

## Rapid and Cost Effective COVID-19 Diagnostic Test Kit

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### Abstract:

COVID-19 or Corona Virus disease spreading since December 2019 starting from Wuhan China has now become a pandemic throughout the globe. For early detection of the infected persons prior to the onset of the disease has become necessary to stop the spreading of this communicable disease. The process of testing of respiratory COVID-19 and the associated SARS- COV-2 virus is proved to be possible only by the detection of antibody produced due to response to the infection. This process of antibody detection can be used for both diagnosis and population surveillance. The testing of generated antibodies due to SARS-COV-2 are antibody isotopes that are to be detected are IgG and IgM . Due to having novel antigens the first responding antigen antibody interactions was IgM. Antibodies that show higher affinity for more specifically binding capable antigens leading to proper immune response is IgG. Due to the infectious reactions IgG antibodies are produced. Positiveness of a sample is considered if both IgG and IgM are present. Whole blood, serum or plasma specimens are used for membrane based immunoassay in a qualitative IgG and IgM test kit. The principle for the testing is same that of a HCG pregnancy test where human glycoprotein is assayed in a rapid chromatographic way. The mentioned process is very useful for mass detection of the COVID-19 infected population in a minimum time and is also safe for the laboratory technicians as it can easily be done without getting infected if proper physical barrier are taken personally.

**Key Words:** COVID19, IgG, IgM antibodies, detection

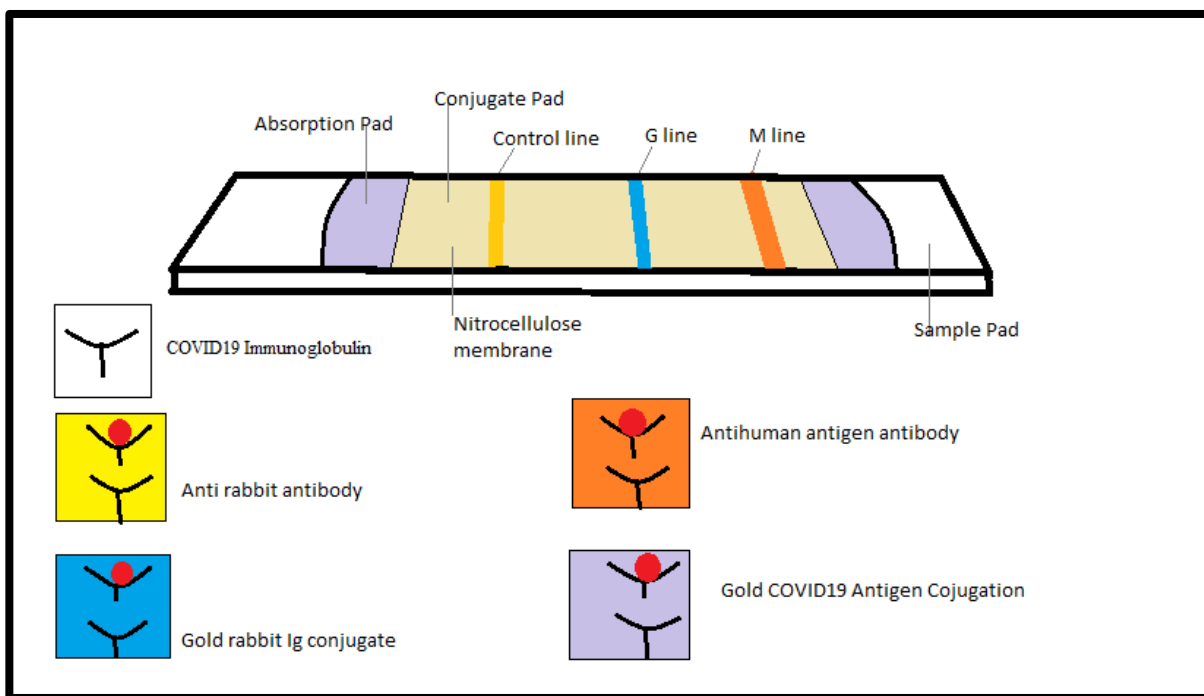
### Introduction:

The spreading of the COVID19 or Corona virus disease 2019 since December 2019 starting from Wuhan China has now become a pandemic throughout the globe. So for early detection of the infected persons prior to the onset of the disease has become necessary to stop the spreading of this communicable disease. (Tanu Singhal .2020) The process of testing of respiratory COVID19 and the associated SARS- COV-2 virus is proved to be possible only by the detection of antibody

produced due to response to the infection.(refer to table 1). RT-PCR which detects the corona virus RNA from samples. This process of antibody detection is able to be used for both diagnosis and population surveillance. Primarily in the COVID breakout in China had 13.8% cases of severe course and there were 6.1% critical course cases. (Ashish B . Budhrani . 2020)The severe cases are presented due to the virus receptor expressing preferentially in the human lungs, the same tropism was decided as causing the pathogenicity and is also used in controlling the pathogenicity in 2003 disease of SARS . Though in COVID19 the patients are observed to grow mild upper respiratory tract infectious symptoms which may lead to oligo symptomatic transmissions.

**Table 1: Immunological response percentage table**

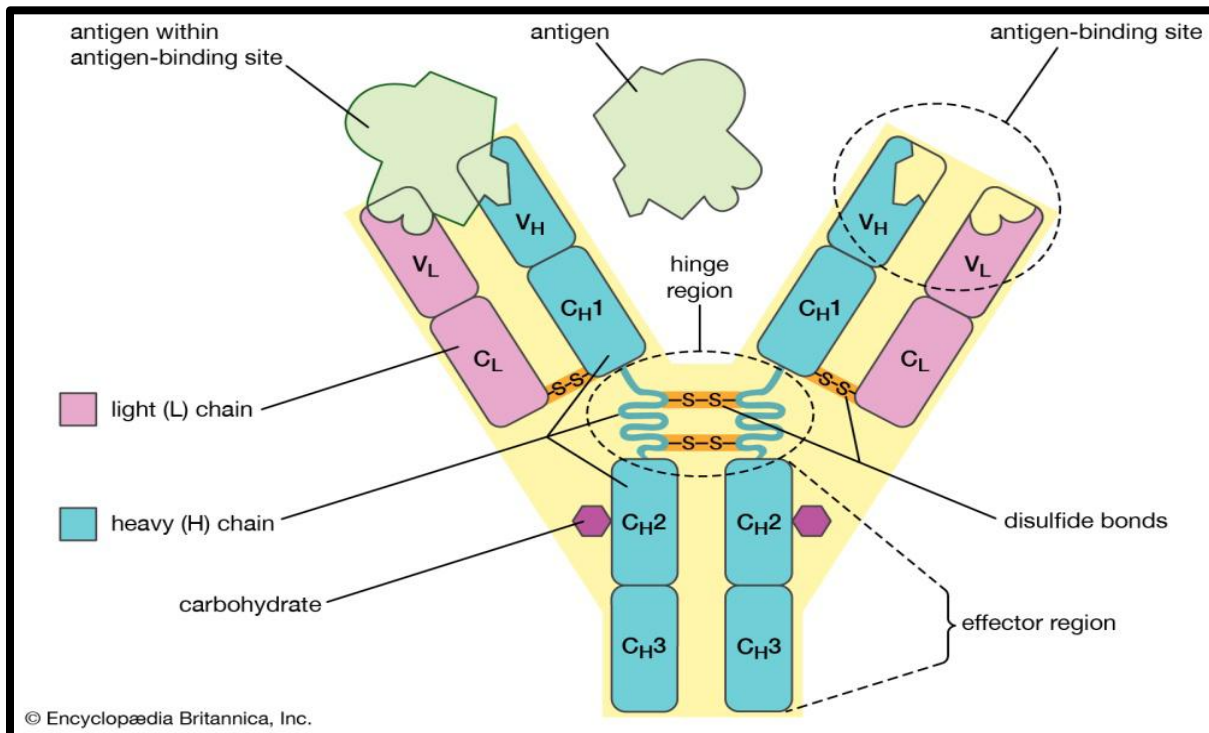
Type of Immunoglobulin.	% of increase than normal	Days to appearance
IgM	85.4%	3 to 6 days to appearance
IgA	92.7%	3 to 6 days to appearance
IgG	77.9%	3 to 6 days to appearance



**Fig:1 Structure and zones of immunoglobulin and antigen antibody reaction zones in the planned designed COVID19 diagnostic test kit.**

### Ideology:

Immunoglobulin (Ig) or antibodies are a special class of glycoprotein produced in response to exposure of healthy human to foreign bodies that is antigens. The immunoglobulins are mainly produced by B lymphocytes and plasma cells. Mainly five basic immunoglobulin are found in humans, IgG , IgM , IgA, IgE ,and IgD.The basic structure of immunoglobulin molecules has 4 Polypeptide chains linked by disulfide bonds along with 2 Polypeptide chains of low molecular weight known as light chains and 2 polypeptide chains with high molecular weight called heavy chains (refer to fig.2).



**Fig :2 The basic structure of immunoglobulin molecules.** (Source: <https://www.britannica.com/science/antibody>)

The functions of antibody or immunoglobulin:

IgG gives protection to human for long period of time through years due to presence and triggering due leading to their production (refer fig 3).

Bacterial, viral toxins induce protein compliment systems and antigens that bind to induce the process of phagocytosis.

IgA is to attach to antigens on microbes surface prior to entering the tissues and aggregation of antigens that leads to the expellation of secretion and antigens.

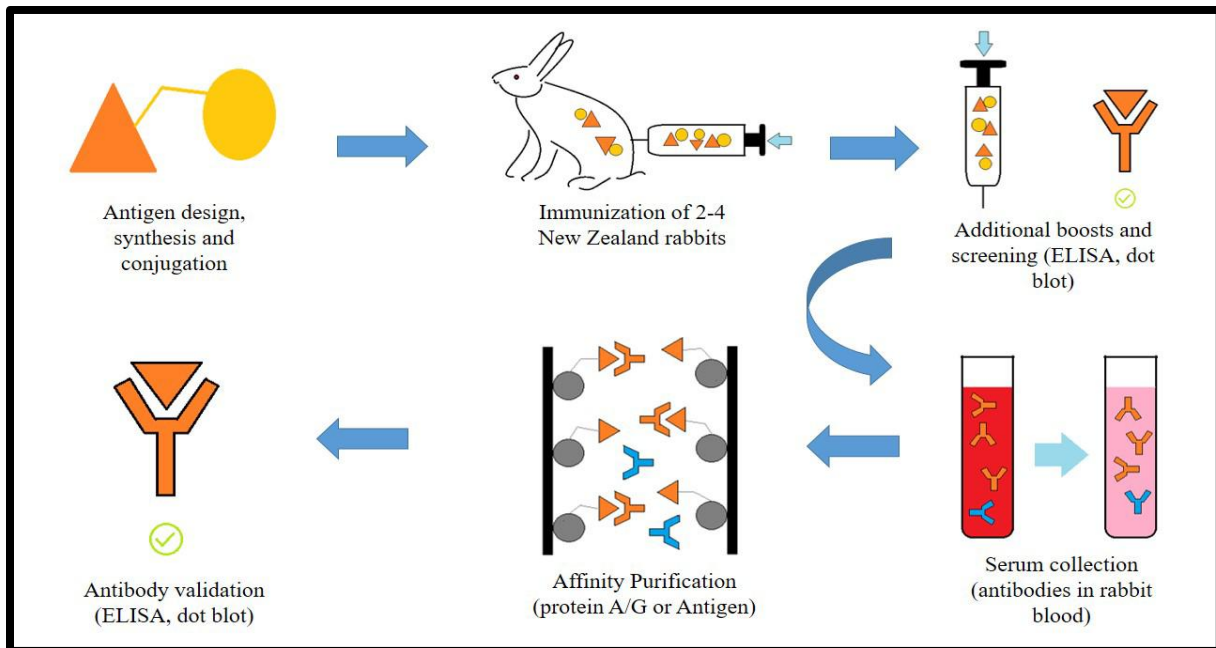
First defense against antigens in mucosa surface of intestines , lungs and nose are done by IgA.

IgM enhance the phagocytotic ingestion process involved in antigens on surface of RBCs ABO blood groups.

Immune response at basophils and mast cells is produced by binding of IgE to them.

IgD produce antibody protection and are present on B cell surface.

The testing of generated antibodies due to SARS-COV-2 are antibody isotopes namely IgG (refer to table 3) and IgM (refer to table 2) are to be detected. IgM antibodies were first to respond in interactions due to novel antigen. IgG antibodies show high affinity for the targeted antigen that are more specifically bind to substances causing immune response. IgG antibodies are produced in process of infection. Positiveness of a sample is considered if both IgG and IgM are present. (Esmail Mortaz et al., 2020) Whole blood, serum or plasma specimens are used for qualitative membrane based immunoassay in a qualitative IgG and IgM test cassette. The principle for the testing is same that of a HCG pregnancy test where human glycoprotein is assayed in a rapid chromatographic way. The test sample containing SARS-CoV-2 IgG antibodies a colored line appears in the region marked for IgG. The test sample containing SARS-CoV-2 IgM antibodies a colored line appears in the region marked for IgM. (refer fig. 1). No colored lines will appear if SARS-COV-2 antibodies will appear, this indicates negative result. But in all cases colored line will appear on C or Control line. (Nicolas Vabret et al., 2020)



**Fig:3 Diagrammatic flowchart of antibody production from rabbit (Self drawn)**

The proportion of truth of positivity which are correctly identified by this test kit (eg. IgG antibodies present in the sample gets detected by the antibodies). So it can be stated as probability of detection.

There are many pathological states in humans where immunoglobulin levels get increased but if studied carefully the % of immunoglobulin increase in COVID-19 infection shows a slight difference in rate of increase. A question may arise how it is relevant in using this test kit for rapid testing through huge population? In this case it may be said that this test kit is for a rapid

COVID-19 infection testing through huge populations at a high speed, which may lead to a reduction in the huge cost for suspected population.(Beeching NJ et al.,2020) This reduction in the huge suspected population can cut off the huge public COVID-19 infection testing cost. The reduced number of suspected population are next available much costly and higher level testing. This test kit is planned for easy and quick production, rapid testing , quick result and low cost testing.(Mayara Lisboa et al.,2020).

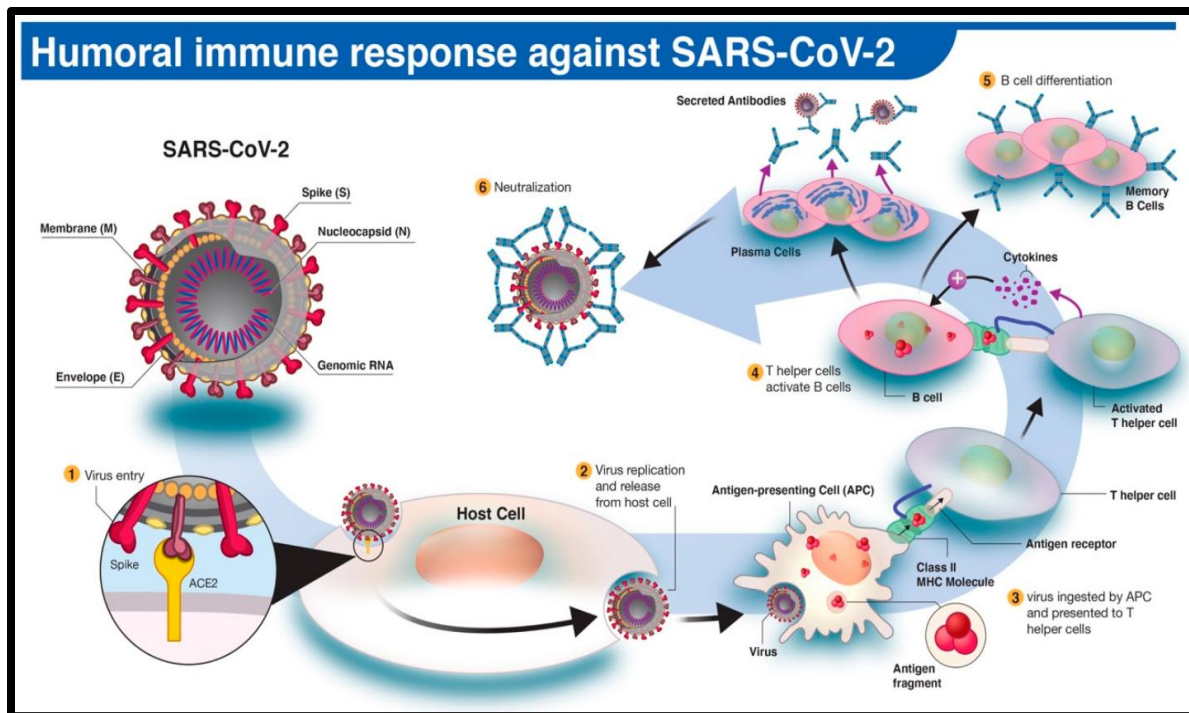
**Table 2: Data collected on study for IgG specificity and sensitivity on COVID-19 study on random individuals.( IgG Relative Sensitivity: 100% ,Relative Specificity :98% , Accuracy : 98.6%)**

Method		PCR		Total Results
2019-n COV gG / IgG	Results	Positive	Negative	
Rapid test	Positive	20	1	21
	Negative	0	49	49
Total		20	50	70

**Table 3: Data collected on study for IgM specificity and sensitivity on COVID19 study on random individuals.( IgM Relative Sensitivity : 85% ,Relative Specificity :96% , Accuracy : 92.9%)**

Method		PCR		Total Results
2019-n COV gG / IgM	Results	Positive	Negative	
Rapid test	Positive	17	2	19
	Negative	3	48	51
Total		20	50	70

The COVID-19 Rapid POC CE-IVD test is a lateral flow immunoassay which is a method to qualitatively assess the presence of analyze from the sample collected from the patient in case of SARS-COV-2 IgG and IgM antibodies are detected .(refer fig. 4)



**Fig 4: Humoral immune response against SARS-CoV-2.**

(Source: COVID-19 Serological Tests: How Well Do They Actually Perform? by Abdi Ghaffari, Robyn Meurant, Ali Ardakani, <https://doi.org/10.3390/diagnostics10070453>)

### Materials required:

Test cassette: test unit which holds the immunoassay strip inside it.

Droppers; for specimen well transferring.

PBS buffer solution: added to specimen well for facilitating the test.

Sample containing containers.

Centrifuge (used for plasma centrifugation)

Capillary tube: for whole blood specimen collection.

Lancets (for finger piercing and blood collection)

Pipettes: for placing the droppers.

Timer: for 10 minutes time measurements.

Capillary tube: used for finger stick whole blood specimen is to be used.

Timer: required to measure the 10 minutes of wait time.

## Methods:

Inside the groove the blood sample (whole blood , serum , plasma ,etc) is added and buffer is added to it for diluting (10 mM Phosphate - buffer saline). (refer to fig. 5).

The sample flows over the sample pad due to capillary action and will hit the conjugation pad.

The conjugation pad is made up of 40nm gold nanoparticile colloid. At this time the COVID-19 sticks to the rabbit IgG conjugated nanoparticles.

The conjugated sample goes on the membrane made of nitrocellulose and reaches the two test lines IgG and IgM and the control line.

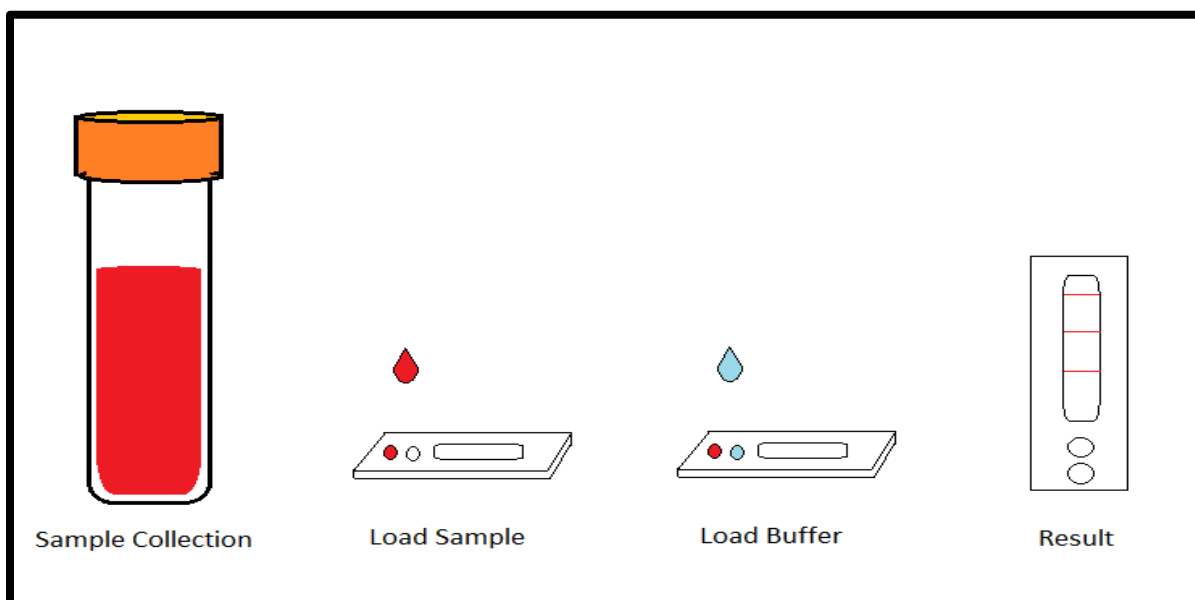
The immobilized antibodies on M line recognizes human IgM as gold nanoparticles complexes only with human IgM and coloured lines will be appearing.

The immobilized antibodies on G line recognizes human IgG as gold nanoparticles complexes only with human IgG and COVID-19 antibodies and coloured lines will be appearing.

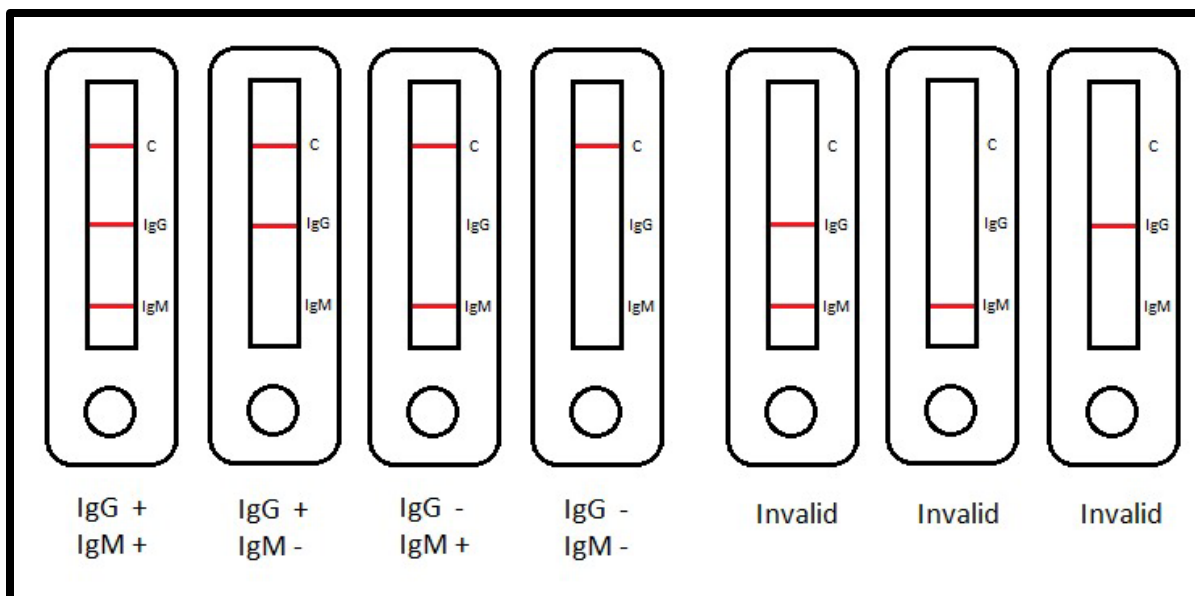
The last line is the control line which the sample touches contains immobilized antibody that are recognized by Rabbit IgG, that control the antibody.

The excess flows through to the absorption pad.

After 10 minutes, the results can be recorded.(refer to fig. 6).



**Fig :5 Methodology of sample loading and using test kit**



**Fig :6 Results on using the COVID19 test kit**

#### **Discussion on prospect of commercialization:**

An approx production pricing while in a large scale production the pricing may range approximately between Rs 500 to Rs 800 if profit is cut down and if profit margin is maintained then it would price between Rs1000 to Rs1200 per COVID19 test kit. So it can be said that it is not impossible to produce a huge number of test kit while the world is in such a pandemic situation and the human race can only be saved if pre detection of the presence or absence of the virus is identified. As the persons who will be tested positive with COVID19 virus are immediately to be taken in intensive care quarantine zones which ultimately save the uninfected population and will generate a huge chance in saving life of the infected persons. The mentioned process is very useful for mass detection of the COVID19 infected population in a minimum time. The process is also safe for the laboratory technicians as this can easily be done without getting contaminated or infected if proper physical barrier are taken personally. So it can be said that it is not impossible to produce a huge number of test kit while the world is in such a pandemic situation and the human race can only be saved if pre detection of the presence or absence of the virus is identified. Thus it can be said undoubtedly that at this pandemic situation maintaining personal hygiene, self quarantine is assertion in personal scale but in huge mass scale the mass detection of COVID-19 positive humans is urgent for saving the human race.



## **Conclusion:**

Thus it can be concluded by stating that COVID-19 is a disease that spreads through human via touch, sneezing droplets, cough droplets, etc. and can be avoided only by maintaining social distance avoiding human to human contact and the isolation of persons having the virus in their systemic circulation from healthy people. For isolation of the infected people the urge for identification of the healthy people and people exposed to COVID-19 virus must be done. The mentioned process can be followed for the detection of the presence or absence of the COVID-19 virus in the human body. This process is not so very costly and for huge production is also possible and requires very less time. The mentioned process is very useful for mass detection of the COVID-19 infected population in a minimum time. The process is also safe for the laboratory technicians as this can easily be done without getting contaminated or infected if proper physical barrier are taken personally. Thus it can be said undoubtedly that at this pandemic situation maintaining personal hygiene, self quarantine is essential in personal scale but in huge mass scale the mass detection of COVID-19 positive humans is urgent for saving the human race. And for mass detection industrial scale production of COVID-19 test kits is necessary.

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## **Conflict of interest:**

The authors declared that they have no conflicts of interest for this study.

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