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Genetic polymorphism of drug metabolizing genes Cytochrome P450 mediated drug interaction in diagnosis of the coronavirus (SARS-CoV-2) using one step RT-PCR followed by RFLP

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ABSTRACT 1: Covid-19 is one of the major pandemics currently posing a severe health problem due to the rapid human-to-human transmission of SARS-CoV-2, a new form of Corona virus that causes fatal pneumonia. SARS-CoV-2 also known as COVID-19 is a fast spreading coronavirus related to the disease that emerged from Wuhan city, China in December 2019 and currently attained the status of a pandemic, spread rapidly worldwide. Both these Corona viruses belong to the Nidovirales tribe, the Coronaviradae tribe and the Coronavirinae subfamily. Doctors use cocktail therapy (a combination of different medicine used to cure malaria, HIV, Pneumonia etc.) to diagnose this disease.
In this analysis we will address the different methods of detection and techniques of the SARS-CoV-2 virus, including:**In the silicon research**, we developed a cheaper and faster diagnostic method based on simple digestion of **PCR**and restriction enzyme, commonly used in polymorphism of restriction fragment length polymorphism **(RFLP**) test, **Immunological assay**, the development of **Genetic tests** aimed to identifying the specific COVID-19, **Serological test** and **Medical imaging.**

ABSTRACT2: Purpose : For COVID-19 pneumonia, to evaluate the diagnostic value of computed tomography (CT) and real time transcriptase polymerase chain reaction (Rrt-PCR).
Methods : This study included all patients with suspected COVID-19 pneumonia who were initially screened for both CT and rRT-PCR.
Conclusion : For the identification of SARS-CoV-2 in clinical samples, the RT-PCRassay has the benefits of high sensitivity and fast applicability. In addition, the method is a promising tool that could theoretically be used to identify low viral load clinical samples.

KEYWORDS: Covid-19, SARS-CoV, Cytochrome P450, RT-PCR, RFLP, Genetic Polymorphism, Genome Sequencing

I. INTRODUCTION :

Coronavirus was first discovered in the 1930s in birds and mice. Later on, human coronavirus were discovered in the mid- 1960s and were known to infect humans and a variety of animals specifically birds and mammals.(In 2003, animal-infected Coronavirus emerged and triggered outbreaks in humans identified in southern China as SARS-CoV (Severe Acute Respiratory Syndrome) and MERS-CoV (Middle East Respiratory Syndrome), identified in 2012 in Saudi Arabia.

The World Health Organization (WHO) will convene in January 2020 renamed the virus as SARS-CoV-2 and the disease as COVID-19[1]. The infection ofSARS-CoV-2 spread worldwide and has been confirmed in more than 170 countries. A total of seven Coronaviruses have been known to infect humans so far; SARS-CoV, MERS-CoV and SARS-CoV-2 may cause severe disease, whereas mild symptoms are associated with HKU1, NL63, OC43 and 229E. SARS-CoV-2 nucleotides have a similarity of 84%, 79.6% and almost 50 percent with bat SARS-like Coronaviruses, SARS-CoV and MERS-CoV, respectively. [2] .SARS-CoV-2 has 96 percent bat coronavirus homology at the entire genome level. [3]. SARS-CoV-2 and SARS-CoV utilize Angiotensin-converting enzyme II (ACE2) as a cell entry receptor [3,4].

Medical symptoms of infected patients included fever, nonproductive cough, shortness of breath [5], myalgia, weakness, anosmia, ageusia, regular or reduced leukocyte and mild flu counts.

Therefore, it is necessary to understand this novel coronavirus at the genetic level to diagnose this pandemic disease in a straightforward way. In order to monitor this pandemic disease, it is

International Journal of Pharmaceutical Research and Applications
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therefore important to understand the genomics of COVID-19 for the production of effective therapeutics in an emergency issue. Extreme pneumonia, acute respiratory distress syndrome (ARDS), respiratory failure, multiple organ failure and eventually death are the complications of this critical stage [6].

(a) Incubation Period :

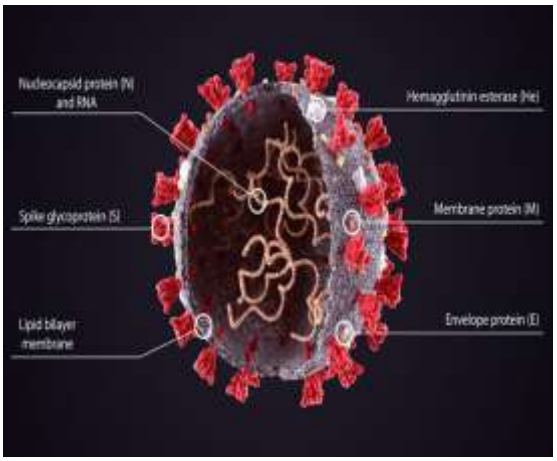
The mean incubation time of COVID-19 is approximately 5.1 days (range 2-14 days), according to the Centers for Disease Control and Prevention (CDC) [7].

(b) Transmission :

Close or direct contact with infected secretions or large aerosol droplets is the main mode of transmission. [8]The virus can exist in nature on surfaces and can last for up to 4hrs. On iron, on cardboard for 24 hours and on surfaces of plastic and stainless steel for up to 72 hours, resulting in transmission of fomite.[9]With vaginal secretions or breast milk, no vertical transmission has been reported in pregnant women from mother to baby. [10,11].

(c) Structure :

Due to the presence of spike glycoproteins on the envelope, the name Coronavirus is derived from the Latin coronam, meaning crown. The coronavirus were named because of the way virus morphology under a microscope. The virus particle, consisting of RNA genetic material surrounded by an envelope and spikes of glycoproteins, is spherical. The viral spike polymers, which are proteins on the surface of the virus and measure approximately 120nm, produce this morphology.



(**Figure : Coronavirus)**

The CoV genome consists of two parts- **(a) Structural proteins-**

- Nucleocapsid protein (N)
- Transmembrane proteins
- Spike protein (S)
- Envelope protein (E)
- Membrane protein (M)

(b) Non structural proteins-

- Protease (nsp5)
- Papain-like protease (nsp3)
- RNA-dependant RNA polymerase (nspl 2,RdRp).

(d) Prevention :

No effective treatment / vaccination is available till date. As the disease spreadsby droplets and near contact, it has been advised to adopt preventive steps.

- **Social distancing** – (Social distancing is a prevention and control technique carried out to minimize / prevent interaction between people who are not to reduce or avoid the spread of disease in a group. The WHO suggests that you and others remain at least 1 metre (3 feet) away.

- **Hand hygiene** – For COVID-19 prevention, frequent and correct hand hygiene is a must. As every other coronavirus, SARS-CoV-2 has a lipid envelope that can tear the fat in the envelope apart when washed with soap, making it difficult or impossible for the virus to infect human cells. Therefore, hand washing with soap and water is the better weapon by far than any other preventive measure.

- **Use of facemask** – Medical masks are classified as surgical or procedure masks that are flat or pleated (some are shaped like cups), according to WHO.

That aim to balance high filtration, adequate breathability and optionally, fluid Penetration resistance.

Wearing a medical mask is one of the prevention measures that can limit the spread of certain respiratory viral diseases, including COVID-19.

A surgical mask provides only “one way protection “ and prevents the spreading of droplets during sneezing and coughing from a wearer to the surrounding areas, must wear a specializedrespirator, N95 which technically is a good fit mask preventing the entry of droplets and

International Journal of Pharmaceutical Research and Applications
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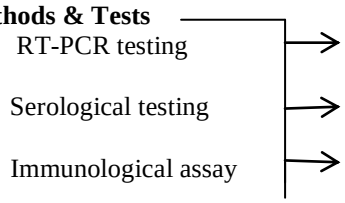
thereby minimizing the chance of acquiring the infection.[7].

2. Current detection & biological diagnostic methods and tools for SARS – CoV -2

The detection of COVID-19 relies either on the proteins from the COVID-19 virus in Respiratory samples (e.g. sputum, throat swab) or detection, in serum of human antibodies generated In response to infection.

Methods & Tests



a. RT-PCR testing :RT-PCR is the most accurate diagnostic test. This method is considered as the 'gold standard' for the detection of some viruses because of its high sensitivity, specificity, and rapid detection. So, real – time PCR (RT-PCR) has been used for the detection of SARS-CoV-2 due to these benefits [12,13]. RT-PCR uses primers in different genes for detection which can be affected by the variation of viral RNA sequences. So RT-PCR should be combined with other diagnostic modality like computed tomography (CT) of the chest in an appropriate clinical setting to best investigate any patient [7].

Comparison of the diagnostic efficacy between CT and rRT-PCR –

- rRT-PCR results often requires 5 to 6 hours, whereas CT examinations results can be obtained much faster. This retrospective study included patients with confirmed COVID-19 pneumonia diagnosed in 'YichangYiling Hospital'. In these patients, we compared the sensitivity of CT imaging and rRT-PCR testing at presentation.
- In a study of 1014 patients in 'Wuhan who underwent both rRT-PCR testing and chest CT for evaluation of COVID-19, investigators found that chest CT achieved higher sensitivity for the diagnosis of COVID-19 as compared with initial rRT-PCR from pharyngeal swab samples. Results, showed indicated that 59% of patients had positive rRT-PCR results, and 88% had positive chest CT scans.
- In patients with negative rRT-PCR results, 75% (n=308) had positive chest CT findings. Besides, analysis of serial rRT-PCR assays and CT scans was performed; the mean interval between the initial negative to positive rRT-PCR results was determined to be 5.1 ± 1.5 days, and the initial positive to the subsequent negative rRT-PCR results was 6.9 ± 2.3 days. Using rRT-PCR results as the references standard, the sensitivity, specificity and accuracy of chest CT in diagnosing COVID-19 were 97%, 25%, and 68% respectively[14].
- In consistency with the previous study, that the sensitivity of chest CT was higher than that of rRT-PCR (98% vs. 71% respectively)[15].
- A total of 36 cases were finally diagnosed with COVID-19 pneumonia. Thirty five patients had abnormal CT findings at presentation, and only one patient had a normal thoracic CT. Using rRT-PCR, 30 cases showed positivity, with 6 cases initially missed. Amongest these 6 missed cases, had a positive result in the second rRT-PCR test (after 2 days, 2 days and 3 days respectively), and the other 3 were positive in the third round of rRT-PCR (after 5 days, 6 days, and 8 days respectively) (fig.1).
- Therfore, sensitivity of CT examinations was 97.2% at presentation, whereas first round rRT-PCR sensitivity was 84.6%.

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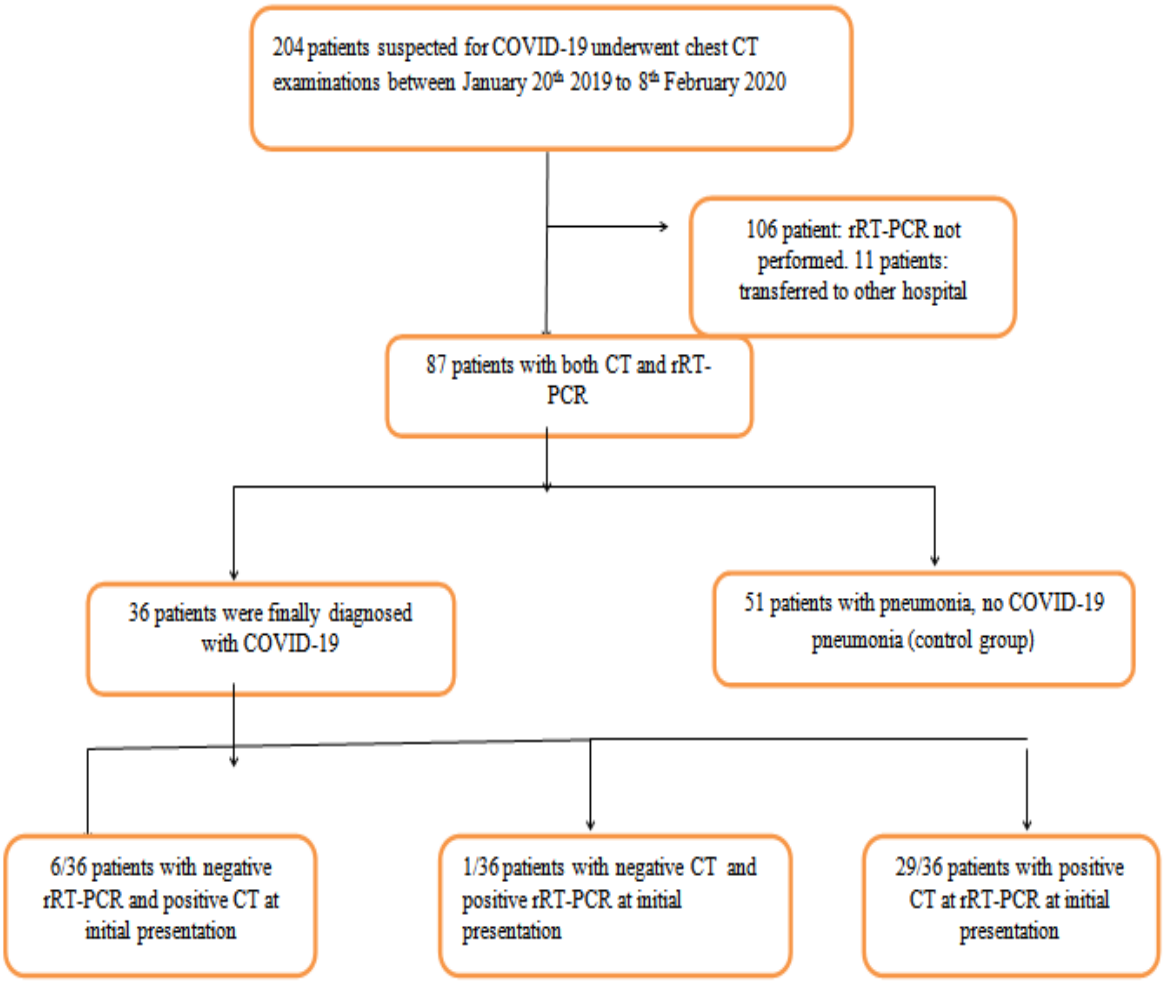

(**Figure1:** Flow chart for patients inclusion)

In this study, we developed and evaluated an rRT-PCR assay for the highly sensitive detection of SARS-CoV-2. Final diagnosis relies on real-time reverse-transcriptase-polymerase chain reaction (rRT-PCR) positivity for the presence of coronavirus [14,15]. Because of the strong infectivity of COVID-19, rapid and accurate diagnostic method are urgently required to identify, isolate and treat the patients as soon as possible, which could reduce mortality rates and the risk of public contamination.

RT-PCR testing in COVID-19 –

- Laboratory confirmed SARS-CoV-2 infection requires the detection of viral nucleic acid in respiratory tract samples by the use of real-time reverse transcription polymerase chain reaction (rRT-PCR) assay. Whereas clinical / radiological diagnosis is based on symptoms, exposure and chest imaging [16].
- The significance of rRT-PCR assay is demonstrated by the fact that it is currently considered the most determining factor for hospitalization decisions and isolation for individual patients [14].
- Targets for RT-PCR assay highly conserved and abundantly expressed genes are considered as attractive targets of SARS-CoV-2 RT-PCR assay, such as, the structural spike (S) and nucleocapsid (N) genes, and the nonstructural RNA-dependent RNA polymerase (RdRp) gene.

What is the PCR test for COVID-19 infection ?

Samples are taken from places likely to have the virus that causes COVID-19, like the back of the nose or mouth or deep inside the lungs. After a sample is collected, RNA, which is part of the virus particle, is extracted and converted to

International Journal of Pharmaceutical Research and Applications
Volume 5, Issue 2, pp: 209-220 www.ijprajournal.com ISSN: 2249-7781

complementary DNA for testing. The PCR test involves binding sequences on the DNA that only are found in the virus and repeatedly copying everything in between. This process is repeated many times, with doubling of the target region with each cycle. A fluorescent signal is created when amplification occurs and once the signal reaches a threshold, the test result is considered positive. If no viral sequences is present, amplification will not occur, resulting in a negative result.

How does PCR testing for COVID-19 work ?

One step RT-PCR reaction was optimized in a total volume of 30µL using One step RT-PCR Kit (QIAGEN, Germany) containing 6µL of RNA template, 3µL of each primer, 12.6µL of molecular grade water, 12µL RT enzyme, 1.2µL dNTPs and 6µL of 5x Buffer. Cycling conditions were –

S. NO.	Temperature	Duration
1.	50°C	30 min.
2.	95°C	15 min.
3.	94°C	30 sec.
4.	50°C	30 sec.
5.	72°C	20 sec.
6.	72°C	10 min.

All reactions were run on the Eppendorf Mastercycler pro PCR system. Amplified products were separated by electrophoresis on 2% agarose gel and visualized under UV light after staining with ethidium bromide (EtBr) for expected sizes of 396 and 344 bp for the fragments of the RdRp gene and the E gene, respectively.

International Journal of Pharmaceutical Research and Applications
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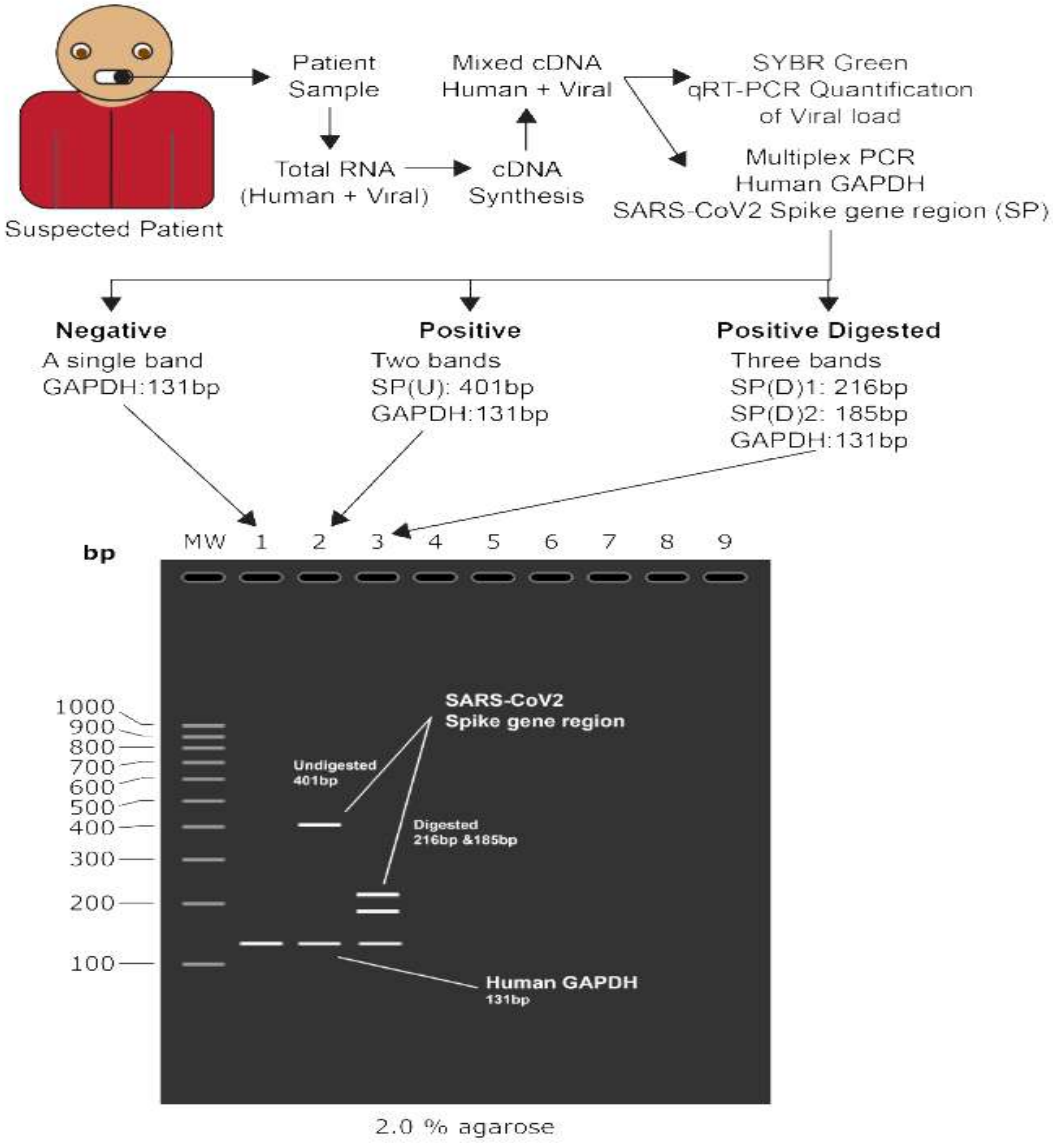

Figure 2: Simulated 2.0% agarose gel electrophoresis, MW: 100bp ladder; Lane 1 : Digested PCR amplicon from virus negative sample; Lane 2 : Undigested PCR amplicon; Lane 3 : Swa1 digested PCR amplicon.

International Journal of Pharmaceutical Research and Applications
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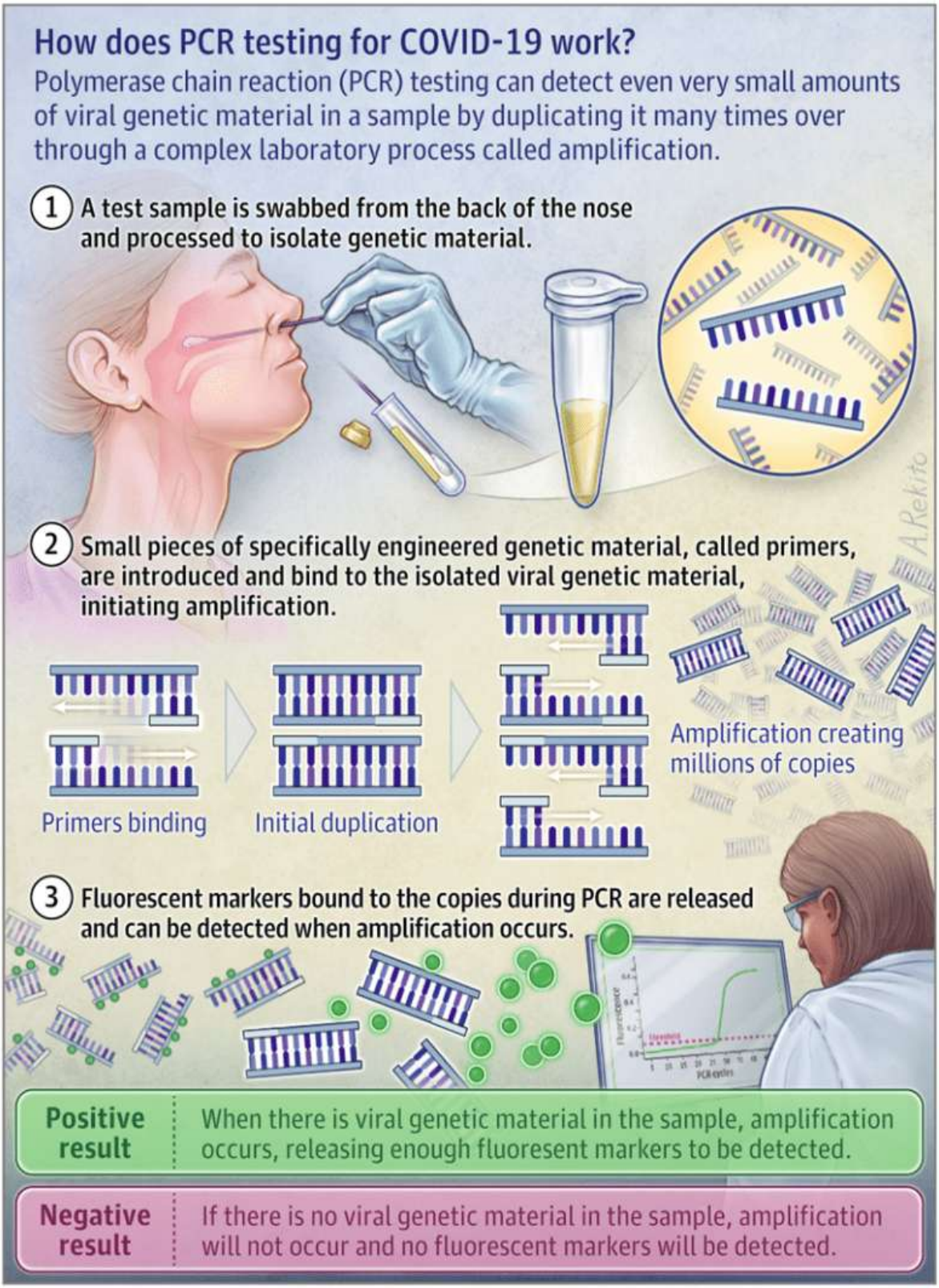

(**Figure3 :** PCR testing work for COVID-19)

International Journal of Pharmaceutical Research and Applications
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Detection of the Virus :

Polymerase chain reaction (PCR) is a process that amplifies (replicates) a small, well defined segment of DNA many hundreds of thousands of times, creating enough of it for analysis. Test samples are treated with certain chemicals[16,17]. that allow DNA to be extracted. Reverse transcription converts RNA into DNA.

Reverse transcription polymerase chain reaction (RT-PCR) first uses reverse transcrription to obtain DNA, followed by PCR to amplify that DNA, creating enough to be analyzed[17]RT-PCR can thereby detect SARS-CoV-2, which contains only RNA. The RT-PCR process generally requires a few hours[18].

Samples can obtained by various methods, including a nasophyrangeal swab, sputum, throat swabs, deep airway material collected via saliva[19,20].Sampling saliva may reduce the risk for health care professionals by eliminating close physical interaction[21].Some studies have found that saliva yielded greater sensitivity and consistency when compared with swab samples[22,23]. On 15 August 2020, the US FDA authorized a saliva test developed at "Yale University", which gives result in hours[24].

(**Fig1:** Demonstration of nasopharyngeal Swab COVID-19 testing)

(Fig2 : Demonstration of throat swab for COVID-19 testing)

(b) Serological testing for COVID-19 :

Serological tests are blood-based tests that can be used to identify whether people have been exposed to a particular pathogen. Serology based tests analyze the serum component of whole blood. These types of tests are often used in viral infections to see if the patients has an immune response to a pathogen of interest, such as influenza. The test can be used diagnose infection.

- **COVID-19 detection and serology –**

Serology testing for COVID-19 is attractive because of the relatively short time to diagnosis and the ability to rest for an active immune response against the virus. While hundreds of serology tests are currently on the market, only 21 have received Emergency Use Authorization (EUA) from the FDA[10]. The results of serology tests can then be used to estimate the true spread of the virus through a population.

But this test is not commonly used to detect the coronavirus but can help to identify who have developed antibodies to the SARS-CoV-2 virus, allowing them to serve as a donor to those currently infected persons with COVID-19.

(c) Immunological assay for COVID-19 –

Immunoassay are methods that rely on the detection on quantitation of antigen / antibody interactions. They can produce valuable data about the dynamic of virus infections and earlier exposures[22].

Some immunological assay have been developed to detect the COVID-19 virus. Peptide based luminescent immunoassay has been developed to detect IgG and IgM antibodies of SARS-CoV-2[23].

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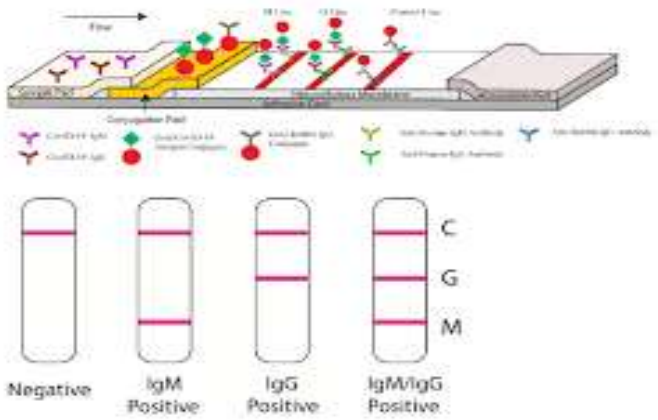

(Figure 4: Immunoassay for the detection of SARS-CoV-2 IgM and IgG)

2. Cytochrome P450 – mediated drug interactions in COVID-19 –

Cytochrome P450 (CYPs) are a superfamily of heme-containing monooxygenase enzymes that have been identified in all kingdoms of life[24]. Cytochrome P450 (CYPs) enzymes are hemoproteins[25,26]. As a result, if CYP enzymes are decreased then the result is decreased heme production [27]. Decreased CYP production decreases heme production and decreases inflammation. Drugs used to treat COVID-19 are CYP inhibitors, which include- Azithromycin, Hydrochloroquine and Chloroquine [28]. Cytochrome in the Electron Transport Chain(ETC), including cytochrome C, are also hemoproteins potentially affected by COVID-19. The binding of COVID-19 may cause dysfunction of the ETC in cells and may translocate cytochrome C into the cytosol from mitochondria.

In humans there are 57 functional members in CYPs families, most of which have specific endogenous functions including the metabolism of arachidonic acid, cholesterol, bile acids, steroid hormones, vitamin D and others [29].CYPs can significantly modulate the overall body exposure to a drug.

Susceptibility of CYPs to the immune response in COVID-19 –

As a main contributor to the metabolic biotransformation of most drugs, CYPs are widely involved in such disease drug interaction [30]. Regulation of CYPs has been linked to inflammation in several disease states such as infectious diseases (including viral infections), cancer, type1 diabetes, rheumatoid arthritis, and inflammatory bowel disease, in addition to age related disorders such as normal aging, metabolic disorder and neurodegenerative diseases[31].

3. Genetic Polymorphism :

In this article, we discuss a hypothesis regarding individual differences in binding-affinity between SARS-CoV-2 and the host cell receptors due to the existence of genetic polymorphism. The historical prevalence of infectious pathogens in a specific geograrphical area has exerted an important influence on genetic variability by influencing the survival of organisms or populations. Genetic variants may influence both behavioural and immune responses to the threat of infectious disease (4). A genetic polymorphism that may influence behavioural patterns of response to pathogens is the short allele of the serotonin transporter promoter region (5-HTTLPR).

This genetic polymorphism codes primarily for immune responses, it may also influence human cognition and social development through effects on central nervous system inflammation (6).

Given the role that these two genetic variants may have played in protecting populations from outbreaks of infectious disease in the past, this pilot study was conducted to examine their potential impact on two measures of the severity of the COVID-19 pandemic.

We also examined the correlation between the frequencies of the two genetic variants of interest in this study : (a) the prevalence, defined as the number of confirmed cases per 1 million population and (b) the crude mortality rate, defined

as the number of deaths caused by COVID-19 per million population.

II. CONCLUSION AND FUTURE PERSPECTIVES -

Collectively, the RT-PCR assay has advantages of high sensitivity and easy applicability for the detection of SARS-CoV-2 in clinical samples. Moreover, the method is a promising tool to be potentially used for the detection of clinical samples with low viral load. There are we discussed different molecular and serological methods for the detection of SARS-CoV-2. RT-PCR can provide good sensitivity and specificity and the result can be obtained in a few hours. It can direct viral DNA in respiratory samples, saliva, blood, urine and stool. However, RT-PCR has some drawbacks including the need for expensive thermocycler and professional staff to perform the assay and interpret results.

Although no direct evidence of pharmacogenomics data in patients with COVID-19 was available at this time. Studies must be conducted in COVID-19 before pharmacogenomics testing can be recommended. These data support the collection of DNA samples for pharmacogenomics studies of the hundreds of currently ongoing clinical trials of COVID-19 therapies. One of the biggest success stories in the field of pharmacogenomics was for a drug used to treat another, highly lethal, infectious disease: abacavir for HIV[32].Thus, the pharmacogenetic test for abacavir is now standard of care in the treatment of HIV. Some large national COVID-19 trials are evaluating pharmacogenomics, which will inform the role of pharmacogenomics markers for future clinical use.

Overall, the study of Genomics of coronavirus is very crucial to overcome the corona pandemic. It is helpful to understand the nature of virus, especially protein coding genes involve in pathogenicity and their proteomics study will be helpful in production of synthetic antidote and vaccines against coronavirus. This is current and future need.

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