A Literature Review on the Efficacy of Test Kits for COVID-19 Diagnosis

By

Saiara Sajita Sajid

16146015

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DECLARATION

It is hereby declared that

1. The thesis submitted is my/our own original work while completing the degree at Brac University.
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 that has been accepted or submitted, for any other degree or diploma at a university or other institution.
4. I/We have acknowledged all main sources of help.

Student's Full Name & Signature:

Saiara Sajita Sajid

16156015

APPROVAL

The thesis titled "A Literature Review of The Efficacy of Test Kits for COVID-19 Diagnosis" submitted by Saiara Sajita Sajid (16146015) of Spring, 2016 has been accepted as satisfactory in partial fulfillment of the requirement for the degree of Bachelor of Pharmacy on 1st July 2021.

Examining Committee:

Supervisor:
(Member)

Dr. Mohd. Raeed Jamiruddin, PhD
Assistant Professor, Department of Pharmacy
Brac University

Program Coordinator:
(Member)

Dr. Hasina Yasmin
Professor, Department of Pharmacy
Brac University

Departmental Head:
(Chair)

Professor Dr. Eva Rahman Kabir
Chairperson, Department of Pharmacy
Brac University

ETHICS STATEMENT

This study comprises no animal or human trials.

Abstract

SARS-CoV-2, a beta coronavirus of the coronaviridae family, is responsible for the ongoing pandemic which has resulted in almost 194 million cases with more than 4 million deaths. The common manifestation of this disease involves fever, coughing, sneezing, etc., which are quite similar as common coronavirus infections. The prognosis of the disease ranges from mild and moderate to severe and ultimately death. Being an RNA virus, extensive mutations result in manifestations of a variety of symptoms which include diarrhea or blood clotting or affecting the central nervous system.

However, early diagnosis can be utilized as a powerful weapon against fighting with this virus, and thus cautious observation of the symptoms and undergoing adequate diagnosis is greatly essential. In this review article, the reliability of the two most commonly used diagnosis methods (Molecular Testing and Serological Testing) for detecting the covid-19 virus are discussed by comparing their diagnostic accuracy, patient compliance, and feasibility. In the case of comparing their diagnostic accuracy, several parameters such as sensitivity, specificity, PPV (Positive Predictive Value), NPV (Negative Predictive Value), and LOD (Limit of Detection) are taken into consideration. According to these parameters, a brief comparison of the test kits' company claim with the authorized governmental laboratory evaluation of different countries (such as the USA, UK, France, Saudi Arabia, Malaysia, etc.) is illustrated. Additionally, a brief idea of the basic mode of action of those test kits and background information about the disease itself is also provided.

DEDICATION

Dedicated to my parents and to all the victims who have died due to COVID-19.

ACKNOWLEDGMENT

First of all, I am grateful to my Almighty Allah for the good health and wellbeing during the project which was vital to complete the project work on time.

It is a genuine pleasure to express my deep sense of thanks and gratitude to my supervisor, Dr. Mohd. Raeed Jamiruddin, Ph.D. (Assistant Professor, Department of Pharmacy, Brac University) for giving me this golden opportunity to do this wonderful project. His prompt inspiration at every stage of my project work and timely suggestion with kindness, dedication, scientific approach, constant guidance, collaboration, and enthusiasm make me enable to finalize the project.

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Also, I take this opportunity to express my gratitude to all the faculty members and lab officers for their help and support.

Last but not the least, I would like to express my greatest regards to my parents for their cooperation, continuous support, unconditional love throughout my life.

CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF ACRONYMS

COVID-19	Coronavirus Diseases-2019
WHO	World Health Organization
USFDA	United States Food and Drug Administration
SARS-CoV	Severe Acute Respiratory Syndrome Coronavirus
MERS-CoV	Middle East Respiratory Syndrome Coronavirus
SARS-CoV-2	Severe Acute Respiratory Syndrome Coronavirus 2
RT-PCR	Reverse transcription Polymerase Chain Reaction
RdRp	RNA dependent RNA polymerase
IgA	Immunoglobulin A
IgG	Immunoglobulin G
IgM	Immunoglobulin M

Chapter 1: Introduction

Although Coronaviruses have originated from an extensive family (Coronaviridae) of viruses, only 4 strains are found responsible for causing infectious diseases in humans. These are 229E, NL63, OC43, and HKU1 (Rucinski et al., 2020). They generally create an infection in the respiratory tracts such as SARS (Severe Acute Respiratory Syndrome) and MERS (Middle East Respiratory Syndrome). The Novel Corona Virus which was most recently discovered was named 2019-nCoV by WHO but was relabeled as SARS-CoV-2 on Feb 11, 2020. (Ahmad, 2020). The human cases infected with COVID-19 were first reported in 2019 (December) by the officials residing in Wuhan City, China. (Bryson Taylor, 2021). These earliest known cases were mostly from an exotic wholesale food market and many of the patients were either visitors to the market or stall owners or employees. (BBC News, 2020). Later, numerous studies with the environmental samples strongly suggested that the Wuhan market could be the source of this pandemic. (Who. Int., 2020)

1.1 Coronaviridae Family

1.1.1 Taxonomy

According to the recent taxonomy, the Coronaviridae family is placed in Nidovirales order. The subfamily Coronavirinae has 4 genera such as the alpha-, beta-, gamma-, and deltacoronaviruses and the Torovirinae subfamily has only one genera that is Torovirus.

Family *Coronaviridae*

Subfamily *Coronavirinae*

Genus *Alphacoronavirus*

Genus *Betacoronavirus*

Genus *Deltacoronavirus*

Genus *Gammacoronavirus*

Subfamily *Torovirinae*

Genus *Torovirus*

(Payne, 2017)

1.1.2 Characteristics

The members of the Coronaviridae family have genomes around 25-32 kb long and the diameter of the virions ranges from 118-140 nm, making them the largest virus among all the other RNA viruses known till now. (Yang et al., 2020) They are enveloped and their RNA is single-stranded. The subfamilies (the Coronavirinae and the Torovirinae) differ by the shape of their nucleocapsids. (Wang et al., 2020) Frequent RNA recombination or also known as 'mutations' are reported which is believed to be caused by the characteristics of their polymerase complex. It tends to transfer from one region located in the templet to a region, which is quite distant from the previous one. (Callaway, 2020) This tendency of dissociation explains the usual mutative nature of the virus in this family.

1.2 Virion Structure

The shape of the virions is roughly spherical with an enveloped nucleocapsid, either flexible (in Coronavirinae subfamily) or doughnut-shaped (in Torovirinae subfamily). (Ghosh, 2020) The nucleocapsid contains a genomic single-stranded RNA that has Nucleoprotein (N). On the surface of the envelope, several structures named 'spikes' are present which are protruded outward covering the virus-like crown. (Chorba, 2020) The spikes have Spike protein (S) in

them, and other notable proteins are membrane proteins (M) and envelop proteins (E). (Artika et al., 2020)

1.3 History of Coronavirus Pathogenesis

The virus was first reported as well as isolated at the Common Cold Research Unit in Wiltshire, UK in 1965 (Myint, 1995). The unknown virus showed to have a propensity to infect the respiratory tract. However, due to the lack of proper understanding of the virus or its morphology the virus was named B814.

Later in 1966, a medical student reported being infected by an unknown respiratory virus which was then named 229E by Dorothy Hamre and John Procknow of the University of Chicago. (Korsman et al., 2012). The next year (1967), a new virus named OC43 was grown by the researchers at the National Institute of Allergy and Infectious Diseases. For this, they cultured the B814 virus that had similar characteristics to the new one (Kahn & McIntosh, 2005). The term 'coronaviruses' was first coined in 1968 by the eight scientists who discovered and imaged B814, 229E, and OC43. They proposed that these all have similar characteristics and pathogenesis patterns. (cdc.gov, 2021)

After a few decades in 2003, a newly emerged virus was reported by some scientists to cause the outbreak of severe acute respiratory syndrome (SARS) in southern China, in late 2002 (Mahase, 2020). The following year (2004), the presence of coronavirus was confirmed in a pneumonia-affected child by the researchers at Erasmus Medical Center in the Netherlands. It was later named NL63 (Weiss & Navas-Martin, 2005). In 2005, another coronavirus named HKU1 was found in two patients suffering from pneumonia in Hongkong. (Williams, 2020)

Several years later in 2012, MERS-CoV, another novel coronavirus was found in a patient living in Saudi Arabia by the scientists at Erasmus Medical Center (Knapp, 2021).

Finally, in 2020, a disease outbreak was noticed in Wuhan in late 2019 and a group of scientists in China first identified the novel coronavirus as the cause of it. Later, the novel coronavirus from 2019 was named SARS-CoV-2. (Meredith, 2020)

Chapter 2: Methodology

To begin with, a searching method was conducted thoroughly by using several authentic websites, journals, and news articles such as WHO, Science Direct, Research Gate, Mayo Clinic, etc. Keywords such that Covid-19 pandemic, test kits for Covid-19, the diagnostic accuracy of test kits, development of antibodies, etc. are used in this matter. The statistically analyzed data were obtained from several authentic governmental websites such that USFDA, GOV.UK etc. Then, all these sources were scanned to select the required information and were compiled in my language. Paraphrasing and summarization techniques were used here. After that, the whole paper was revised numerous times and modified accordingly. In addition, all the pictures are drawn manually based on the images available on authentic websites. Paint Software was used to draw these pictures. Finally, the paper was screened properly by the software Turnitin to check for plagiarism and any further potential inconvenience was removed.

Chapter 3: COVID-19 Pandemic

The COVID-19 pandemic has led people all over the world to witness a condition of a medical emergency where more than 140 million people have been infected by this deadly virus with reported deaths of around 3 million (worldometers.info, 2021). Till now, a total of 7 various strains of Coronavirus have been discovered that invaded human physiology, and among them MERS-CoV, SARS-CoV, and SARS-CoV-2 are responsible for severe outbreaks. According to several genealogical analyses, it is assumed that the genomes of SARS-CoV found in bat and pangolin have been recombined to give rise to the origin of SARS-CoV-2 which eventually caused this global pandemic (Rehman et al., 2021).

3.1 Symptoms of COVID-19

Symptoms usually manifest around 2-14 days after the viral exposure. Frequently reported symptoms of COVID-19 are cough, fever or chills, difficulty breathing or shortness of breath, headache, muscle or body aches, fatigue, sore throat, loss of taste or smell, congestion or runny nose, vomiting or nausea, and diarrhea. Symptoms like these usually get improved after proper treatment, self-care, and healthy habits and lifestyles. However, if symptoms such that, persistent pain or pressure in the chest, trouble breathing, inability to wake or stay awake, confusion, pale, blue, or gray-colored lips, skin, or nails occurs then immediate medical emergency is required (cdc.gov., 2021).

3.2 Mechanism of Action of COVID-19 Infection

A biochemical component named receptor-binding domain (RBD) was found in the novel coronavirus SARS-CoV-2 that closely resembles the original SARS-CoV which was initially evolved in 2002. (Cherry & Krogstad, 2004). Other essential structural proteins such as spike (S), membrane (M), envelope (E), and nucleocapsid (N) proteins, and functional proteins such

as ORFs (1a and 1b) were also discovered. (Schoeman & Fielding, 2019). Based on the previously conducted researches, M and E proteins are vital for the assembly of the virus and S protein is essential for attachment and affinity towards the host cells. (Neuman et al., 2011). The main structural protein of SARS-CoV-2 named N protein, is necessary to transcript and replicate the RNA of the virus, package the genome which is encapsulated into virions, and also interact with the host cell cycle. (Afzal, 2020)

3.3 Development of Post Infection Immune Response

Within 1-3 weeks of manifestation, antibodies are found to be developed in most of the patients recovering or recovered from COVID-19. (obgproject.com, 2020) The level of the presence of antibodies usually depends on the severity of the infection. Thus, severely affected people tend to have more antibodies present whereas people with mild symptoms have fewer antibodies present. (Ibarrondo et al., 2020) It is believed people who recovered from a mild viral infection, have their innate immune response and T-cell response used to eradicate the viral infection. However, findings from recent researches have proven the disappearance of neutralizing antibodies after around 3 months. (Guan et al., 2020)

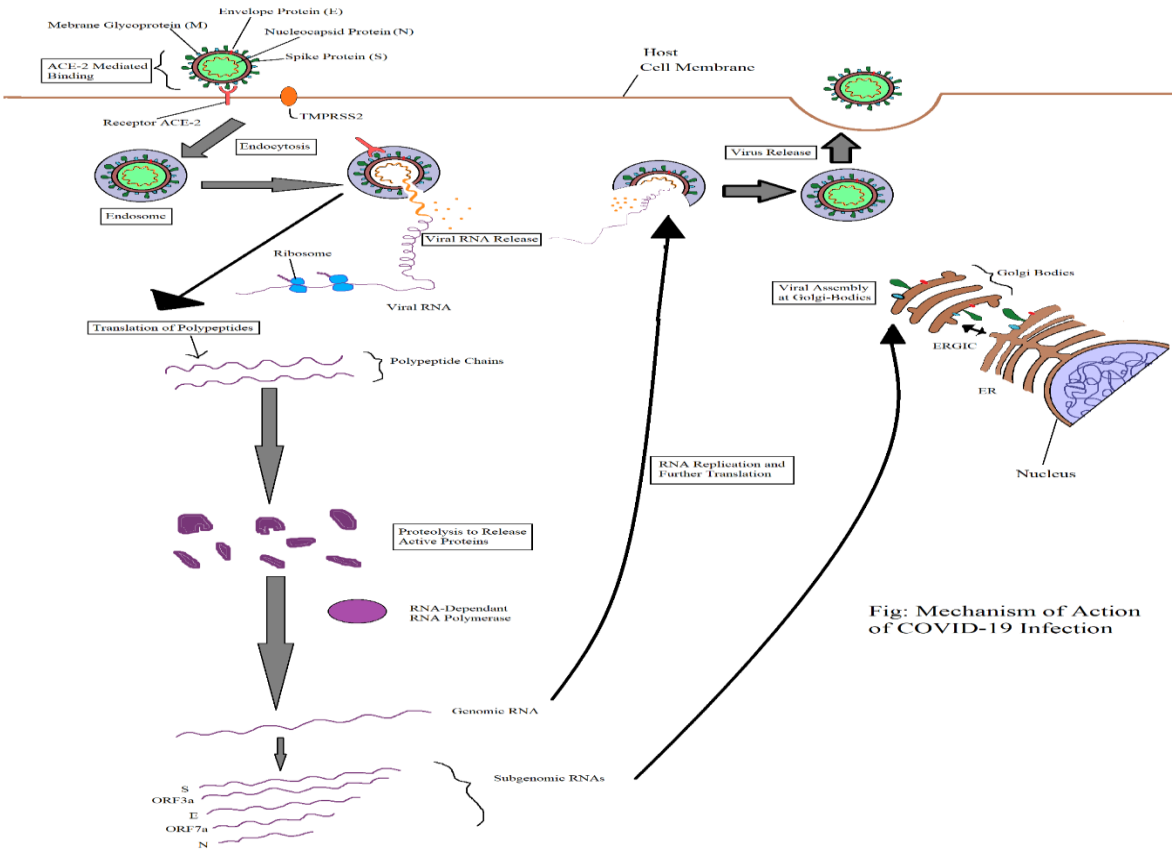

Fig. 1 Schematic Representation of COVID-19 Infection Mechanism of Action (Tocris Bioscience, 2021)

In Fig. 1, the mechanism of action of COVID-19 is illustrated schematically, such that;

1. Attachment of the virus to the host cell occurs by binding to the receptor named ACE 2 receptor through ACE-2 mediated binding
2. After that, endocytosis and release of viral RNA occur followed by the translation of polypeptides.
3. The polypeptide chains are then undergoing proteolysis to release active proteins which are then converted into genomic RNA and sub-genomic RNAs
4. The genomic RNA is then replicated and further translated, whereas the sub-genomic RNAs get assembled at the Golgi bodies and convert into full virus
5. Finally, the fully grown virus gets released from the host cell to invade other cells

Chapter 4: Diagnosis of COVID-19

In order to minimize the spread of this highly contagious virus, effective testing is necessary on a large scale. Most of these tests target the basic molecular structure or serological features of the virus and the derived results are later interpreted.

Currently, there are numerous tests available all over the world among which mostly fall under two categories: a) Molecular b) Serological (Gurbuz, 2020)

The first one incorporates RT-PCR technology that involves observing the molecular structure of virus RNA. (Jawerth, 2021) The other one measures how the antibody reacts to the virus present in the plasma. (The Conversation, 2020)**.** These antibodies may or may not help our defense mechanism against the virus based on their corresponding characteristics. (Acosta et al., 2013)

There are basically two methods of diagnosis, these are, by molecular testing or by serological testing. (La Marca et al., 2020)

4.1 Terms of Diagnostic Tests

Ensuring the highest accuracy is the greatest challenge in preparing a test method for use. The accuracy of the tests can be determined by several parameters such as specificity, sensitivity, LOD (Limit of Detection), PPV (Positive Predictive Value), NPV (Negative Predictive Value), etc. (Mandrekar, 2010) To understand these terminologies, concepts of false/ true positive and false/true negative should also be comprehended.

4.1.1 Sensitivity and Specificity

The capacity of the diagnostic method to determine the presence of the virus correctly is known as sensitivity. For instance, if a test kit shows results where all the actual infected people are shown as covid-19 positive, then its sensitivity is 100%. (online. stat, 2021)

The capacity of the diagnostic method to determine the absence of the virus correctly is known as specificity. For instance, if a test kit shows results where all the uninfected people are shown as covid-19 negative, then its specificity is 100%. (xlstat.com, 2020)

Now, to portray the whole concept, an example is discussed here.

400 students of college X underwent a particular molecular diagnostic test 'M' for covid-19 for this experiment and the pre-risk factor is set as 14%, meaning 14% of the population was previously tested positive for COVID-19 and that is the pre-assumed number of infected people.

Here, on average, the test accurately detects 95% of people who are infected (sensitivity) and 98% of people who are not infected (specificity). (Labtestsonline.org.au., 2020)

4.1.2 Other Terminologies

If a person tests positive for COVID-19 despite being uninfected by the virus, then the value will represent a false positive data point value. (Shendruk, 2020)

Similarly, if a person tests negative for COVID-19 despite being uninfected by the virus, then the value will represent a false negative data point value. (MedicineNet, 2021)

If the results can detect the presence and absence of the virus correctly then the value will be represented as True Positive and True Negative, respectively. (Datascience, 2020)

Moreover, if someone randomly takes the test now and gets a positive value, then the chances of its accuracy are known as positive predictive value or PPV (Sphweb, 2021) and if he gets a negative value then the percentage of accuracy is known as negative predictive value or NPV. (Parikh et al., 2008)

The limit of detection (LOD) is usually defined as the lowest concentration or amount or quantity of a substance that can be reliably detected by using a specific analytical procedure is known as the Limit of Detection or in short LOD. (Boqué & Heyden, 2009)

4.2. Molecular Testing

4.2.1 What is it?

The samples required for Molecular diagnostics usually are obtained from a subject's nasopharynges or sputum. (Marty et al., 2020). It utilizes the procedure of real-time reverse transcriptase quantitative polymerase chain reaction (rRT-PCR) therefore the majority of them are labeled as rRT-PCR. (Carter et al., 2020). In the case of quantitative information, quantitative (q)PCR is incorporated instead of PCR alone. (Thermofisher.com, 2020).

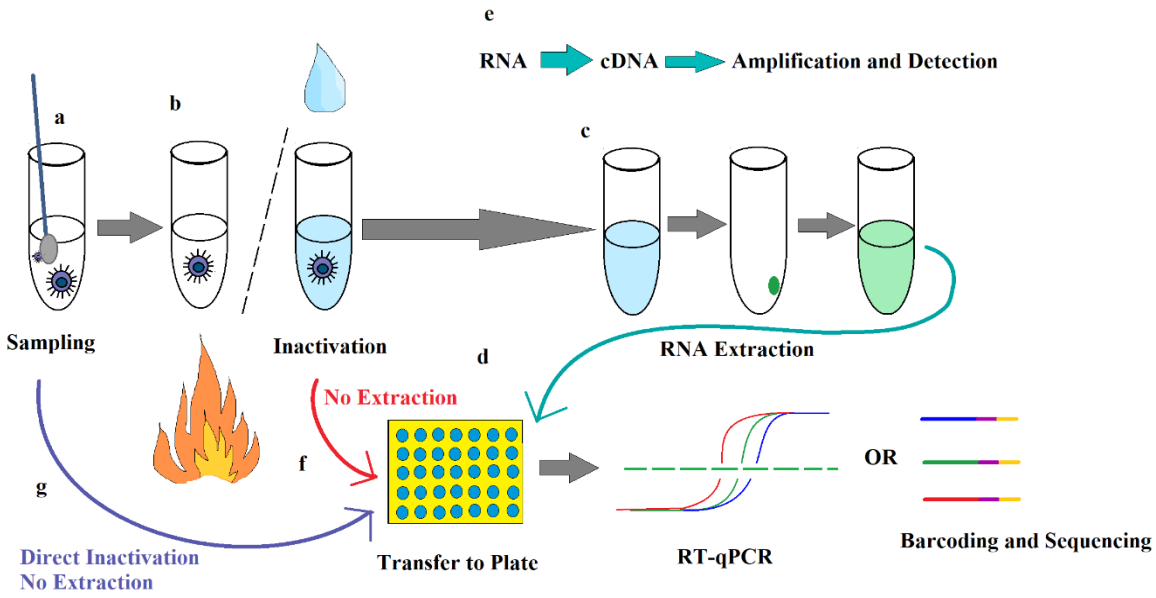

Fig. 2 Diagram of an Overview of Molecular Testing method (Smyrlaki et al., 2020)

In Fig. 2, total 7 steps of the method of Molecular Testing are shown schematically; which are illustrated below:

a) First, the sample of a potential COVID 19 affected patient is collected

b) Then the potential viral components are inactivated using either heat or other solvents e.g. aqua

c) After that, RNA is extracted from the inactivated material

d) Finally, the derived substance is transferred to the plate and RT-q PCR or Barcoding and Sequencing is conducted

e) Conversion into c DNA followed by amplification and detection can also be performed primarily to the transferring

f) The inactivated material, however, can also be directly transferred to plate without RNA extraction

g) Furthermore, inactivation can also occur directly while sampling and shifted right into the plate

4.2.2 Basic Principle

RT-qPCR test, which is also known as a molecular test, is based on the detection of RNA-dependent RNA polymerase (RdRp), envelope (E), and nucleocapsid (N) of SARS-CoV-2. (Mathuria et al., 2020). It acts by detecting the unique RNA sequences of SARS-CoV-2 found in the genetic material extracted from the specimens of patients through collecting tracheal

aspirate, sputum, or bronchoalveolar lavage, swabs from pharynx and nasopharynx, urine, blood, or stool. (Kubina & Dziedzic, 2020)

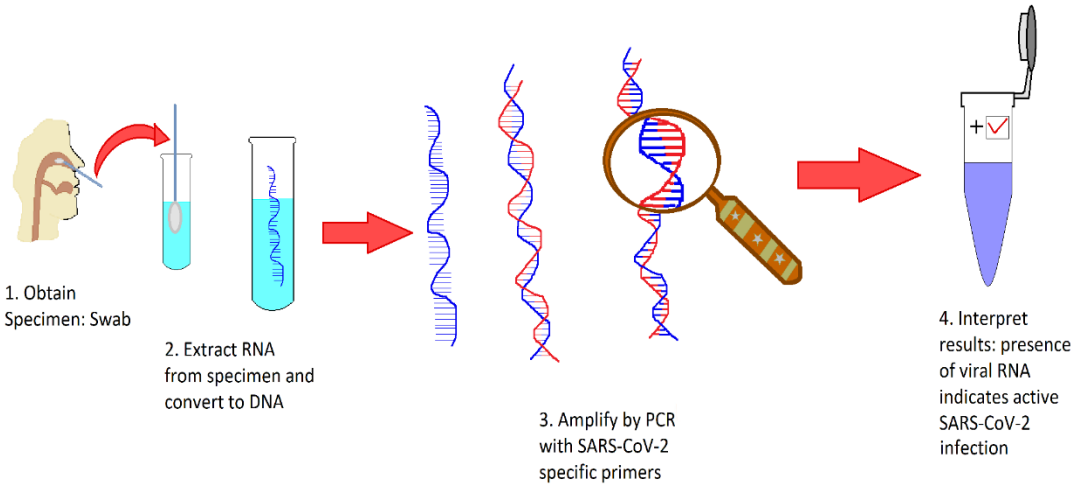

Fig. 3 Schematic representation of the Molecular Testing Process for SARS-CoV-2 infection (asm.org, 2020)

In Fig.3, the basic process of molecular testing for Covid-19 is illustrated through pictographic representation. To begin with, the specimen of a subject is collected by swab method which is conducted by incorporating a long narrow tool inside the nasopharynx. (Torretta et al., 2020) After that, RNA from the sample is obtained and is converted into DNA through a reverse transcriptase process. (britannica.com, 2020) Then the genetic material is amplified through PCR techniques by using SARS-CoV-2 specific primers and thus the specific characteristic of that DNA is revealed and detected. (Eboigbodin et al., 2017) Finally, the results are interpreted and evaluated where the presence of viral RNA refers to the presence of active SARS-CoV-2 infection. (Wölfel et al., 2020)

4.2.3 Method of Test Development

The molecular method identifies the presence of the virus by detecting its genetic materials or its characteristic markers and through amplification, the results are converted into a readable format to make it easier to derive accurate conclusions. (Udugama et al., 2020)

The test is developed based on the method as follows:

1. Virus-specific genetic material (e.g. DNA or RNA) detection
2. The detected region or location of the viral genetic material is then amplified by creating multiple copies
3. Finally, a result is produced that involves the quantified amount of amplified genetic material if any exists in the aforementioned sample. (Center for Health Security.org, 2020)

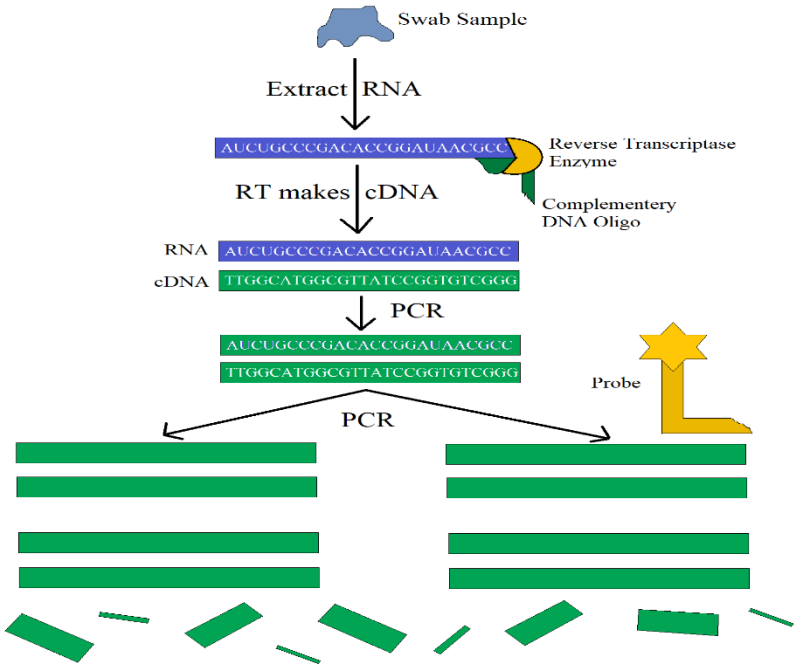

Fig. 4 Process of Genetic Material Amplification by PCR technology (centerforhealthsecurity.org, 2021)

In Fig. 4, the amplification process of genetic material is illustrated as follows:

1. At first, the RNA is extracted from the swab sample and reverse transcriptase enzyme (RT) is incorporated into it
2. Thus, complementary DNA (c DNA) is prepared by the action of RT followed by insertion into PCR machinery
3. Finally, the DNA strands are amplified with the help of a probe and the sequence is later detected and quantified

4.2.4 Drawbacks

1. Quite a time-consuming process, often take several days to show results and inform patients
2. POC (Point on Care) test is not available, so self-diagnosis is impossible
3. Requires greater expertise to perform the techniques
4. Do not provide a previous medical history of viral infection

5. The efficacy of vaccines can not be tested as the number of antibodies produced can not be detected

4.3 Serological Testing

4.3.1 What is it?

The test detects two types of antibodies (IgG and IgM) that are present in a patient's body infected by COVID-19. (Clinisciences.com, 2021) Antibodies are an essential part of our immune system to prevent or fight an invasion of foreign unwanted materials or microbes. (Ncbi.nlm.nih.gov, 2021) There are many isotypes of antibodies and IgG and IgM are two among them. (Sinobiological.com, 2021) IgM was the first one to respond to the coronavirus antigen but IgG shows greater affinity for that antigen. (Hou et al., 2020). IgG antibodies are more specific in case of binding to the viral protein but they are generated later in the body while the infection starts to grow. (Marklund et al., 2020). If either of these two antibodies or both of them are present in a sample, then the result can be interpreted as the subject being COVID-19 positive. (Assay Genie, 2020)

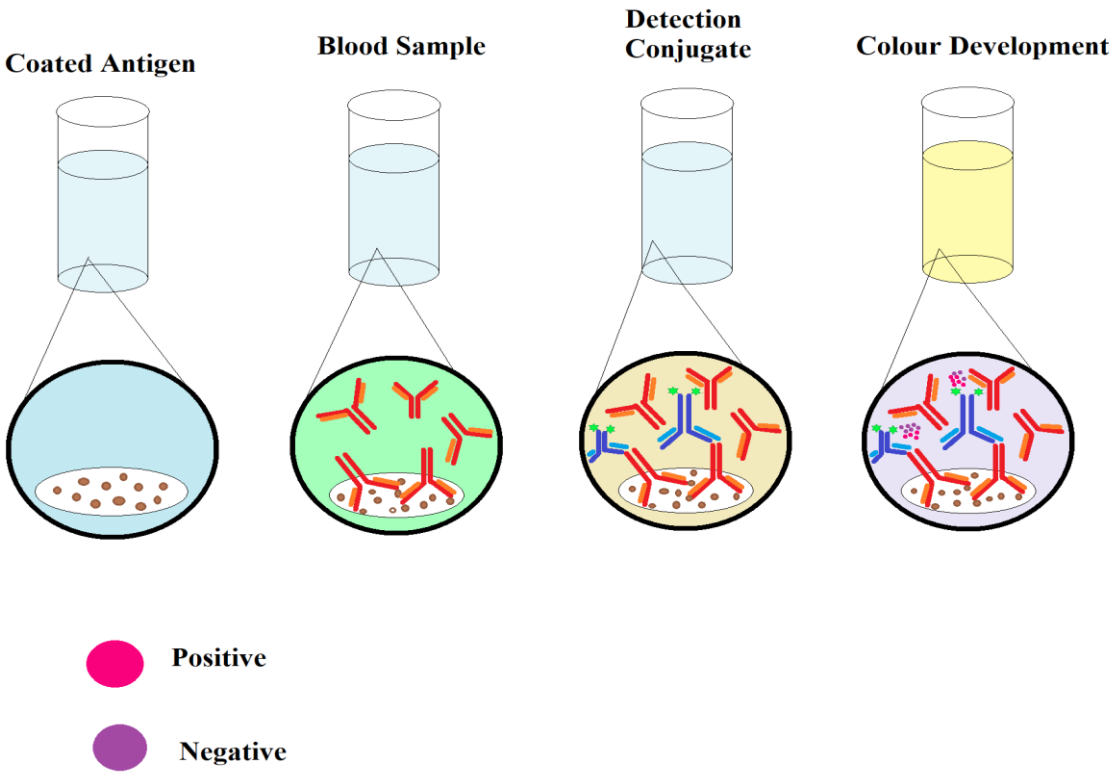

Fig. 5 Diagram of an Overview of Antibody (Serological) Testing Method (enzolifesciences.com, 2021)

In Fig. 5, a brief process of Antibody (Serological) Testing Method is showed in a simple diagram which is illustrated below:

1. The first test tube from the left contains a solution coated with antigen
2. Then the blood sample is added to the first test tube, the antibodies of the blood sample bind to the coated antigen shown in the second test tube in the picture
3. The third test tube showed detection of the antibodies by fluorescent probe
4. Finally, color is developed specifically for COVID-19 positive (pink) and negative (purple) patients and corresponding results are generated

4.3.2 Mechanism of Action

Serological Tests (Antibody tests) act by detecting immunoglobulins which are generated after exposure to foreign antigens by the host's plasma B cells. (Ghaffari et al., 2020) For infection and replication, approximately 25 proteins are necessary which are encoded by the SARS-CoV-2 genome. (Bianchi et al., 2020) These proteins include four major structural proteins, such as

envelope (E), spike (S), nucleocapsid (N), and membrane (M). (Mousavizadeh & Ghasemi, 2020) The S protein contains C-terminal S2 subunits, an N-terminal domain (NTD), and an N-terminal S1 receptor-binding domain (RBD) which facilitate the fusion and entry into the host cell. (Katsnelson, 2020) During viral replication, SARS-CoV-2 N protein (NP) pack and bind the viral RNA genome into a helical nucleocapsid structure. (Zeng et al., 2020) Several types of research imply that host-neutralizing antibodies present in the serum of previously infected COVID-19 patients, work against N and S proteins. (Bošnjak et al., 2020) Therefore, the prediction of immunity conditions might be elevated in serological (antibody) tests that focus on different locations of N or S proteins. (MacDonald, 2020) Thus, the humoral response can facilitate the depiction of precise SARS-CoV-2 antigen domains which has become a significant portion of the serological (antibody) test evolution. (Ni et al., 2020)

4.3.3 Classification of Serological Test Kits

There are four main types of serological diagnostic tests, such as:

a) The rapid diagnostic test (RDT),
b) Enzyme-linked immunosorbent assay (ELISA),
c) Chemiluminescence immunoassay (CLIA), and
d) Neutralization assay

a) The Rapid Diagnostic Test (RDT)

An RDT is a quick and easy test that incorporates lateral flow immunoassay (LFIA) technology, which is quite familiar with pregnancy test kits. (Ko et al., 2020) It has the potential to be utilized as a self-test or point-of-care (POC) test. (Jacobs et al., 2020) The RDT test strips generally utilize a drop of blood to determine the existence of antibodies such as IgM, IgG, or IgA which are generated in a patient body against a specific viral

antigen. (Clinicaltrials.gov, 2021) As this test only takes 10-30 minutes with minimal effort and easy procedure, it can potentially be proven as a reliable diagnostic method for large-scale serological researches. (Olalekan et al., 2020)

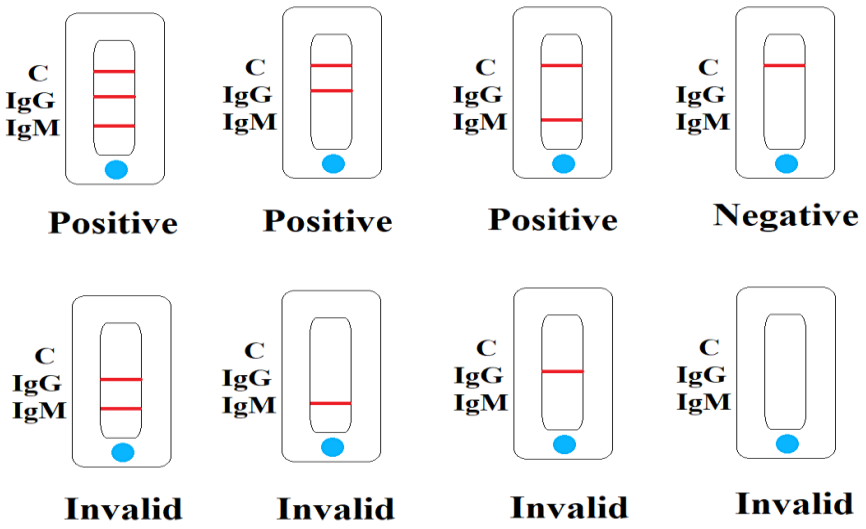

Fig. 6 Working Principle of RDT for COVID-19 Infection (generon.co.uk, 2021)

In Fig. 6, several possible outcomes of RDT are shown schematically, for instance;

There are three lines designated for each binding affinity outcome. Such as, if a particular line is signaled as red color then it will show its result accordingly.

Here, the control line must always be signaled in order to get a valid result. Any result having other lines signaled except for the control line or none signaled at all, is considered invalid.

Four possible outcomes are there for a valid result having at least the control line signaled, for example;

a) All three lines (IgG, IgM, and control lines) are signaled, or

b) Either IgG along control line or

c) IgM along with control line

d) Or only the control line is signaled

b) Enzyme-linked immunosorbent assay (ELISA)

The most common serological technique used during the current situation is ELISA assay, which is also based on laboratory testing that takes around 2-5 hours to derive a result. (Nguyen et al., 2020) It usually utilizes a surface on which specific viral antigens are coated. (Yuen et al., 2020) These antigens detect the corresponding antibodies (IgA, IgM, IgG) after binding to them. (Racaniello, 2020) By incorporating a second antibody and either a fluorescent-based signal or a substrate that generates a specific color, the derived antigen-antibody complex is then detected. (MacMullan et al., 2020) ELISA assays can be utilized in various methods such as competitive, direct, and, the most widely known, sandwich, or double-antigen-bridging assay (DABA). (rndsystems.com, 2021)

b) Chemiluminescence immunoassay (CLIA):

CLIA method is quite similar to the ELISA technique except for the way of determining the presence of the viral infection. (Coste et al., 2021) Instead of a fluorescent signal or a color-generating substrate, it incorporates chemical probes that undergo a chemical reaction that causes the emission of light. (Padoan et al., 2020) This light is then interpreted as a positive signal for viral presence in the blood. (Cinquanta et al., 2017) This technique takes around 1–2 h to derive a result. (centerforhealthsecurity.org, 2021) Both CLIA and ELISA are high-efficiency assays that are conducted in laboratories and have an analytical agreement of high level. (Zhao et al., 2020)

c) Neutralization assay

Neutralization assay incorporates live virus and cell culture procedures to determine the ability of a subject's antibodies to prevent viral infection in vitro (Perera et al., 2020) The whole

procedure is conducted in laboratories and the result can be derived within 3 to 5 days (Focosi et al., 2020).

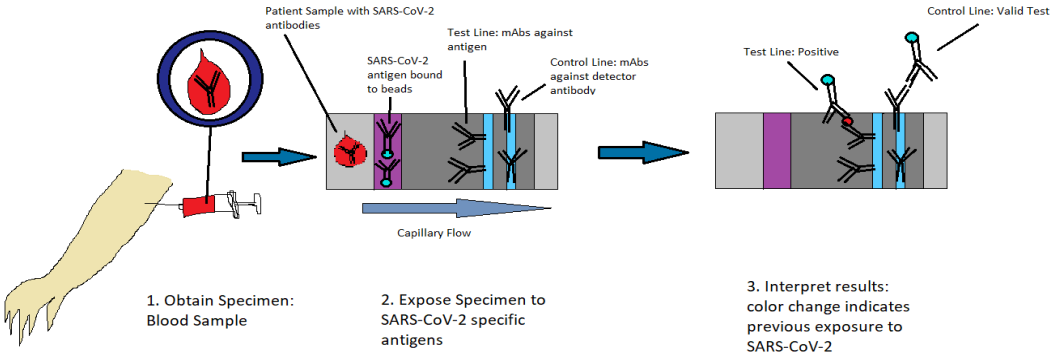

Fig. 7 Schematic representation of the Serological Testing Process for SARS-CoV-2 infection (asm.org, 2021)

In Fig. 7, the basic process of serological testing for SARS-CoV-2 infection is illustrated through pictographic representation. At first, Specimen is collected from an individual as a blood sample which may or may not contain the antibody formed against active or formerly active SARS-CoV-2 infection. (Mayoclinic.org., 2021) Then the specimen is exposed to SARS-CoV-2 specific antigen and later through capillary flow, bound to test line and control line as denoted in the diagram. (Chan et al., 2020) Finally, the results are interpreted, where the change of the color of the specimen indicates previous exposure to SARS-CoV-2 infection. (Assay Genie, 2020)

4.3.4 Method of Test Development

Among many of the serological test methods available currently, the method of COVID-19 Rapid Point of Contact CE-IVD test is discussed below:

The test contains two components such as IgG and IgM and the area of the M test line in the IgM component is coated with anti-human IgM. (finddx.org, 2021) There is a conjugation pad in the test cassette where gold nanoparticles are present (AuNP). (MyBio, 2021) These

nanoparticles are coated with viral antigens and react with the sample during the test process. (Rapidmicrobiology.com, 2021) If any of the antibodies are present in the sample, it will bind to the Antigen-AuNP complex by recognizing the MK201027 SARS-CoV-2 antigen. (González et al., 2020) Then parallel transportation of the mixture occurs by capillary action throughout the membrane. (GlobeNewswire News Room, 2021)

The mixture is called human antibody/antigen/AuNP complexes and as they flow further, they are attached either at the anti-human IgG 'G' Line or the anti-human IgM 'M' Line, or both, based on the specimen's antibody composition. (Westburg, 2021) The presence of any of these two antibodies will color the test lines in pink or red based on the amount of antibody present. (Kanik, 2021) Most importantly, these test lines are only specific for human IgM or IgG antibodies, for any other kind of human antibody, these lines will remain colorless. (Assay Genie, 2020)

4.3.5 Drawbacks

1. Not sufficient tests available to prove diagnostic accuracy
2. However, according to available tests information, it shows less diagnostic accuracy
3. Early diagnosis is quite impossible as it takes at least 1-3 weeks for the antibodies to be developed and to be detected

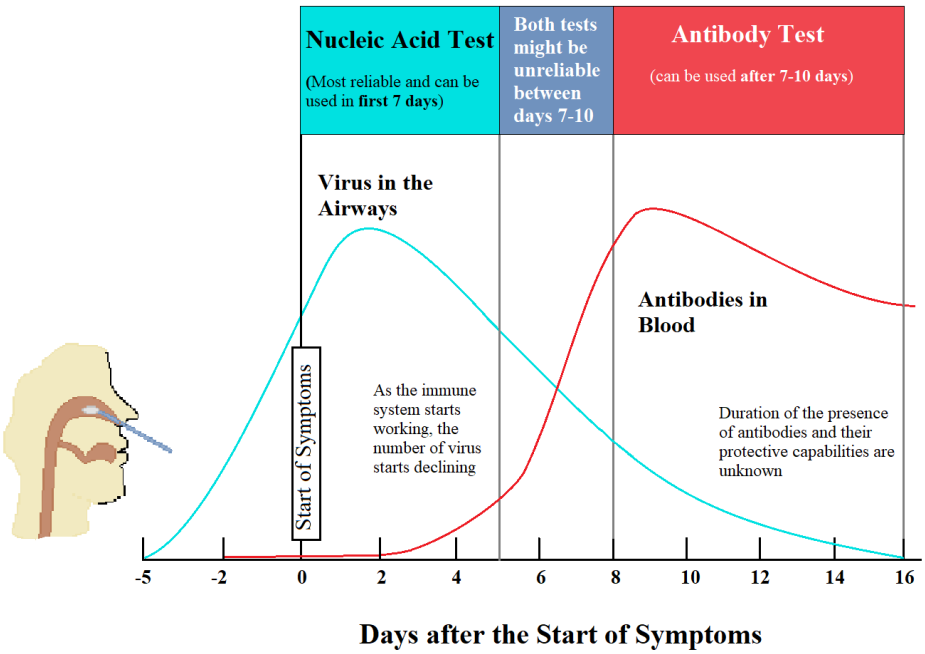

Fig. 8 **Comparison of the Probability of Accurate Detection of COVID-19 Infection** (UKRI, 2020)

In Fig. 8, an overall estimated timeframe for COVID-19 diagnostic testing can easily be comprehended. Here, a comparison is shown by a graph regarding the reliability of getting accurate testing depending on the time of testing after the viral exposure.

In the case of molecular testing, it is highly reliable for early diagnosis as it detects the presence of the viral genetic materials by nasal swab and as the virus remains in the airways for the first 7 days, there is a higher probability of getting an accurate result by this testing. However, between 7-10 days the probability for accuracy get lessened and after 10 days, this test is not recommended.

On the other hand, antibody testing detects the presence of antibodies in the human body due to viral exposure which is developed after around 7 days and lasts for quite a long period. Therefore, early diagnosis is not possible in the case of antibody testing but can be reliable for detecting past viral invasions as the antibodies remain in the body even after the subject overcomes the COVID-19 infection.

4.4 Evaluation of the Test Kits

In order to evaluate the diagnostic accuracy of the test kits, a comparison between the information (sensitivity, specificity, PPV, NPV, LOD) provided by the manufacturing company and the several authentic pieces of research under several countries' governments is conducted. In case of the evaluation of the serological test kits, studies under the countries such as USA, UK, France, Czech Republic, Netherlands, Malaysia and in case of molecular test kits, Saudi Arabia, UK, Poland, France researches are taken into account. Diagnostic evaluation of a total of 40 test kits is analyzed here, where all of their company claims are compared with USFDA and some compared with some other countries as well.

4.4.1 Molecular Test Kits Evaluation

Among the 20 molecular test kits discussed in this paper, only 2 of them have complied with the authentic study in Saudi Arabia Government, and these are Abbott RealTime SARS-CoV-2 and BioGX SARS-CoV-2 Reagents (for BD MAXTM System). (Kubina & Dziedzic, 2020), (Afzal, 2020)

Moreover, both of these test kits have the highest possible rate of accurate diagnosis. (Kubina & Dziedzic, 2020)

However, information was scarce regarding the sensitivity and specificity of molecular tests in USFDA but according to their evaluated LOD values (Limit of Detection), Abbott RealTime SARS-CoV-2, Xpert Xpress SARS-CoV-2, and Lyra SARS-CoV-2 Assay have comparatively the lowest value of LOD, meaning they are the most prone to be able to detect the possible presence of the virus. (U.S. Food and Drug Administration, 2020) On the other hand, TaqPathTM COVID-19 Combo Kit has the highest value of LOD meaning it is the least susceptible to detecting the virus. (U.S. Food and Drug Administration, 2020)

Furthermore, according to Saudi Arabia studies, BioGX SARS-CoV-2 Reagents (for BD MAXTM System) has the lowest value of LOD which is 40 copies/ml (even lower than Abbott RealTime SARS-CoV-2), and the others such as Real-Time Fluorescent RT-PCR Kit for Detecting SARSCoV-2 and Lyra SARS-CoV-2 Assay are ranked below it. (Afzal, 2020)

4.4.2 Table for Molecular Test Kits

Table. 1 Comparison with US Govt. Evaluation (USFDA) and Saudi Arabia:

Name of the Kits	**IFU (Company Claim)**		**Saudi Arabia**			**USFDA**		
Parameters	Sensitivity	Specificity	Sensitivity	Specificity	LOD (copies/ml)	Sensitivity	Specificity	LOD (copies/ml)
Abbott RealTime SARS-CoV-2	100	100	100	100	100	N/A	N/A	5400
UltraGene Combo2Screen SARS-CoV-2 Assay	100	100	80	100	N/A	N/A	N/A	N/A
Bosphore Novel Coronavirus (2019-nCoV) Detection Kit	N/A	100	80	100	N/A	N/A	N/A	N/A
iAMP COVID-19 Detection Kit (isothermal amplification)	100	100	100	99	N/A	N/A	N/A	N/A
BioGX SARS-CoV-2 Reagents (for BD MAXTM System)	100	100	100	100	40	N/A	N/A	N/A
Real-Time Fluorescent RT-PCR Kit for Detecting SARS-CoV-2	88.1	99.6	100	99	150	N/A	N/A	N/A
Xpert Xpress SARS-CoV-2	100	100	99.5	95.8	N/A	N/A	N/A	5400

EurobioPlex SARS-CoV-2 Multiplex	N/A	N/A	100	100	N/A	N/A	N/A	N/A
Lyra SARS-CoV-2 Assay	97	100	75	0	800	N/A	N/A	6000
cobas SARS-CoV-2 Test (for cobas 6800/8800 system	N/A	N/A	100	95	N/A	N/A	N/A	N/A
EURORealTime SARS-CoV-2	98.2	100	100	98	N/A	N/A	N/A	N/A
ePlex SARS-CoV-2 Test	N/A	N/A	91.4	100	N/A	N/A	N/A	N/A
TaqPathTM COVID-19 Combo Kit	100	100	87.5	100	N/A	N/A	N/A	180000
COVID-19 genesis Real-Time PCR assay	N/A	98.2	100	100	N/A	N/A	N/A	N/A
YouSeq Multiplex Covid19 qPCR Kit	100	100	81.2	100	N/A	N/A	N/A	N/A

(Kubina & Dziedzic, 2020), (U.S. Food and Drug Administration, 2020), (Afzal, 2020)

4.4.3 Serological Test Kits Evaluation

Half of the evaluated 20 test kits in this paper completely comply with the laboratory researches for the approval of USFDA and these are Abbott Alinity i SARS-CoV-2 IgG, Assure Tech. Assure COVID-19 IgG/IgM Rapid Test Device, Bio-Rad Platelia SARS-CoV-2 Total Ab, Cellex qSARS-CoV-2 IgG/IgM Rapid Test, Healgen COVID-19 IgG/IgM Rapid Test Cassette, Roche Elecsys Anti-SARS-CoV-2, Thermo Fisher Scientific OmniPATH COVID-19 Total Antibody ELISA Test, and Xiamen Biotime Biotechnology BIOTIME SARS-CoV-2 IgG/IgM Rapid Qualitative Test. (U.S. Food and Drug Administration, 2020)

Among them, Abbott Alinity i SARS-CoV-2 IgG, Assure Tech. Assure COVID-19 IgG/IgM Rapid Test Device, Bio-Rad Platelia SARS-CoV-2 Total Ab, Healgen COVID-19 IgG/IgM Rapid Test Cassette, and Roche Elecsys Anti-SARS-CoV-2 have the highest sensitivity and specificity values promising the highest accuracy of diagnosis. (U.S. Food and Drug Administration, 2020)

However, according to UK researchers, Abbott Alinity i SARS-CoV-2 IgG has a sensitivity of 92.7% and specificity of 100%. (GOV.UK., 2020)

On the contrary, in the research conducted in Netherlands and Czech Republic, Access Bio CareStart COVID-19 IgM/IgG showed both the sensitivity and specificity of 100%, which is greater than the company's product claim mentioned in their IFU (Instruction for Use). (Ecdc.europa.eu., 2020)

In addition, according to Malaysian studies, the test kit that was formulated under Roche Pharmaceuticals named Roche Elecsys Anti-SARS-CoV-2, showed a sensitivity of 87.9%, which is far less than the company's product claim, which is 99.8%. (centerforhealthsecurity.org, 2020), (GitHub, 2020)

4.4.4 Tables for Serological Test Kits

Table.2. 1 Comparison with USA Govt. Evaluation (USFDA)

Name of the Test Kits	**IFU (Company Claim)**				**USFDA**			
Parameters	Sensitivity	Specificity	PPV	NPV	Sensitivity	Specificity	PPV	NPV
Abbott Alinity i SARS-CoV-2 IgG	100	99	N/A	N/A	100	99	84	100
Abbott Architect SARS-CoV-2 IgG	100	99.63	N/A	N/A	100	99.6	93.4	100
Access Bio CareStart COVID-19 IgM/IgG	100	97.5	67.8	100	98.4	98.9	82.5	99.9

Assure Tech. Assure COVID-19 IgG/IgM Rapid Test Device	100	98.8	80.8	100	100	98.8	80.8	100
Beckman Coulter Access SARS-CoV-2 IgG	N/A	N/A	92.7	99.6	96.8	99.6	93.5	99.8
Beckman Coulter Access SARS-CoV-2 IgM	91.3	99.9	N/A	N/A	96.7	99.9	97.3	99.8
Beijing Wantai Biological Pharmacy Enterprise WANTAI SARS-CoV-2 Ab ELISA	96.7	97.5	67.1	99.8	96.7	97.5	67.1	99.8
Bio-Rad Platelia SARS-CoV-2 Total Ab	98	99.3	N/A	N/A	98	99.3	88.6	99.9
BioCheck SARS-CoV-2 IgG Antibody Test Kit	99.09	100	100	100	99.1	100	100	100
BioCheck SARS-CoV-2 IgM Antibody Test Kit	95.5	97.2	N/A	N/A	95.5	97.2	64.2	99.8
Biohit Healthcare (Hefei) Biohit SARS-CoV-2 IgM/IgG Antibody Test Kit	96.7	95	50.4	99.8	96.7	95	50.4	99.8
bioMérieux VIDAS SARS-CoV-2 IgG	100	100	N/A	N/A	100	99.9	98.1	100
Cellex qSARS-CoV-2 IgG/IgM Rapid Test	93.8	96	N/A	N/A	93.8	96	55.2	99.7
Healgen COVID-19 IgG/IgM Rapid Test Cassette	100	97.5	N/A	N/A	100	97.5	67.8	100
Roche Elecsys Anti-SARS-CoV-2	99.8	99.5	N/A	N/A	99.8	99.5	96.5	100
Siemens Healthcare Diagnostics ADVIA Centaur SARS-CoV-2 IgG (COV2G)	100	99.89	N/A	N/A	100	99.9	98	100
Thermo Fisher Scientific OmniPATH COVID-19 Total Antibody ELISA Test	96.7	97.5	N/A	N/A	96.7	97.5	67.1	99.8
Xiamen Biotime Biotechnology BIOTIME SARS-CoV-2 IgG/IgM Rapid Qualitative Test	100	96.2	58.4	100	100	96.2	58.4	100

(U.S. Food and Drug Administration, 2020)

Table.2. 2 Comparison with some European Countries and Malaysia

Name of the Test Kits	**France**		**UK**		**Netherlands**		**Czech Republic**		**Malaysia**	
Parameters	Sensitivity	Specificity	Sensitivity	Specificity	Sensitivity	Specificity	Sensitivity	Specificity	Sensitivity	Specificity
Abbott Alinity iSARS-CoV-2 IgG	96.5	96.5	92.7	100	N/A	N/A	N/A	N/A	N/A	N/A
Abbott Architect SARS-CoV-2 IgG	80	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Access Bio CareStart COVID-19 IgM/IgG	N/A	N/A	N/A	N/A	100	100	100	100	N/A	N/A
Roche Elecsys Anti-SARS-CoV-2	N/A	N/A	100	99.8	N/A	N/A	N/A	N/A	87.9	100

(GOV.UK., 2020), (Ecdc.europa.eu., 2020), (centerforhealthsecurity.org, 2020), (owid/covid-19-data, 2020)

Chapter 5: Discussion and Remarks

5.1 Comparison between the Test Kits Options

After observing the data regarding the evaluation of the test kits (both serological and molecular), it was found that most of the available testing methods do comply with their claimed accuracy of detection in comparison with the governmental evaluation tests conducted in various countries. Therefore, it can be implied that these test kits show high reliability for use in case of diagnostic accuracy. However, the evaluating tests conducted by Saudi Arabia govt. do show dissimilarities in the data in most of the test kits but most of these differences are too insignificant to be considered. (Afzal, 2020) For instance, UltraGene Combo2Screen SARS-CoV-2 Assay and Bosphore Novel Coronavirus (2019-nCoV) Detection Kit are commonly used these days but their results deviate from the accuracy and their company claims. (U.S. Food and Drug Administration, 2020) Proper caution should therefore be taken to judge which test kits to be used.

In the case of determining the type of testing method, which is more reliable, the data for their accuracy level is again the most significant parameter. According to the previously mentioned data, molecular test kits indeed show a higher level of accuracy in detecting the presence and absence of the infection. However, the process is quite time-consuming and by the time the results reach the suspected subject, it may take 2-3 days. (Parker-Pope et al., 2021) For rapid results, serological test kits may become better options but they do have a lesser probability of providing greater accuracy than the molecular ones according to the derived results discussed above. (Memorial Healthcare, 2021)

A table is provided below to compare the reliability of both of the test kits:

	Features		Molecular Test Kits		Serological Test Kits
1.	Diagnostic Accuracy	**1.**	Provide greater diagnostic accuracy with sufficient tests available to validate the reliability of the results	**1.**	Less diagnostic accuracy found from the scarce tests conducted
2.	Sensitivity	**2.**	Highly sensitive	**2.**	Quite sensitive
3.	Specificity	**3.**	Highly specific	**3.**	Quite specific
4.	Detection Time	**4.**	Take longer time to conduct the overall procedure and derive the results and inform the patients	**4.**	Provide rapid results with less lengthy procedure
5.	Early Diagnosis	**5.**	Early diagnosis is possible as highly sensible	**5.**	Early diagnosis is improbable as it takes at least 1 weak after the viral exposure for the antibodies to develop and to be detected
6.	History of the Previous Infection	**6.**	Previous viral infection can not be traced	**6.**	Previous medical history of viral infection can be traced along with the number of antibodies produced. It makes it suitable to test the efficacy of the vaccine whether it can generate a sufficient immune response or not.

7.	Ease of Use	**7.**	Requires greater expertise and efficient laboratory instrumentation and POC (point on care) tests is usually not available	**7.**	Can be used for self-diagnosis as well as POC, as the procedure is quite easier than PCR tests

5.2 Reliability of the Test Kits

Despite being highly sensitive and specific, molecular testing certainly does have several drawbacks. For instance, there is no way to tell if a person has been previously infected with the virus after its elimination from the body. (cdc.gov, 2021). Besides, this test is quite time-consuming as it requires a laboratory to conduct the essential procedure. (Reuters.com, 2020). It may take around 2-7 hours depending on the technical features, due to transport, sample collection, and sample processing, 2-3 days it may take to let the patient know the results. (The Conversation, 2020)

Antibody testing (Serological testing) is capable of providing a medical history of past Covid-19 invasions as antibodies produced in the body in response to the infection tend to remain much longer in the blood than the virus itself. (idsociety.org, 2020). This kind of technique is highly reliable in case of determining the efficacy of a particular vaccine. (Rose, 2021)

However, due to the scarcity of the required tests to validate the diagnostic accuracy of the antibody testing, WHO still recommends RT-PCR technology as the most reliable option for COVID-19 diagnosis. (Who. Int, 2020)

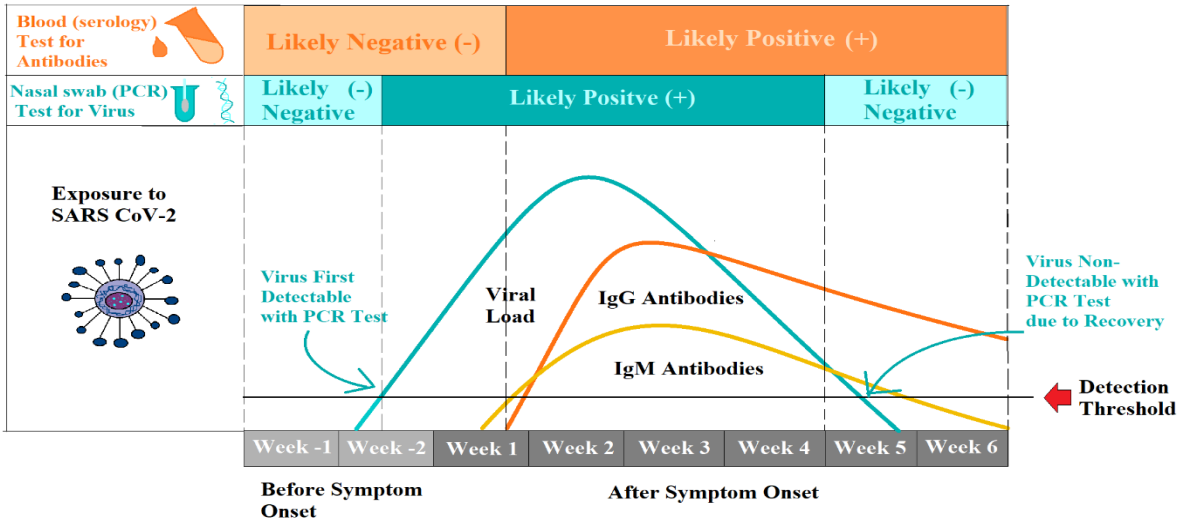

Fig. 9 Graphical Representation of Test Kits Duration of Action (siemens-healthineers.com, 2021)

A similar representation as Fig. 8 is provided in Fig. 9. In this figure, the amount of viral components and antibodies before and after the symptom onset is indicated. Before 1-2 weeks of symptom onset, both the tests are likely to show negative results as neither the virus is detectable nor the antibodies for the virus are produced yet. PCR test may start detecting the presence of the virus from several days before symptom onset to around 4 weeks after the symptom onset. Whereas, the antibody testing start detecting IgM antibodies before detecting the IgG antibodies and the detection time is around from 1st week after the onset of the symptoms. Both of the antibodies are detectable by antibody testing long after the recovery of the patients but due to the absence of virus in the recovered patient, PCR can not detect the virus after around week 4-week 5.

5.3 Recommendations

1. There is a scarcity of tests available to validate the diagnostic accuracy of the newly developed serological test kit options. Hence, sufficient tests should be conducted to eradicate this problem.
2. Even though serological test kits provide lesser diagnostic accuracy, this test can be highly useful in determining the efficacy of a vaccine by quantitative determination of the level of antibodies produced specifically against that vaccine.

3. Duration of the overall process of Molecular Test Method can be increased by incorporating more efficient instrumentation and techniques
4. All the effective test kits should be affordable and available for people through proper funding and necessary monitoring.
5. Government should ban all the test kits that do not meet the criteria of an effective diagnosis and should keep proper monitoring regarding this issue.
6. The manufacturing companies should also be cautious enough to retain the integrity of their test kits and be transparent and honest about their claims.

Chapter 6: Conclusion

To sum up, this whole review has delivered positive feedback regarding most of the popular test kits (Molecular and Serological) available till now which have shown results almost near to the accurate values. Moreover, this review can be utilized for future research purposes as an overview of the probable reliability of the widely used test kits available in various countries. Furthermore, the govt. of every country should be sincere enough to ban the test kits which do not provide results with an accepted range of accuracy and the companies should also be careful providing accurate information.

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