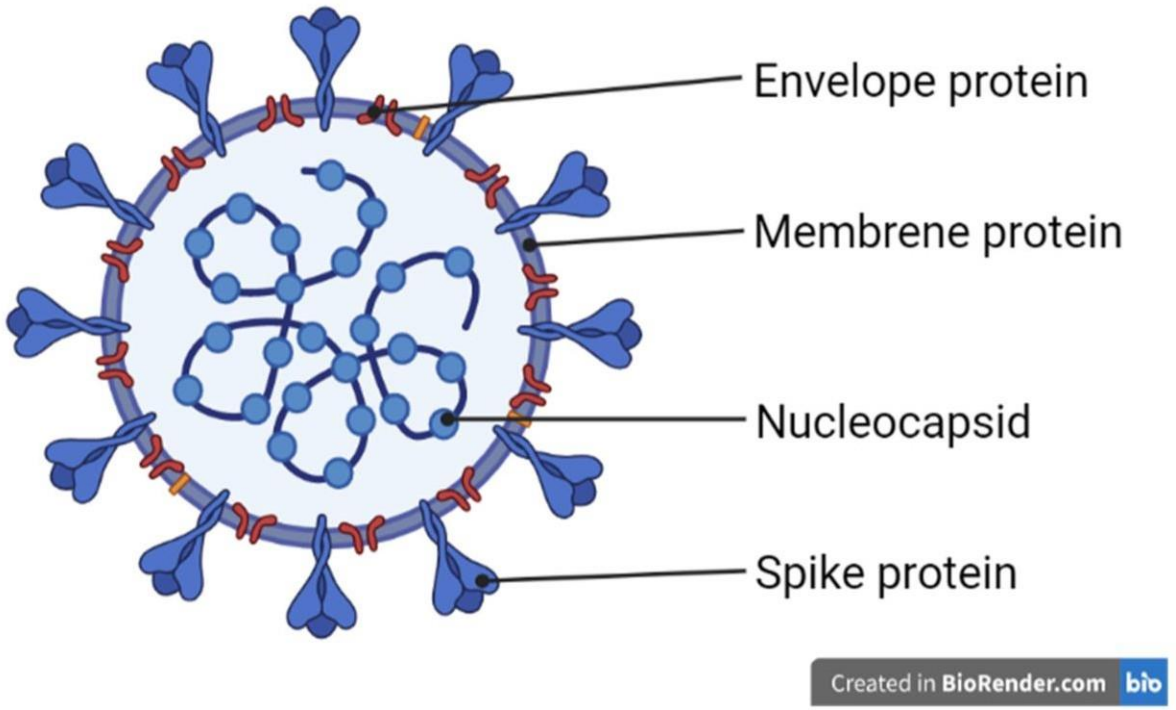

Figure 2: The molecular structure and structural proteins of SARS-CoV-2. The causative agent for Coronavirus-2019 has four structural proteins: (a). envelop protein that interacts with host membrane protein, (b) membrane protein forms the viral membrane, (c) Nucleocapsid attached with an RNA genome, viral to form viral envelope, (c) Spike protein helped to bind with the receptor for host cell entry. "Created with BioRender.com".

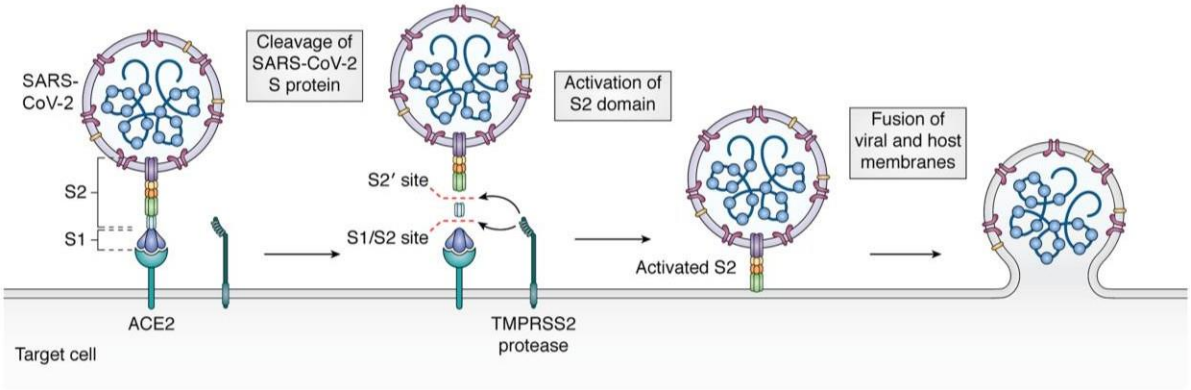

Figure 3: SARS-CoV-2 viral entry. The viral spike protein attaches to the host target site to be activated. The activated spike protein binds to the cell membrane and initiates fusion followed by viral entry to the cell (Hartenian et al., 2020).

3.2 Diagnostic tests for SARS-CoV-2

RT-PCR

Reverse Transcriptase PCR (RT-PCR) is a molecular technique that produce cDNA from mRNA using the enzyme reverse transcriptase and utilize PCR principle for exponential amplification of the genetic material. The applications of RT-PCR include, genetic disorder diagnosis, gene expression, pathogen detection, etc. RT-PCR find its extensive usefulness in detecting RNA virus from patient sample (Bachman, 2013; S. A. Bustin, Benes, Nolan, & Pfaffl, 2005). RT-PCR is considered the gold standard confirmatory test for detecting recently emerging pathogenic RNA virus SARS-CoV-2 (Stephen A. Bustin & Nolan, 2020; Pereira-Gómez et al., 2021; Y. Wu, Xu, Zhu, & Xia, 2020).

The method mimics a natural process of RNA organisms to replicate in host cell, making double stranded DNA from RNA as starting material. The process starts with sample preparation for RNA extraction. Nasopharyngeal or oropharyngeal swab collected from the patient is treated with lysis buffer and mechanically disrupted to bring protein, enzyme and genetic materials in solution. The lysis buffer contains protein kinase, $MgCl_2$, PCR grade water, etc. Lysis buffer in the solution degrades protein, enzymes and DNA present in the solution and only RNA remains intact. The solution is further placed on a spin column containing magnetic beads to separate viral RNA. The RNA is then taken from the buffer solution and treated with target specific primer, reverse transcriptase enzyme and master mix. The master mix is a premeasured and prepared solution contains all the necessary reagent and chemicals

for PCR amplification, such as heat stable DNA polymerase, $MgCl_2$, dNTP, buffer, water for PCR, etc. Primer binds to the specific region of RNA and reverse transcriptase enzyme make a complimentary copy DNA (cDNA) from the target RNA (*Figure: 4*). Primers is classified into two based on the binding region, oligo(dt) and random hexamer. Oligo(dt) primers attaches to the poly A tail of the mature mRNA and make complimentary copy. It is only specific for mRNA and make cDNA regardless region of interest. Random hexamers are approximately 20 nucleotide long single stranded molecule specifically designed to bind at the target of interest. Primer design is one of the major steps in PCR procedure. Fine technical, bioinformatic skill as well as knowledge on the structure and nature of the desired region is required to design a primer specific and complementarily to the region of interest. Random hexamer primers bind and act as initiation site for reverse transcription enzyme to make cDNA. The cDNA is amplified in a PCR cycler for further utilization. PCR amplification is often checked after the amplification step with Agarose Gel Electrophoresis: band, intensity, and size of the band (Bachman, 2013; Stephen A. Bustin & Nolan, 2020; Green & Sambrook, 2018).

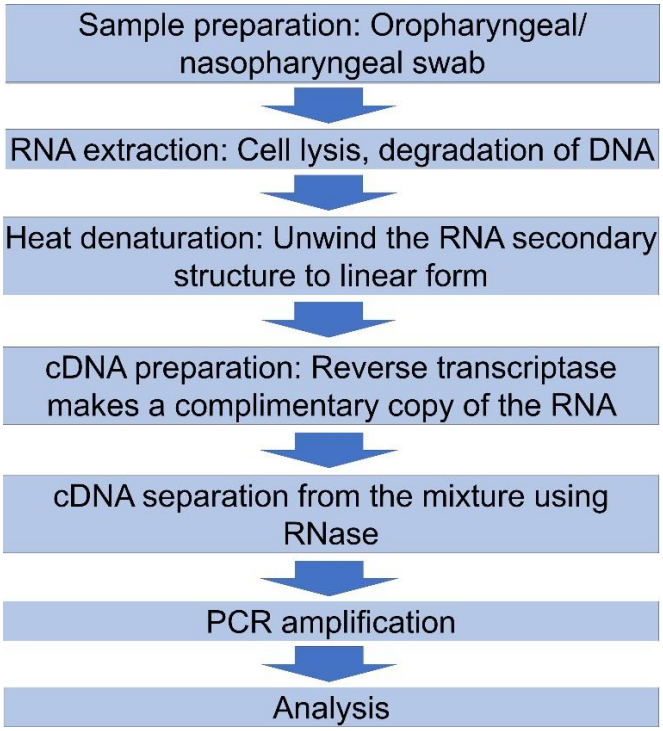

Figure 4: RT-PCR process. The first step is sample preparation. Cells are lysed to bring the genetic materials. DNA and other substance other than RNA is degraded using the RNase enzyme. The RNA is then separated for further procedure. RNA is treated with reverse transcriptase enzyme to make the complementary DNA. The complementary DNA is amplified with PCR reaction. Finally, the amplified product is used for further analysis (Bachman, 2013; S. A. Bustin et al., 2005; Green & Sambrook, 2018).

Agarose Gel Electrophoresis is only applicable at the end of the amplification while the real-time reverse transcriptase polymerase chain reaction (RT-qPCR) opens up the possibility for quantitative detection directly during the amplification cycles. Fluorescent marker molecules are integrated to the RT-PCR method that specifically binds to the PCR product and emit fluorescence (S. A. Bustin et al., 2005). Exponential amplification of genetic material implies proportional increase in fluorescent intensity (*Figure:5*). The relative fluorescent intensity plotted against number of PCR cycles quantifies the increasing genetic materials (Nolan, Hands, & Bustin, 2006).

Based on the binding specificity, there are two classes of fluorescent reporter molecules (Pereira-Gómez et al., 2021).

Non-specific detection (SYBR green): The reporter molecule is sensitive to the DNA double strand, binds to the major groove. The amount of DNA is amplified in each PCR cycle, enables more reporter marker to bind and emit fluorescence. Although, non-specific detection specifically identifies amplification, it fails to confirm if the amplified genetic material is the target of interest or not (Giglio, Monis, & Saint, 2003; Pereira-Gómez et al., 2021; Watzinger, Ebner, & Lion, 2006).

Specific detection with detector probe (TaqMan probe): A single stranded DNA molecule with binding site for fluorophore and quencher acts as the fluorescent reporter. Fluorophores are

small organic or synthetic molecules that absorb light at a specific wavelength and emit fluorescence. Quencher is a molecule when close to fluorophore, it cancels out the light emitting property. The single stranded detection probe is generally 20 nucleotides long and specifically designed to bind to the target of interest. In PCR buffer fluorophore and quencher stays together and does not show any light property. However, as the PCR reaction moves forward, more and more detection probe attach to the DNA and fluorophore and quencher binds to the complementary ends for the probe resulting light emission. Again, the DNA polymerase displaces the probe from its binding state, termination of light follows (Pereira-Gómez et al., 2021; Watzinger et al., 2006).

RT-qPCR finds its enormous applications on, RNA quantification, molecular biochemistry, biotechnology, microbiology, etc, (Nolan et al., 2006). The RT-qPCR is widely used as a diagnostic test for the current COVID-19 pandemic (Pereira-Gómez et al., 2021). Nasopharyngeal or oropharyngeal swab is collected from the subject for RNA extraction. The extracted RNA is taken in a PCR tube containing reverse transcriptase enzyme, fluorescent reporter, and PCR master mix and placed in a PCR cycler. Relative fluorescent intensity is observed along with PCR reaction progression. When the fluorescence rises above the detectible level it is called the threshold and the number of PCR cycle is called threshold cycle (C_t). C_t value is predefined parameter for a specific target sequence. C_t value 35 is taken as threshold for SARS-CoV-2 detection. The sample is called SARS-CoV-2 positive if the relative fluorescence exceeds the threshold fluorescence level before reaching C_t value 35. Similarly, if the relative fluorescence stays below threshold level in C_t value 35 the sample is marked as SARS_CoV-2 negative sample *(Figure: 5)*. The required number of cycles for detection is dependent on genetic material present in the sample or viral load. If the sample contain higher viral load, it takes less PCR cycles to cross threshold and require more cycles if the sample has lower viral load (Stephen A. Bustin & Nolan, 2020; Nolan et al., 2006). However, C_t value do

not directly interpret the viral load, bioinformatic and melting curve analysis is required to determine the viral load in the sample (Han, Byun, Cho, & Rim, 2021).

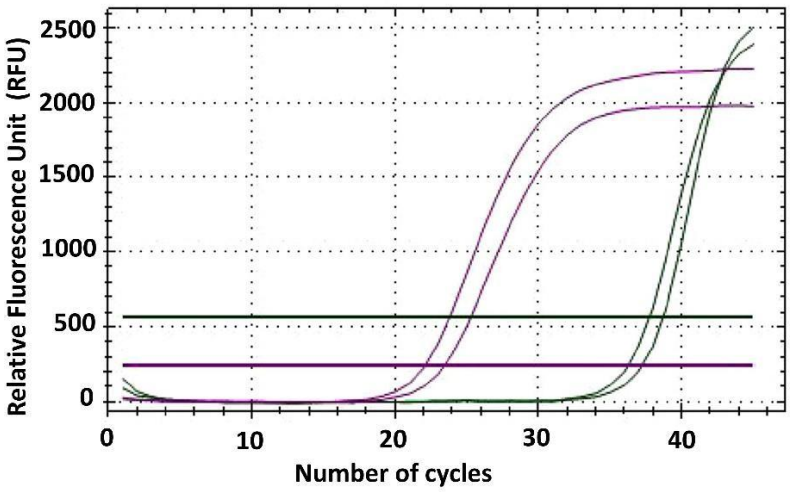

Figure 5: Threshold cycle Vs Relative Fluorescence Unit (RFU), PCR curve. The purple curve indicates gene A and the green curve indicates gene B. The purple and green horizontal lines represent the threshold for genes A and B respectively. Two separate curves represent two different samples. Adapted from (Viszlayová et al., 2021).

SARS-CoV-2 antigen detection

An antigen is a foreign body to the host that can induce immune and antibody response. SARS-CoV-2 infection can be detected during the active replication stage utilizing antigenic detection assay. The patient specimen is collected from swabs and placed on specialized paper strips. The strips have antibodies embedded in the surface that comes in contact with the sample specimen. The antigen-antibody reaction produces a distinct and detectable signal. The assay is only applicable at the very early infection stage where most of the viruses are actively replicating and the amount of dead viral particles is very low (Chaimayo et al., 2020; Parvu et al., 2021; Weitzel, Pérez, Tapia, Legarraga, & Porte, 2021; WHO, 2021).

3.3 Limitations of current SARS-CoV-2 diagnostic tests

Although several methods of SARS-CoV-2 detection is available, current effort on SARS-CoV-2 surveillance greatly depends on the RT-PCR, chest CT and X-ray. Chest CT and X-ray is implied alone or in association with the gold standard RT-PCR test. The RT-PCR tests require several hours to days to provide the results as well as requires a skilled technician to collect sample and perform the experiment (Kralik & Ricchi, 2017; Munne et al., 2021). In the current pandemic situation, testing as many samples as possible and isolation of SARS-CoV-2 positive patients are dire needs to ensure safety in emergency sectors, hospitals, airports as well as public life. Additionally, chest X-ray, CT scan, etc. demonstrate lung infections well but fail to confirm SARS-CoV-2 infection, the tests are only limited to additional examination with or without RT-PCR to screen out potentially infected patients (J. Liu, Yu, & Zhang, 2020; López-Cabrera, Orozco-Morales, Portal-Diaz, Lovelle-Enríquez, & Pérez-Díaz, 2021; Vasilev et al., 2021). Moreover, Nucleic Acid Amplification Test is a potential alternative to Rt-PCR. However, it is not available at a clinical set-up as the result depends on the skill of the technician, sample collection, and it also takes time to generate a result (Kashir & Yaqinuddin, 2020). Finally, Antigen-antibody dependent tests are specific to a certain period of infection and the patient physical condition affects test outcome. The SARS-CoV-2 antibody is detectable from serum at least a week post-infection and antigen testing is only applicable at the initial stage of infection where the virus is actively replicating. Therefore, these tests are not applied for surveillance purposes or in a clinical setting (Kevadiya et al., 2021; G. Liu & Rusling, 2021).

3.4 Limitations of Traditional RT-PCR

The conventional gold standard RT-PCR method has a few limitations such as long wait time, need for trained personal, bioinformatic tools, etc, (Daum & Fischer, 2021; Kevadiya et al.,

2021). Sample preparation is the initial step for RT-PCR, the collected sample is treated with buffer and mechanical force to bring out genetic materials. RNA is extracted with DNase enzyme and reverse transcription enzyme produce cDNA for further procedure (Kralik & Ricchi, 2017; Munne et al., 2021). This preparation step is time consuming and also error prone. Error introduced in preparation step propagates throughout the process and finally deviate from actual result. Sample preparation accuracy greatly depend on quality of used materials and reagents as well as excellence of the technician. Additionally, PCR curve and melting point analysis is performed to confirm presence of SARS-CoV-2 in patient sample. The analysis requires fine expertise on bioinformatic tools and interpretation (Giglio et al., 2003; Nolan et al., 2006). Therefore, result interpretation is also dependent on technician on biometric and interpretation expertise. Furthermore, standard laboratory setup is required for conventional RT-PCR which is occasionally unavailable in poor and remote areas (Y. Wu et al., 2020).

Chapter 4

Fast PCR techniques (LIAT, MIC PCR, etc.)

Early and accurate detection of SARS-CoV-2 is essential for suppressing the spread; isolation of infected patients, inpatient admission, handling time-sensitive outpatient care, and safeguarding public life (Blackall et al., 2021; Hansen et al., 2021; Mahmoud et al., 2021; Soares et al., 2021). Identification of variants is making it harder to contain the infection (Paton, Marr, O'keefe, & Inglis, 2021). RT-PCR is the preferred method of detection on point of care (POC) facilities (Perlitz, Slagman, Hitzek, Riedlinger, & Möckel, 2021). However, testing requirements such as trained technician, expensive equipment, reagents, laboratory setup, etc. hinders the RT-PCR detection process. Additionally, turnaround time (TAT) is also quite long for RT-PCR: several hours to days that result in poor public health response (Paton et al., 2021). Moreover, the risk of cross-contamination between samples also increases with a longer turnaround time. Furthermore, existing facilities lapse testing capacity for running tests on mass quantity. Insufficient testing facilities add an extra psychological burden on common people (Rezaei et al., 2020). High-income countries have drastically boosted their testing capacity expanding SARS-CoV-2 detection laboratory expenditure, sample handling, and transportation. In contrast to the wealthy countries, underdeveloped, developing countries and regional areas are lagging on race to test on a mass scale (Soares et al., 2021).

Other SARS-CoV-2 diagnostic tests: rapid antigen testing lacks sensitivity and is only applicable at the initial days of infection while the virus is actively replicating, antibody testing is detectible at least a week before infection, radiographic and imaging tests do detect lung infections but do not confirm SARS-CoV-2 infection alone. Taking the strengths and weaknesses of the currently available SARS-CoV-2 detection methods, searching for a faster, mobile, economic, and efficient equivalent of RT-PCR remains a challenge (Kevadiya et al., 2021; Paton et al., 2021). A few faster PCR-based instrument have recently been developed

that detects SARS-CoV-2 within 30 minutes. The faster techniques trade sensitivity for time, however, they are of comparable sensitivity and accuracy to be alternative to the gold standard RT-PCR. Critical public health decisions concerning infection control are not taken only based on the faster PCR techniques, an additional RT-PCR test is performed on the suspected discrepancies. The assays are especially useful as a screening method and in resource-limited regions (Blackall et al., 2021; Hansen et al., 2021; Mahmoud et al., 2021; Soares et al., 2021).

Among the newly developed faster SARS-CoV-2 assays, Cobas Liat developed by Roche Molecular Systems, Inc. and Magnetic Induction Cycler (MIC) qPCR developed by Bio Molecular Systems is two prominent instruments that can detect SARS-CoV-2 in 20 minutes (BioMolecular Systems, 2022a; FDA, 2020).

4.1 Cobas Liat test

Cobas Liat is an automated NAAT tool developed and manufactured by Roche Molecular Systems, Inc. that detects SARS-CoV-2 as well as Influenza A and B within 20 minutes *(Table: 1)*. The system automates and multiplexes the RT-PCR detection steps of the mentioned viruses together and makes a unit diagnostic test. The FDA has approved emergency use authorization of Cobas Liat as a diagnostic tool for SARS-CoV-2 in the USA in September 2020 (FDA, 2020). The test has shown proficiency in both laboratory detection and POC facilities, a study demonstrated Cobas Liat to detect SARS-CoV-2 with 98% accuracy with no false-negative cases (Daum & Fischer, 2021). During the COVID-19 pandemic, RSV targets were replaced by SARS-CoV-2 targets, ORF-1 a/b, and N gene. Liat amplifies viral target regions so that, samples with very low viral content are also detectible (Blackall et al., 2021; Hansen et al., 2021).

The patient samples are first pretreated to inactivate the virus and make the genetic material stable (Perlitz et al., 2021). The Liat instrument automates sample preparation, RNA extraction,

PCR amplification and result generation. The test sample is added to the test kit with the pipet provided with the kit. The kit is designed to perform one test each and has all the necessary reagents in premeasured quantity separated from each other into sections. The kit is scanned for serial numbers and patient information is entered to get an automated result for the test before completion. Following the pretreatment procedure, the sample is deposited into the kit and inserted into the Liat instrument. The sample is placed in the first section and will flow through the sections to complete the reaction *(Figure: 6)*. Automated sample preparation and RNA extraction reduces time requirement as well as reduce risk of error from personal. It takes approximately 20 minutes to amplify the genetic material to a detectable level. An automated test result is shown upon completion of the test to summarize virus detection status, minimizes the bioinformatic and interpretation expertise requirement. Therefore, it is able to produce more efficient and accurate results within a smaller time limit (FDA, 2020).

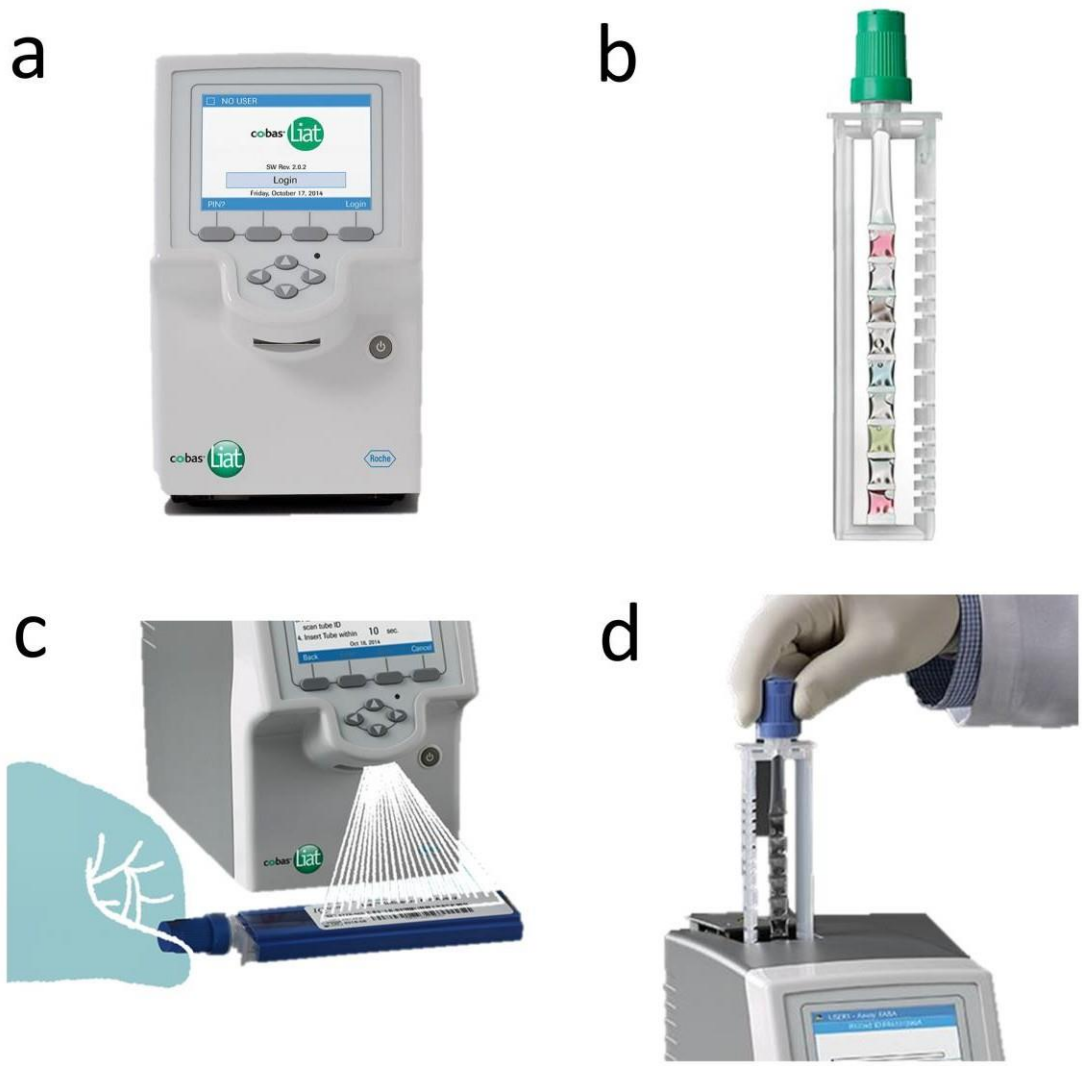

Figure 6: SARS-CoV-2 detection with Cobas Liat instrument. (a) The Cobas Liat instrument is a small, tabletop device, (b) The test kit is prepackaged for testing one sample, (c) the sample Is inserted into the kit and scanned for identification, (d) the kit is inserted on the instrument for running the test (FDA, 2020).

The Cobas Liat of Roche Molecular Systems, Inc. is a small tabletop instrument. The instrument is suitable for laboratory use as well as in POC and emergency departments as it does not require an expensive laboratory environment, equipment, reagents, etc. to run tests. Comparatively lesser chemical handling keeps the environment less hazardous even in the busy and crowded test centers. Outpatients seeking immediate medical attention requires to perform an RT-PCR test before consultation with a physician: the world still has not recovered from

the social distancing and travel ban, most of the countries require the passenger to have a molecular test confirming the person is not carrying SARS-CoV-2: offices and institutions functioning in-person require frequent diagnostic testing to ensure the safety of the staffs. Cobas Liat may prove to be an immediate and accurate screening test for the above-mentioned emergencies. A study on seasonal influenza by Perlitz, Slagman, Hitzek, Riedlinger, & Mockel, 2021 established a link to influenza detection test with Cobas Liat with enhanced therapeutic response and significantly sick leaves. Researchers expect the trend to be true for SARS-CoV-2 tests too (Perlitz et al., 2021). The test kit is a unit package for a test and all the required reagents are pre-measured and loaded on the cartilage and the final result output is automated on the test completion reduces reliability on technician's superiority.

False-positive results are one of the major concerns with Cobas Liat. A study on 8 community hospitals of Oregon province, they performed 12000 COVID-19 tests with Cobas Liat instrument revealed a 9.6% false-positive rate and they hinted, leaky test cartilage is one of the probable reasons for this discrepancy (Blackall et al., 2021). False-negative results are not noticed with the Cobas Liat system, researchers showed Cobas Liat to detect SARS-CoV-2 positive cases with 100% accuracy. FDA has also issued an alert on false-positive results obtained by Cobas Liat. Additionally, they also suggested a repetitive molecular test, RT-PCR for all the positive samples (Hansen et al., 2021).

4.2 Magnetic Induction Cycler (MIC)

Magnetic Induction Cycler is also called MIC and MIC qPCR cycler. BioMolecular Systems developed MIC qPCR cycler as a portable and lightweight tabletop alternative for RT-PCR especially in remote areas *(Figure: 7)*. The device attains an accelerated heating-cooling cycle of conventional RT-PCR experiment for saving time and achieving 35 cycles within 25 minutes (MIKROGEN DIAGNOSTIK, 2019). The Therapeutic Goods Administration (TGA), the

regulatory authority for medicine and therapeutics in Australia, one of the prominent regulatory authorities in the world has approved MIC qPCR as a diagnostic instrument for detecting nucleic acid in a sample (Table: 1). MIC has also received a CE-IVD mark, a requirement from the European directive imposed on marketing medical devices in Europe. MIC is approved to be marketed and sold in several countries of the European Union, Asia-Pacific, and North America (BioMolecular Systems, 2022b; genomeweb, 2021).

Alike RT-PCR the MIC qPCR workflow also constitutes three major steps, sample collection, genetic material extraction, and amplification. The standard PCR sample collection and RNA extraction procedure are followed for SARS-CoV-2 detection. The conventional PCR cycles between high and low temperatures exponentially amplify genetic material to a detectable threshold. Time loss between the adjustment of temperature is reduced in MIC by accelerated induction heating and forced cooling (Paton et al., 2021). Electric current is passed through a coil of wires to produce a magnetic field that heats a metal plate bearing the sample and a fan is employed on the instrument to speed up the cooling process. The forced acceleration enables MIC qPCR to perform 35 cycles within 25 minutes (Dong et al., 2021; Rezaei et al., 2020).

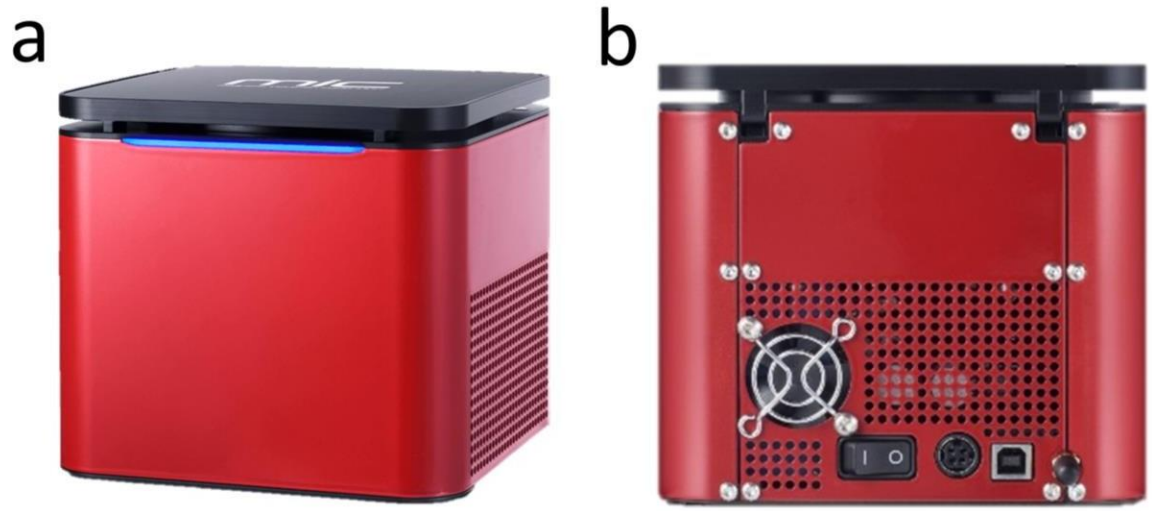

Figure 7: Magnetic Induction Cycler (MIC). (a) front and (b) back of the MIC device (BioMolecular Systems, 2022a).

The magnetic induction Cycler is a compact, lightweight, and portable tabletop instrument for molecular testing in both laboratories and public diagnostic facilities. The device is even smaller than a notebook and stackable up to three one on another. Mobility, ease of transportation, and no requirement for special calibration: make MIC qPCR suitable for detecting SARS-CoV-2 in emergency departments and remote areas (BioMolecular Systems, 2022a; Paton et al., 2021). The emergency departments, a patient seeking immediate expeditious medical attention: consultation with a physician: before performing surgery: discharging patients, offices continuing in-person activities: require routine diagnostic tests: require tests before joining after sickness for the safety of all the staffs, immigration: traveling: tourism: etc. demand a fast, reliable, sensitive and economic mode of SARS-CoV-2 detection. Remote and underprivileged regions lag in terms of development, infrastructure, communication, and coordination. A similar trend is noticed in pandemic management and diagnostic testing effort. Wealthy and developed countries have extrapolated diagnostic testing facilities to continue mass testing however, developing and underdeveloped regions are at the bottom of the list. Limited testing facilities, reagent supply, want for trained and skilled technicians are the root behind the drop back. Convenient transportation, ease of operation features of MIC qPCR will minimize the existing limitations in SARS-CoV-2 diagnostic detection (MIKROGEN DIAGNOSTIK, 2019; Paton et al., 2021).

Table 1: General information on Cobas Liat and MIC qPCR

Features	Cobas Liat	MIC qPCR
Manufacturer	Roche Molecular Systems Inc.	BioMolecular Systems
Dimension	19.0x 11.4x 24.1 cm	13.0x 15.0x 15.0x cm

Weight	3.76 kg	2 kg
Turnaround time	20 minutes	25 minutes
Operating temperature	15˚C- 32 ˚C	18 ˚C- 30 ˚C
Relative humidity	15-80%	20-80%
Samples in one run	Unit sample	48 samples
RNA extraction	Not required	Required
Chemical handling	Minimum	Moderate
Approval from regulatory authorities	FDA (emergency use authorization)	TGA, CE-IVD
Detection and result generation	Automated result generation	Signal detected on photodiodes

Chapter 5

Sensitivity and Specificity

Accuracy of a method is validated with the measure of sensitivity and specificity (Swift, Heale, & Twycross, 2020). When a method is being tested with known samples can have four probable outcomes, True Positive (TP), False Positive (FP), True Negative (TN), and False Negative (FN) (*Figure:8*). True positives are the positive samples that the test has also identified as positive and true negative are the negative samples also marked negative. True positive and true negative are desired outcome as they give correct prediction and false positive and false negative are undesired for the opposite. False positive outcomes are the negative samples that the test marked as positive and false negative are positive outcomes that the test marked as negative.

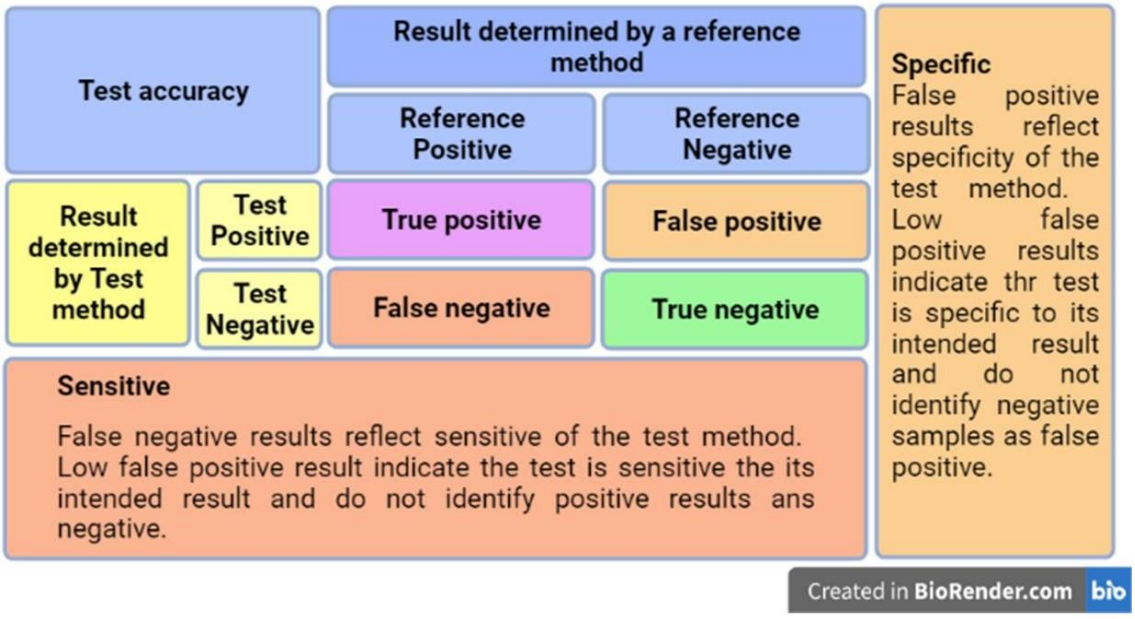

Figure 8: Test outcomes and their relation with sensitivity and specificity. Known samples examined with the test method may have four outcomes, True Positive, True Negative, False Positive, and False Negative. Specificity is the ability of a test to detect positive samples correctly. Sensitivity is the ability to detect a negative sample correctly (Forthofer, Lee, & Hernandez, 2007; Trevethan, 2017). "Created with BioRendrer.com".

Sensitivity is the probability for identifying a positive sample correctly. Sensitivity of a test can be determined mathematically by dividing True positive by total number of positive samples.

$$\text{Sensitivity} = \frac{TP}{TP + FN} \times 100$$

A test is said highly sensitive if it identifies most of the positive samples as true positive and has a few or no false negative outcome (Swift et al., 2020; Trevethan, 2017).

Specificity is the probability for identifying a negative sample correctly. Specificity of a test can be obtained mathematically by dividing true negative outcomes by negative samples.

$$\text{Specificity} = \frac{TN}{TN + FP} \times 100$$

A test is said highly specific if it identifies most of the negative samples as true negative and has a few or no false positive outcomes (Forthofer et al., 2007; Parikh, Mathai, Parikh, Sekhar, & Thomas, 2008; Swift et al., 2020; Trevethan, 2017; Wikramaratna, Paton, Ghafari, & Lourenço, 2020).

A valid and highly accurate test should be both fully sensitive and specific, however in reality a test might not be both fully sensitive and specific. If the detection threshold is set too low it will be highly sensitive, detect all the positive samples. However, it will also detect a portion

of negative samples as positive, loose specificity. Again, if the detection threshold is set too high, it will detect most of the samples as negative outcome, high specificity but low sensitivity.

Hence, a optimum threshold is chosen to balance between sensitivity and specificity (*Figure:9*) (Mattocks et al., 2010; Swift et al., 2020; White, 2020).

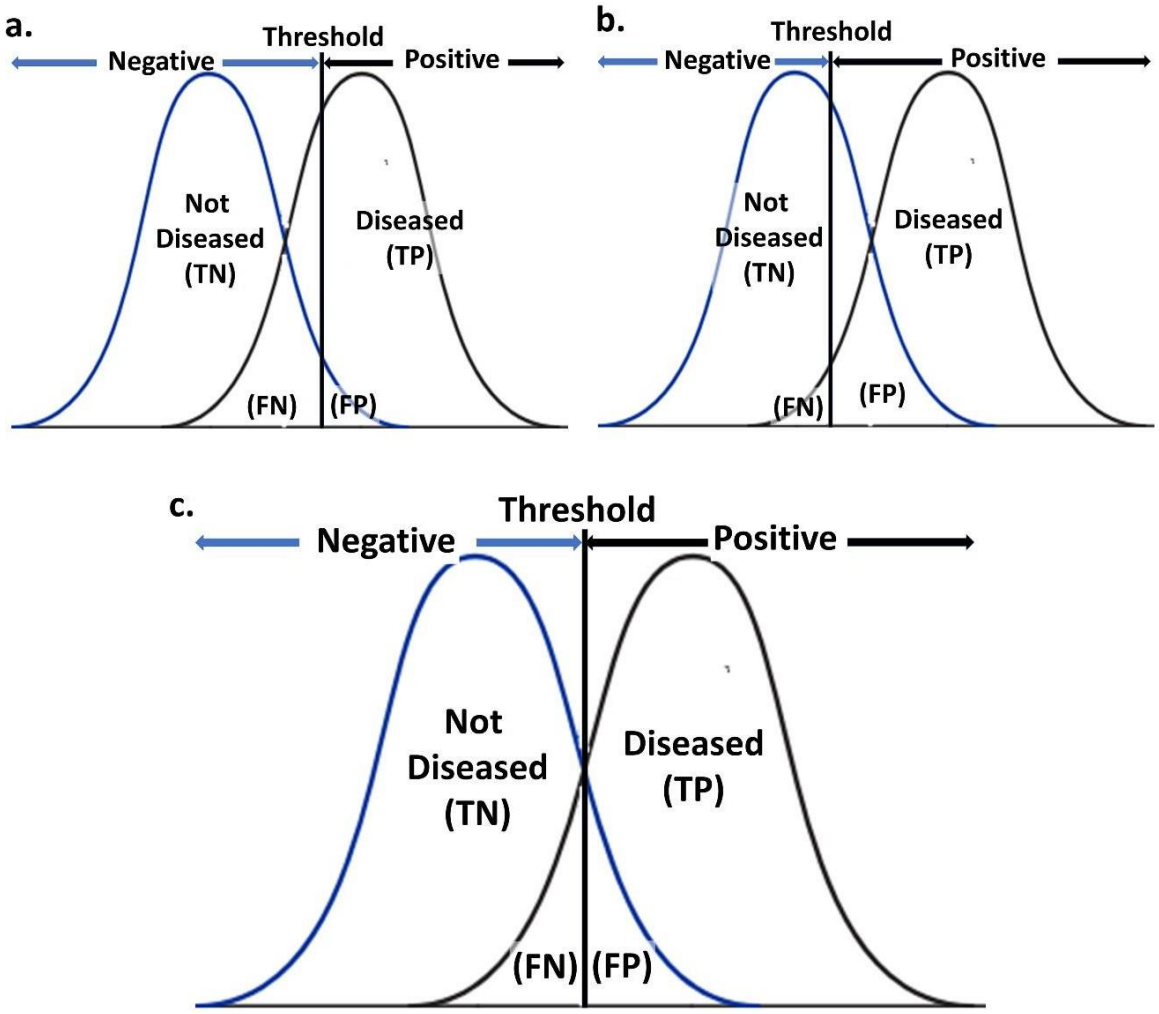

Figure 9: Relationship between threshold and test outcomes. (a). If the threshold is placed too high, the test will identify most of the samples including some negative samples as False Positive. (b). If the threshold is placed low, it will identify most of the samples including some positive samples as False Negative. (c). A fit between both is most preferable for threshold. Adapted from (White, 2020).

The RT-PCR method is highly specific and sensitive to SARS-CoV-2 with approximately 99%, 68% respectively (Kostoulas, Eusebi, & Hartnack, 2021). An error introduced during sample preparation, PCR steps, result interpretation, contamination from other samples, etc. lead to

incorrect results (Giglio et al., 2003; Nolan et al., 2006). The fast PCR techniques have higher accuracy then the conventional RT-PCR tests. The cobas Liat instrument performs all the steps automatically and minimize the personal induced errors. False negative results are not observed with cobas Liat. A proportion of false positive results are by leaky test cartridges and can be eliminated by testing all the positive samples with another method such as RT-PCR (Blackall et al., 2021; Hansen et al., 2021). Similarly, MIC automates the PCR steps, reduce human contact and provide relatively more accurate results (BioMolecular Systems, 2022a; Paton et al., 2021).

Chapter 6

Implementation

The COVID-19 epidemic has spread initially via clustered transmission from symptomatic and asymptomatic individuals especially in family clusters. Symptomatic patients often infect in close vicinity, family member, caregiver in hospital, neighboring patients in hospital, etc. While, asymptomatic as well as pre-symptomatic patients are mostly involved in the community transmission from social gatherings (Ghinai et al., 2020; Jiang et al., 2020; Klompas et al., 2021; Li et al., 2020; Pan et al., 2020; Sun et al., 2020). * The nature of rapid COVID-19 spread in Bangladesh points toward clustered transmission (Al Nahid & Ghosh, 2021; Parvin et al., 2021). The existing diagnostic detection facilities are not sufficient to screen and isolate at mass level. The fast PCR tests are an immediate, accurate, faster, and sensitive alternative to the gold standard RT-PCR. Compared to several hours to days turnaround time for RT-PCR, the faster PCR devices generate decisive results from collected samples within 20 to 25 minutes. Longer turnaround time limits the daily testing capacity which in turn destabilizes patient satisfaction and care. The fast PCR instruments (Cobas Liat, MIC qPCR) minimize several limitations of the standard testing procedure and has a great potentiality to

improve healthcare at the POC level (BioMolecular Systems, 2022a; FDA, 2020; Rezaei et al., 2020). The shorter turnaround time will reduce patient suffering on getting admitted to hospitals and consultation with a physician. The implementation of faster detection techniques will boost work efficiency in emergency departments. Faster and immediate detection will help isolate the infected from the suspected population. Faster screening ensures immediate and proper safety measures with proper medical attention further reduce the viral spread. The whole world went through a travel ban during the COVID-19 pandemic to minimize the cross-border spread of the virus. Although currently many countries have lifted the ban, the immigration department has imposed stringent entry requirements including a SARS-CoV-2 negative confirmatory test 8 to 12 hours prior traveling. Many airports across the globe have installed RT-PCR facility, travel medicine center that perform PCR test on passengers to screen out infected individuals. Nevertheless, airports in Bangladesh have no such facility, passengers have to complete PCR test requirement on their own and carry report during travelling. They often struggle to meet the requirements on time and for this reason, the passengers sometimes have to cancel the flights (Hasan, 2022; Karim, 2021). The faster PCR solutions if installed on airports would provide instantaneous test results for safe traveling (Paton et al., 2021; Perlitz et al., 2021). The developing and undeveloped regions have a limited testing facility with limited human and material resources. The faster methods are also expected to minimize the existing gap in pandemic management between the developed with developing and undeveloped parts of the globe. Both Cobas Liat and MIC qPCR offers mobile and easy-to-use PCR solution without an expensive laboratory setup. Additionally, technician's skills for the proper testing procedure and accurate result are also eliminated. Faster and accurate results will improve the overall testing capacity of a country, ultimately reflecting the pandemic management ability (Jacobs et al., 2020).

The fast PCR methods are of comparable sensitivity and specificity and the generated result sometimes deviate from the conventional RT-PCR tests. Therefore, these fast methods are not applied in critical decision making, the standard RT-PCR tests are performed. However, the faster approaches might be employed as an initial screening test and an additional RT-PCR or other diagnostic tests might be performed on the suspected discrepancies (Blackall et al., 2021; Hansen et al., 2021). The fast PCR instruments could be installed on POC and airports communicating and collaborating with the concerned departments of the government and experts. Initially, a feasibility study might provide with the insight on the necessity, scopes and limitations of the fast PCR instruments. Small scale pilot projects on few POC or airport can be carried out for further assessment. Finally, based on the success on small scale installation, the method can be implemented on mass level.

Chapter 7

Conclusion and Future Works

COVID-19 has deeply impacted public life. mass infection on both individual and community levels has made it difficult to handle the spread. Global effort to detect the causative pathogen identified SARS-CoV-2 early 2020. Although several methods have been developed for diagnostic testing of SARS-CoV-2, RT-PCR is considered the gold standard. RT-PCR have been crucial in pandemic management, isolation and therapeutic interventions. However, long turnaround time, material and human resource requirement, etc. make RT-PCR less effective and slow in some situations. The newly developed fast PCR techniques promise a faster and more convenient mode of SARS-CoV-2 detection. Implementation of the mentioned strategies in POC; airports and departments with special PCR testing need, is expected to aid in reducing public sufferings. Further studies should focus on optimizing the existing PCR methods and invent new diagnostic detection tools to withstand the current pandemic and necessarily prepare for future situations.

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