Insights into the SARS-CoV-2 Diagnosis in India: Present Status and Future Prospects

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ABSTRACT

COVID-19, the infectious pandemic disease is caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). This deadly disease was unknown before its catastrophic outbreak of the infection in Wuhan city of China, in December 2019. The pandemic situation has increased the demand of rapid enhancement of the *in-vitro* diagnostic assays which would enable the mass screening and testing. Several molecular and serological diagnostics assays such as direct viral antigen tests, nucleic acid amplification tests and serological tests were developed. Nucleic acid

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tests such as RT-PCR. TrueNAT, Feluda Test, loop-mediated isothermal amplification (LAMP) etc. detect the presence of RNA virus in the nasal or throat swab or from saliva. Antigen tests detect the presence of a virus as the antigen, which is a surface protein. Antibody tests such as enzyme-linked immunosorbent assays (ELISA), lateral flow assays (LFA), chemiluminescence assays (CLIA) etc. detect the presence of antibodies generated against SARS-CoV-2 in the blood samples.

Keywords: *COVID-19; RT-PCR; rapid antigen test; antibody test; biosensor.*

ACRONYMS

1. *CB-NAAT - Cartridge Based Nucleic Acid Amplification Test*
2. *CCMB - Centre for Cellular and Molecular Biology*
3. *CLIA - Chemiluminescence assays*
4. *CLIA - Clinical Laboratory Improvement Amendments*
5. *CRISPR - Clustered Regularly Interspaced Short Palindromic Repeats*
6. *CSIR - Council of Scientific and Industrial Research*
7. *ELISA - Enzyme-linked immunosorbent assays*
8. *EP - Envelope protein*
9. *EUA - Emergency use authorization*
10. *FDA – Food and drug Administration*
11. *IAs - Immunoassays*
12. *ICMR – Indian Council of Medical Research*
13. *Ig – Immunoglobulin*
14. *IGIB - Institute of Genomics & Integrative Biology*
15. *LAMP - Loop-mediated isothermal amplification*
16. *LFA - Lateral flow assays*
17. *MP - Membrane protein,*
18. *NAAT - Nucleic acid amplification test*
19. *NGS - Next-generation sequencing*
20. *NP - Nucleocapsid protein*
21. *PHEIC - Public Health Emergency of International Concern (PHEIC)*
22. *qPCR - Quantitative polymerase chain reaction*
23. *RAD - Rapid antigen detection*
24. *RT-PCR – Real time - PCR*
25. *SHERLOCK - Specific High Sensitivity Enzymatic Reporter UnLOCKing*
26. *SP - Spike protein*
27. *ssDNA – Single stranded DNA*
28. *WHO – World Health Organization*

1. INTRODUCTION

Corona, the most infectious pandemic of the decade, is still breaking records each day. This novel coronavirus is responsible for inducing pneumonia. WHO has declared coronavirus disease 2019 (COVID-19) outbreak as a Public Health Emergency of International Concern (PHEIC) on 30th January 2020 [1]. As per the WHO (11th February, 2020), disease has rapidly increased in epidemic scale [2]. As on 11th December 2020, according to www.statista.com, the worldwide total cases stand 70,735,868, followed by 1,588,759 deaths. The worldwide recovery stands 49,166,194. As compared to world scenario, India stood second position after USA with 9,796,992 cases and 9,290,834 recovery cases followed by 142,222 registered deaths followed by Brazil, Russia and Colombia (Fig. 1a). After analysis, it is found that the ratio of total infection against the total population is highest in case of USA followed by France, Brazil, Russia and India and the ratio of total death against the total infected or diseased is highest for Brazil followed by France, USA, Russia and India (Fig. 1b). This analysis revealed that though India is in second position for COVID-19 infection but the ratios of total infection against the total population and total death against the total infected are comparatively less when compared with USA, France, Russia and Brazil, the top countries present in the COVID-19 infected lift, worldwide [3]. Previously India somehow could control the spread at the second stage due to its timely action, judicious policy implementation and quick and progressive testing intention. India tested over 151 million samples for the coronavirus (COVID-19) as of December 10, 2020 [4]. The number of people infected with the virus was growing across the south Asian country and the government swung into action to curtail further spread of the outbreak. The country went into lockdown on March 25, making it the largest lockdown in the world, restricting 1.3 billion people. After extensions of the lockdown, the country started easing restrictions by dividing districts into red, orange and green zones. Furthermore, economic activities had slowly begun to resume since the end of May. This was made happen due to adaptation of different testing techniques like RT

PCR rapid antigen test, antibody test, CRISPR technology, next-generation sequencing, Biosensor technology etc. To complement these molecular assays for the diagnosis of COVID-19, a wide range of serology immunoassays have also been invented by various scientists. Among them, the most efficient immunoassays are automated chemi-luminescent IA (CLIA), manual ELISA, and rapid lateral flow IA (LFIA). The above mentioned immunoassays (IAs) methods are meant for the detection of the antibodies like immunoglobulin M (IgM) and immunoglobulin G

(IgG) that are produced in response to SARS-CoV-2 infection [5]. According to a report [6], India has tested over 151 million samples for the coronavirus (COVID-19) by December 10, 2020 (Fig. 2). The number of people infected with the virus was growing in the beginning of the year across the south Asian country and the government took a major step to contain further spread of the outbreak. The country went into lockdown on March 25, making it the largest lockdown in the world, in the view of restricting the spread of the virus.
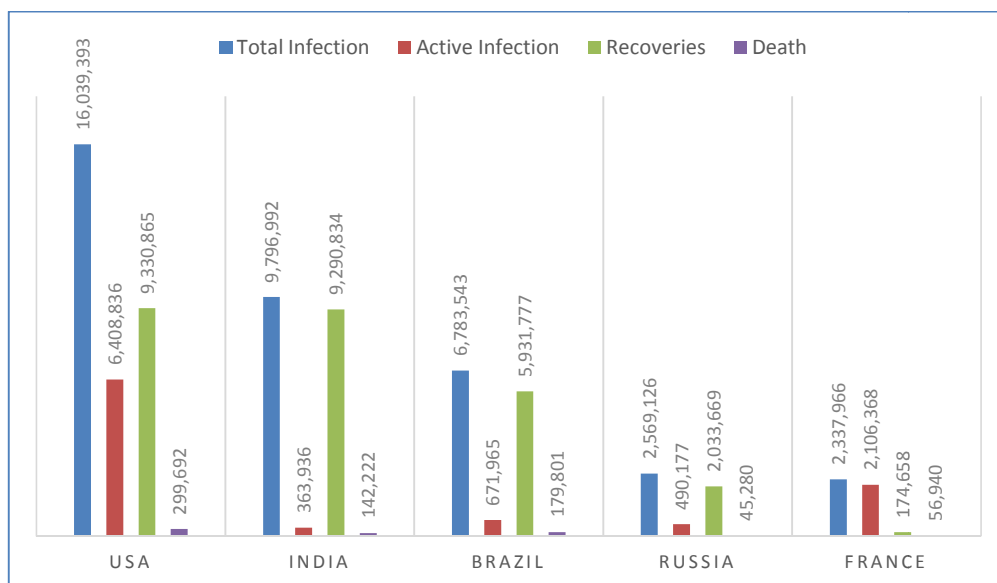

Fig. 1a. COVID-19 scenario of some major infected countries as on 11th December 2020
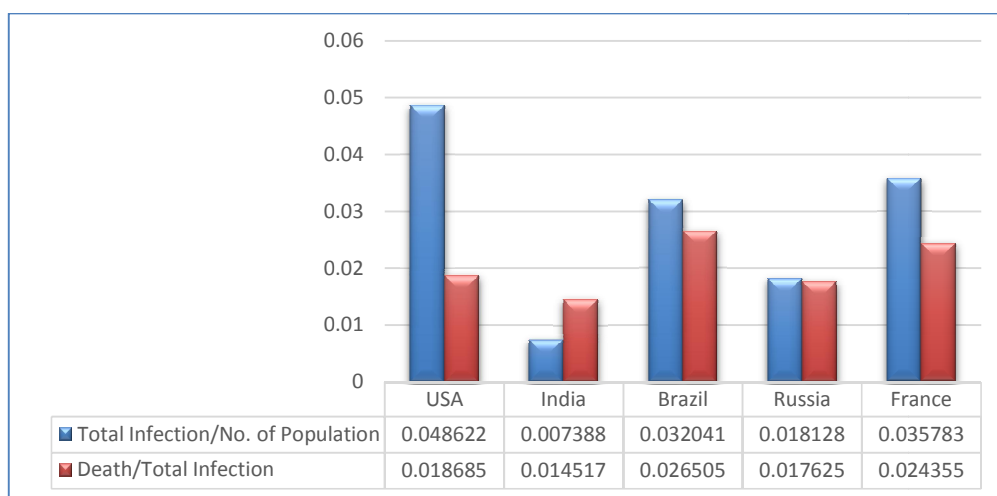

Fig. 1b. Ratio of total infection with total population and total death with total infection of some major infected countries as on 11th December 2020
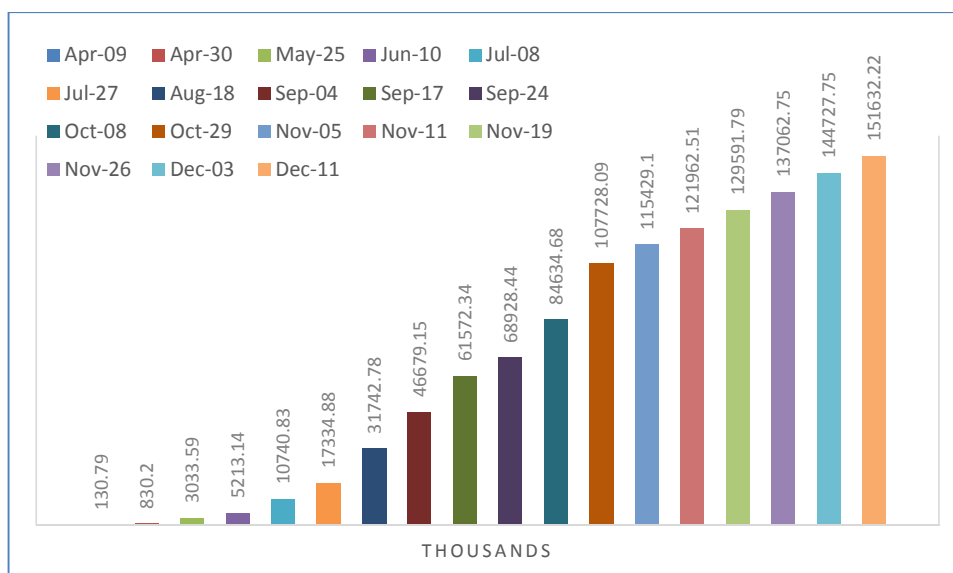
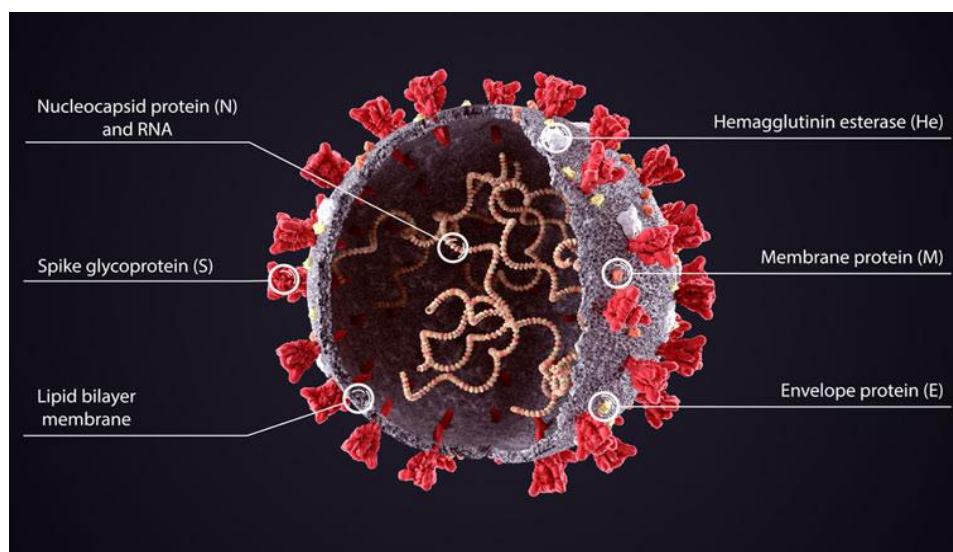**Fig. 2. Cumulative COVID-19 tests in India as on 3rd Dec 2020**

Fig. 3. SARS-CoV-2 virus structure (adapted from https://www.theweek.in/)

The country has been divided into various zones like red, orange and green zones on the basis of the severity of the disease. Furthermore, economic activities had slowly begun to resume since the end of May. ICMR (Indian Council of Medical Research) has approved different testing kits of both foreign and indigenous origin based on the above methods. Ultra-Sensitive and High-Throughput CRISPR technology-based kits are also available for the fast identification of COVID [5]. Among all these above mention diagnostic assays RT PCR test is the most trusted and popularized one due to its accuracy. Other methods are also suitable, which give quick result and cost effective when considered over larger public interest and for mass testing. However, it may give false result due to lack of accuracy. Instead of the flaws, all these played a crucial role in mass testing and contact tracing. When the safety of health personals is considered and also the shortage of protection kits, saliva test also played a significant role; still, we are in the midst of this catastrophic situation. With more understanding of the virus, furthermore advanced technique will be developed in future and most importantly, the

vaccine. The present study focused on the different testing assay developed so far to detect the COVID-19 virus effectively and the mechanism of its action.

2. GENOMICS OF CORONA VIRUS

The unprecedented COVID 19 pandemic situation which just put all worlds to stand and is still playing with men. Human evaluation, which earlier faced different catastrophic situation is now somehow incapable of controlling the spread of just a nanometre length RNA particle. The nuclear material of SARS-CoV-2 is a positive-sense single-stranded RNA virus which has four structural proteins (Fig. 3), out of which one is nucleocapsid protein (NP) that helps to hold the viral RNA and other three major structural proteins such as spike protein (SP), envelope protein (EP), and membrane protein (MP), that create the viral envelope. The viral particle's diameter lies within 50–200 nm and spikes are located on the surface (up to 20 nm in length) and provides it with the crown-like appearance which is a distinct characteristic of coronaviruses (CoVs) [7,8]. The investigation about genome sequence of SARS-CoV-2 has revealed that about 82% of the sequence shows homology with the human SARS-CoV and around 89% with bat SARS-like-CoVZXC21 [9]. This is disease highly dangerous for aged people (above 65 years), and persons with weaker immunity power or persons with chronic disease issues are at a higher risk of corona virus infection. So, utmost precautions and effective preventive measure should be taken to fight against this pandemic COVID-19 [7].

3. RESPONSE OF HUMAN IMMUNE SYSTEM TO COVID-19

Whenever we come in contact with any symptomatic and asymptomatic people and get exposed to the virus particles, viral particles enter into our body through openings of nose, eyes or mouth. Thereafter breathing air containing viral particles to the lower respiratory tract where the viral spike proteins act as key which get locked into the epithelial cells lining of the respiratory tract as along with those in the air sacs in the lungs. The spike proteins of the virus are able to get an entry by unlocking of the Ace2 protein and surface glycoproteins on the lung cells. Thus, they are able to take over the cells' machinery after that start replication multiplication and further infection to the subsidiary cells. Just like the Ace2 protein on the epithelial cell viruses too have specific proteins on their surface called antigen which gets identified by our body which kicks start the immune system into action and the production of antibody [10]. These also trigger a class of chemicals known as cytokines and chemokines that work for sending alert signals to activate the immune system and initiate the production of different kinds of cells in destroying viral particles. However, these cytokines and chemokines trigger inflammation in the cells, majorly in the nose and upper region of the respiratory system. Further, this inflammation triggers a fluid build-up in the lungs. Thus, depending on the degree of infection, it can even lead to pneumonia, followed by breathlessness. However, the high cytokines level can cause extensive damage to the lungs and leading to condition called acute respiratory distress syndrome.

4. DIFFERENT METHODS FOLLOWED FOR DETECTION OF CORONA VIRUS

4.1 Thermal Scanning Method

These scans are used to identify potential patients and preventing them from entering into crowded places. According to the US national library of medicine, the average normal body temperature is 98.6^oF. A temperature over 100.4^oF is most often presumed to be a fever caused by an infection or illness. The thermal imaging technology is being widely used to screen the body temperature. However, Julian Hall outlook highlights the use of thermal screening for identifying the potential cases of coronavirus, and explores the potential uses and drawbacks of this method [11]. Thermal imaging scanner is a combination of a detector and lens that detects temperature and gives a visual representation of the infrared radiation emitted by humans and surrounding objects which is otherwise invisible to naked eyes. The camera of thermal imaging scanner uses a unique lens that captures infrared light onto an infrared detector array, which then converts it into electric signals, and processes to create a thermal image and detect the temperature [12].

4.2 Saliva Test Method

Apart from all techniques, another such game-changing method is the saliva test for COVID detection. This test used the saliva of a patient sample instead of the swab. Saliva is the most dangerous thing and can have an essential role

in the human to human transmission, and the non-invasive salivary test may offer a suitable and cost-effective way for the quick and initial detection of COVID-19 infection [13]. In this test, saliva is used as sample instead of swab. The testing method also replaced the PCR with a low-priced PCR alternative well-known as loop-mediated isothermal amplification (LAMP), which was already used to detect ZICA and EBOLA virus. This test was invented by Yale school of public health which was directed by Nathan Grubaugh and Anne Wyllie team. In this method of testing, people can collect their own samples into a sterile tube or bottle and send it to the lab, which ultimately reduces the physical contact between the health worker and the patients. This test mostly lowered the entire bottleneck faced during the PCR method to detect and diagnose COVID 19. Instead of all benefits, some risk factors are there, such as it required purified saliva for the diagnosis. In this method, the focus was given on uses bits and pieces of DNA that attach to short and distinctive parts of RNA of the virus rather than amplification process of RNA. Finally, linear strand alters to loop for easy detection in LAMP. To identify the total six possible different sequence of target DNA it required LAMP DNA polymerase and a set of 4 to 6 primers. The whole detection process is based on displacement amplification by different primer and formation of until the DNA achieves dumbbell shape structure. Then the accumulated by-product got easily detected. A compatible study (Table 1) of different COVID-19 diagnostic tool revels the significance of the test for rapid detection of Corona virus.

4.3 Rapid Antigen Test

Rapid antigen diagnosis methods are frequently taken for consideration to detect pathogens attacking the respiratory system, including influenza viruses and respiratory syncytial virus (RSV). The emergency use authorization (EUA) has been authorized by FDA for antigen tests that can identification of SARS-CoV-2 [14]. Rapid antigen testing technology is quick and reliable techniques. Some previous study revealed that during early infection period the coronavirus nucleoprotein is the predominant, and the structural proteins are released in large amounts into the serum, nasopharyngeal aspirate, throat wash samples, faecal material, and urine [15]. So, the detection of NP antigen methods may be the efficient line of attack for the early detection of virusin suspected SARS-CoV or MERS-CoV-infected patients [16]. Ihling and his team [17]

have performed the mass spectrometric analysis that showed that NP is present in gurgle solution samples of infected patients. Rather than this SARS-CoV-2 NP may be found in NS (nasal) specimens of COVID-19 patients after three days or more after fever symptom [18]. This method of detection test diagnosis the SARS-CoV-2 rather than the antibody formed by the body. The significant advantage of this technique is the fast results and more affordable than reverse transcription polymerase chain reaction (RT-PCR) for detection of SARS-CoV-2.This testing technology is ready to use testing methods which have rapid and qualitative detection of viral antigen in nasopharyngeal secretion and also have the ability to give results in 30 mins [19]. The antigen is the protein molecule that is found on any viral pathogen. This antigen triggers the immune response in our body leading to the production of antibody. As antibody test detect the antibody, the antigen test detects the antigen in our body.

The swab samples are collected and processed in the chemical to expose the viral nuclear protein and to test the antigen. Rapid antigen detection (RAD) technology is analyzed for detection of SARS-CoV and SARS-CoV-2 nucleoprotein antigen which is highly conserved. This diagnostic assay uses monoclonal antibodies detection method in this assay using colloidal gold nanoparticles which are conjugated to the antibodies. These antibodies are then fixed onto the nitrocellulose membrane due to their immobilization nature. Then the detection was carried out according to the guidance of the manufacturer's company. Generally, 100 μL of nasopharyngeal secretions with four drops of LY-S dilution buffer mixed in a tube and then the mixture is added to the strip. To calculate the exact migration of the samples control line is built-in in the strip. Then after 15 min, visual analysis of the result is enforced. Two versions of the test are generally analysed out of which in the second version, the conjugate is coupled on a diverse way to optimize the control [20]. Although antigen detection assay sounds better than another standard testing because of faster results, but it was found that results are not as sensitive [21]. One investigation suggested that SARS-CoV-2 test kit developed by a Belgian company show higher specificity, at 99.5% but the sensitivity of the test was quite low, only 57.6%. Therefore, it was a clear indication that a large percentage of diseased patients may not be captured with the antigen test [22]. Rather than this another major drawback of this RAD

test can only give appropriate results if the person is *recently* infected with SARS-CoV-2.According to an article of Financial Express published on 11th September 2020, Govt. of India advised to use RT PCR for best results as between June 18 and July 21, 15.2% infections were missed in India by the rapid antigen tests, as per the health ministry.

There has been a rapid inflation in the number of infections from 2.2 million on August, 2020 to 4.5 million on September, 2020, which has compelled the government to make a change in the testing strategy. While increasing the testing numbers seemed to be wise option, but it still did not help as most of the tests were done by Rapid Antigen Tests and a very less tests were performed by RT PCR. The rapid antigen tests constitute the majority of the total tests done in all the states *viz.* 75% in Delhi, 90% in Bihar, 66% in Kerala (Fig. 4a).The false negative results of the rapid antigen tests might have made the infection rate fall relative to the RT-PCR. According to an RTI filed by The Indian Express, out of 6,054 symptomatic people tested between June 18 and August 27, 699 i.e. 11.5% were found positive through RT-PCR testing. Similarly in Maharashtra, data released by the government last month found that RAT had missed 23.9% infections. In most of the cases the negative RAT results were subsequently found positive when tested through RT-PCR (Fig. 4b).

Table 1. A comparative study of different COVID-19 diagnostic tool

| Properties	Molecular test RT-PCR and Saliva test	Antigen test	Antibody test
Other names	Diagnostic test, viral test, molecular test, nucleic acid amplification test (NAAT), RT-PCR test, LAMP test	Rapid diagnostic test (Some molecular tests are also rapid tests.)	Serological test, serology, blood test, serology test
Sample for the test	Nasal or throat swab (most tests) Saliva (a few tests)	Nasal or throat swab	Blood sample
Waiting time for result	2-7 days (depending on the distance from the testing lab)	Within 30 mins to 1 hr	Within 30 mins to 1 hr
Accuracy of the test	Typically, highly accurate without any flaws	Positive results are usually highly accurate but negative results may need to be done with a molecular test for further conformation	Not much accurate further confirmation required
Result shows	Presence of active virus particles	Presence of viral antigen	Responsive antibody produces by week long infection
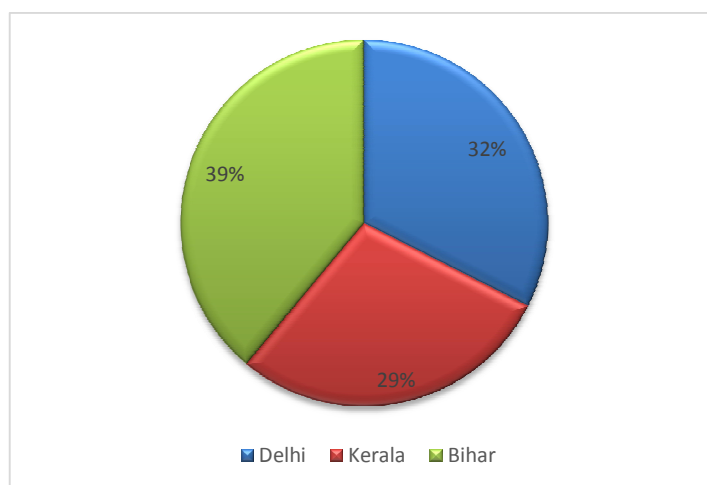
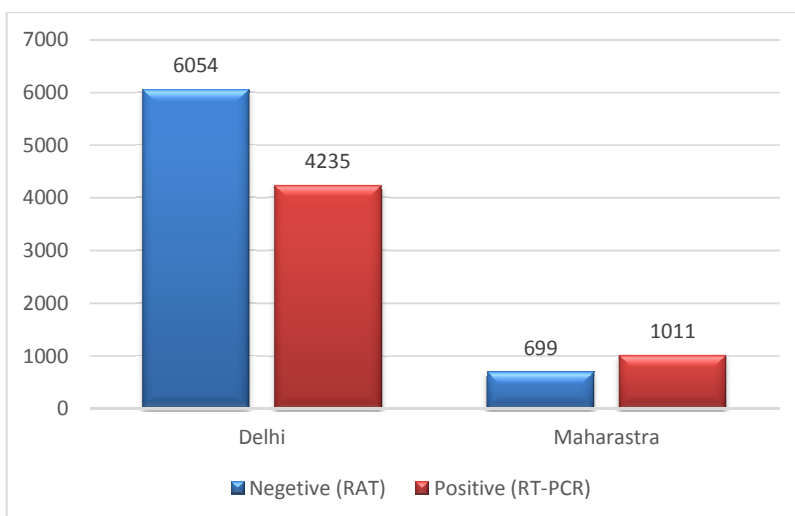**Fig. 4a. Preference of RAT (case study)**

Fig. 4b. Defectiveness of RAT (case study)

These statistics reveal the significance of RT PCR tests for COVID-19 rapid testing and detection of virus. The 'case infection rates' shown by the daily tests are absolutely different from the results portrayed by the sero-surveys. The case infection rate in Delhi, shown by these daily tests is around 10.8% which contradicts to 29.1% positive infection rate that is suggested by the sero-surveys. Similar status is found in case of Mumbai. The sero-survey is more reliable as it involves vivid sampling technique. Due the higher number of false negative data, the Union Health Ministry has advised the states to strictly abide by the rules advocated by ICMR on testing. According to the ICMR guidelines, all the symptomatic persons should undergo RAT, if found negative, they need to further screening through RT-PCR testing. This can help us in early detection and isolation of infected persons by eradication of false positive results.

4.4 RT PCR Test

The reverse transcription-polymerase chain reaction-based test is recommended as a testing method. This test detects the presence of viral RNA in human nasal and throat swab samples. It is preferred due to its higher sensitivity and specificity rate [23,24]. PCR stands for polymerase chain reaction in which a few copies of DNA are amplified for production of millions of copies. This test requires primers, probes, polymerase enzymes, nucleotides and buffer solution. Primers are short stretches of DNA which are targeted viral genome specific and are complementary. Whereas the probes are labelled with fluorescent molecules that binds with the

target region in between the primers (forward and reverse). WHO has recommended use of at least two target regions for the diagnostic detection of the virus, because different types of antigen are present in virus such asE, S, N, ORF1a, ORF1b & RDRP, among these entire E antigens is common to all coronavirus strains [25]. These targeted antigens differ from country to country, so two antigens apart from E, and another region-specific strain of the virus is tested for detection. In this method first the viral RNA is converted to DNA through reverse transcriptase, then amplified with the help of polymerase and nucleotides and also the primers which get bound with each copy of DNA during the repeated cycle and extension. Probe binds to DNA which is the product of PCR after the completion of PCR cycles. This allows the amplification of single molecule of DNA, which is the main aim of PCR technique, so even virus particles in single digits can also be detected. RT–PCR technique is the quick, super sensitive, qualitative and specific [26].

This method provides reliable diagnostic results within three hours but took eight hours to produce final results [27]. In comparison with other methods, this assay for detection of the virus is relatively faster and very fewer chances for contamination or avoids errors during the diagnostic process due to automation [28,29]. By considering the above advantage of this technique, the 'criterion-referenced' this assay as a major tool for detection of the causative agent like COVID-19, SARS-CoV-2 [29]. Despite of so many advantages, still it has some significant drawback such as RT-PCR can only detect

ongoing infection with SARS-CoV-2 [27]. Another significant problem with this assay is the real-time RT-PCR diagnostic is the threat of removing false-negative and false-positive results [30]. Lots of factors have been proposed to be linked with the inconsistency of real-time RT-PCR [31]. RT-PCR assays need specific molecular equipment with sophisticated laboratory with trained person, which ultimately results in the more time-consuming process [27]. According to ICMR, a cumulative total of 7,56,19,781 samples have been tested up to 30 September 2020 and according to the same report of ICMR on 1st October 2020, 255 RT PCR kits were evaluated out of which 126 kits give satisfactory results and the top six companies providing the kits are listed in Fig. 5.

4.5 Antibody Test

Even though social distancing and staying at home is being practised, the concentration has now changed to prevalent antibody (serologic) testing of the population to fight against this pandemic. This serological test is based on the presence and absence of antibodies of the suspected blood sample. Antibody testing method developed to find out IgG only, both IgG and IgM, or total antibodies [32]. If the suspected person opens to the SARS-CoV-2 then immunoglobins (Ig) will be detected in the sample, because of immunoglobin produced as an immune response to SARS-CoV-2 infection. US Food and Drug Administration (FDA) gave permission on April 30, 2020 for ten antibody tests by the under-emergency use authorizations. FDA-approved antibody tests then declared as average sensitivity and specificity of is 84.90% and 98.63%, respectively [33]. As said earlier antibodies are the proteins developed within the body to fight against an antigen or foreign entity when that person is exposed to that particular disease. The coronavirus affected person developed antibody such as IgM and IgG, which were detected by this method. These methods work similarly like pregnancy detection kits. Generally, when someone gets infected, two kinds of antibodies are produced. Antibody IgM is produced first within a week of infection. Thenafter two-three weeks, the IgM level gets decreased and changed by IgG, which remains for a long time and provides a long duration of immunity. By this way, COVID specific antibody can be diagnosed on the basis of its presence and absence which was described in the Fig. 6 [34], which follows blood sample collection, addition of diluents to

the samples and using the antibody testing kits. This product is based on the capture and solid-phase immunochromatography methods for determination. The specimen (whole blood/serum/plasma) flows from the blood separator through to the conjugate release pad (which occurs when the conjugation reaction between IgG/IgM antibody in the specimen and the antigen colloidal gold of 2019-nCoV to form an immune complex of IgG/IgM and colloidal gold-labeled antigen) due to capillary action. The sample then migrates to a capture zone of nitrocellulose membrane-immobilized antibody (mouse-anti-human IgM antibody) to form an immune complex of colloidal gold-labeled antigen, IgM antibody, and mouse-anti-human IgM antibody, thereby generating an IgM red line. The unreacted immune complex continues to flow upward, will be captured by the mouse-anti-human IgG antibodies to form an immune complex of colloidal gold-labeled antigen, IgG antibody, and mouse-anti-human IgG antibody, thereby generating an IgG red line. The remaining uncaptured immune complex moves upward, combining with the C line (quality control line) to indicate the completion of this reaction (https://www.assaygenie.com/vazyme-2019ncov-igg-igm-detection-kit-colloidal-gold-based/).

Antibody test gives significant results only after 7 to 10 days of infection, but do have a larger percentage of error than swab tests. Before five-day SARS-CoV-2 antibody response found meagre seroconversion rates after onset symptom. But after 15 days, there is a very high seroconversion rate in SARS-CoV-2 antibody response found in confirmed COVID-19 patients [35,36,37]. Currently available many diagnostic kits are showing false-negative and false-positive results [38,39]. Some results show false negative because after exposing to the COVID-19 that individual is inefficient to produce antibodies. Similarly, if an individual has another, very similar virus in the body, then the diagnostic assays may show a 'false positive' results. The researchers found that antibody tests detected only 30 per cent of positive COVID-19 cases one week after the first symptoms appeared only. Whereas it also detected that the accuracy increased to 70% and 90% in the second and third week of infection respectively. According to reports of ICMR, around 126 RT PCR test kits, 20 IgG ELISA ans CLIA test kits and 5 Antigen based rapid test kits (Fig. 7) have given satisfactory results till date, which sounds good during this pandemic.

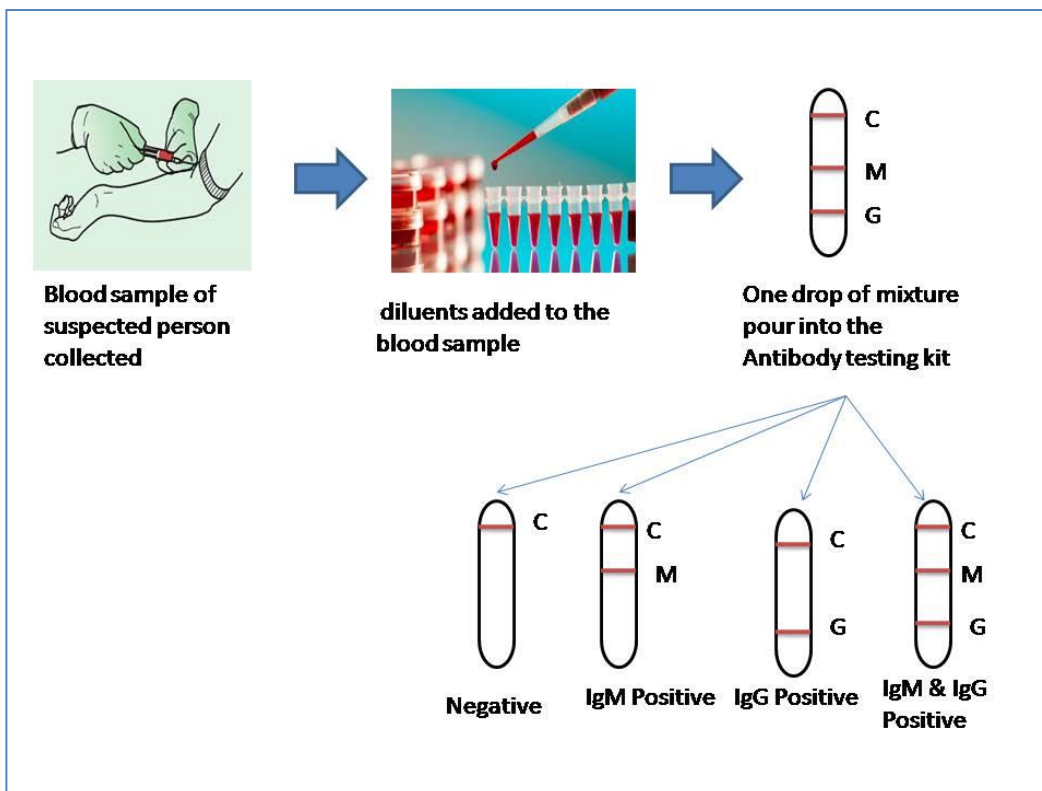**Fig. 5. Top 10 biotech companies providing satisfactory RT PCR kits (ICMR)**
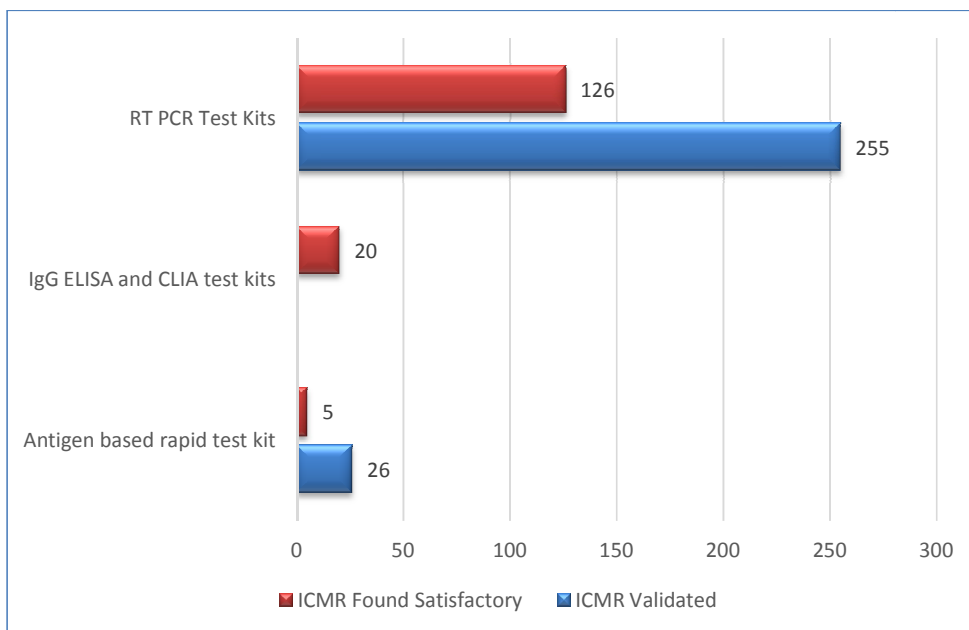

Fig. 6. General protocol of antibody test

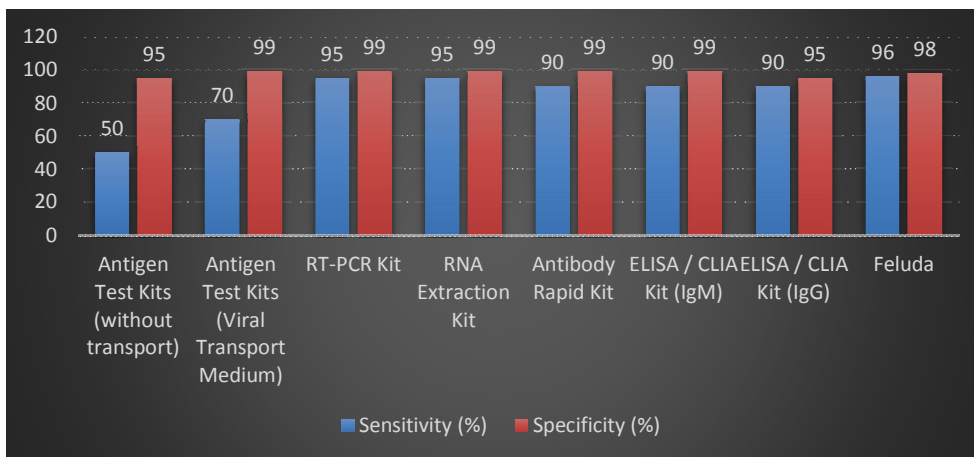**Fig. 7. Kits providing satisfactory results after analysis (ICMR)**

Fig. 8. SARS-COV2 diagnostic commodities acceptance benchmark (ICMR)

4.6 Crispr Based COVID-19 Testing

According to FDA- CRISPR based diagnosis technology for COVID-19 is very first and rapid. Recently the company Sherlock Biosciences made one kit based on CRISPR machinery which has the ability to provide results within one hour with higher specificity. Some finding suggests that a CRISPR-based fluorescent application has potential to improve current COVID-19 screening efforts [40]. CRISPR-based SHERLOCK (Specific High Sensitivity Enzymatic Reporter UnLOCKing) technique is based on synthetic COVID-19 virus RNA fragments [15]. The test can be carried out with the RNA purified from the disease patient and further undergo qRT-PCR assays. Basically, the kit functions by CRISPR machinery which detects the presence and absence of specific genetic materials of SARS-CoV-2 from the patient's sample. Total framework of the kit uses CRISPR nucleases is basically planned to get the target and defined gene sequence. This kit uses two Cas protein, one is Cas13 to cleave and degradation of neighboring ssRNA and activates a fluorescent reporter and the second one is Cas12a with ssDNA used for detection.

48

First of all, the nuclease activates a cleavage capability to cleave a reporter DNA strand after that it creates a fluorescence signal, which ultimately finds the target sequence. After the sequence found the CRISPR enzyme get activated and releases a detectable signal. The CRISPR enzyme generates a fluorescent glow if there is any presence of virus materials [41]. Recently, the Drugs Controller General of India has approved the commercial launch of 'Feluda' which is the Tata CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) COVID-19 test. According to the CSIR this is based on CRISPR technology for detection of the genomic sequence of SARS-CoV-2 virus. As per ICMR guidelines, this diagnosis testing achieves the high-class benchmarks with 96 per cent sensitivity and 98 per cent specificity for detecting novel coronavirus. It is a paper-based Diagnostic kit programmed with CRISPR which is now called as Feluda. The Feluda test kit took 45 minutes time duration to produce results for COVID [41]. Feluda test kit (Fig. 8) is highly effective as it is time and cost effective and gives accurate results with about 96-98 % sensitivity.

4.7 Chip Based COVID-19 Testing System

Indian Council of Medical Research (ICMR) approved the use of the TrueNAT system for screening and confirmation of COVID-19 on 19th May, 2020. This system is a chip-based test which detects the SARS-CoV-2 E-gene, which is responsible for building the envelope and activate the gene the enzyme (RNA-dependent RNA polymerase) that permits the virus to replicate. In TrueNAT tests, the sample preparation is automated and read out the results within 30 min [42]. In this test nose or throat swab samples of suspected patients was put in a solution that inactivates the virus. Then few drops of that solution put on a cartridge, which is inserted into a machine with a pre-programmed reaction set up to extracts the nucleic acids or the target genetic material from the samples - whole process followed by an RT-PCR. Then purified nucleic acid put into a micro-tube having freeze-dried RT-PCR reagents, and then solution left for a minute. After one minute, the solution put into a microchip which is then inserted into another machine, where the reverse transcription and PCR was taken place [43,44]. The significant advantage of this test is quick and portable. This test avoids the transportation problem while sending the sample to the diagnostic laboratory. This test grants teams to locate up mobile testing centres or kiosks in containment zones. This is

an indigenously developed, battery operated and portable version of CB-NAAT (Cartridge Based Nucleic Acid Amplification Test) which is otherwise popularised as the Genexpert test, initially developed to diagnosis tuberculosis disease. According to a report on June 10th, 2020, totally, 30 confirmed SARS CoV-2 positive and 45 confirmed negative swab samples were tested. All positive and negatives were correctly detected on Truenat™ SARS CoV-2 assay indicating 100% sensitivity, specificity and 100% overall concordance to reference gold standard assay.

4.8 Next-Generation Sequencing for COVID-19 Testing in India

According to news briefing made by Nature biotechnology on 8th July 2020,the US Food and Drug Administration has granted Emergency Use Authorization for Illumina's next-generation sequencing (NGS) test for COVID-19, which is the first such authorization for a NGS diagnostic. Through the proper exploitation of the NGS, the COVID Sequence test can be increased to a higher volume screening. The modified version of the test developed by the ARTIC Network; a Welcome Trust collaborative set up employs 98 DNA fragments, or amplicons, to cover the roughly 30 kilobases in the SARS-CoV-2 genome. Around 3,000 tests of nasopharyngeal or oropharyngeal samples can be run at one go in a day by multiplexing the reactions. Company literature states that the test requires at least 1,000 copies of the viral genome per millilitre and displays 98% sensitivity and 97% specificity. Although the test has the authorization, it isyet to receive approval from the FDA. There are certain limitations to the technology since there is lack of equipment to run the tests; along with the Clinical Laboratory Improvement Amendments (CLIA) certification, labs ought to be trained on Illumina's NovaSeq6000 sequencing platform.

However, there is a planning from the company's side to increase the number of sites in the coming months which shall be facilitated by Ginkgo Bio works. In May, Illumina has led a $70 million series E funding from which Ginkgo creates the infrastructure for large-scale testing at its Boston lab. Rapid and large-scale testing is considered key to for safer opening of schools and workplaces. The sequence information, further shall aid in providing information to the public health officials on the route of transmission and mutation rate of the virus. With almost 6.31 million cases[*] of COVID-19, India has the second

highest number of cases worldwide and higher testing capacity is essential to mitigate the continued spread of the virus. According to a report on 13th August 2020 [43], the Institute of Genomics & Integrative Biology (IGIB), a premier Institute of Council of Scientific and Industrial Research (CSIR) in New Delhi, has assessed the ability of Illumina's COVIDSeq™ research assay for detection of SARS-CoV-2.COVIDSeq, a high-throughput next-generation sequencing based assay, typically generates results within 24 hours using the NovaSeq™ 6000 Sequencing System and is complimented with the highly accurate and ultra-rapid analysis performed using the COVIDSeq Test installed on a local DRAGEN™ Bio-IT platform. IGIB generated data that demonstrated enhanced sensitivity compared to quantitative polymerase chain reaction (qPCR) test, with an overall 8-10 percent increase in diagnostic yield. If the next generation sequencing is being discussed, Fulgent molecular diagnostic test is FDA-authorized for emergency use authorization (EUA) during the COVID-19 pandemic. Clinicians can now directly order Fulgent FDA EUA-authorized molecular test, NGS test, or antibody test through a safe, straightforward, and streamlined process. According to the latest report of the Economics Times on 1st October 2020, Syngene International joins global consortium of19 healthcare organisations on COVID-19 testing. It has collaborated with the Centre for Cellular and Molecular Biology (CCMB) to deliver a high throughput net generation sequencing (NGS)-based genomic screening assay that can test 5,000-10,000 samples simultaneously.

4.9 Possible Application of Biosensor for the COVID-19 Virus Detection in India

According to one report of ScienceDaily on April 21st, 2020, researchers from ETH Zurich and Zurich University Hospital, Empa have been successful in developing a new sensor to detect the novel coronavirus [45]. This can be used to measure the concentration of the virus in the future. But presently qRT-PCR is in maximum use to detect the virus from various biological specimens but it has many shortcomings like it is time consuming, labour intensive and not rapidly deployable. These short comings can hinder in getting the realistic data of infection and community spread of SARS-CoV-2 in the population. Novel biosensors that are used to detect RNA-viruses areCRISPR-Cas9 based paper strip, nucleic-acid based, aptamer-based, antigen-Au/Ag nanoparticles-based electrochemical biosensor, optical biosensor and Surface Plasmon Resonance. These can be efficient tools for rapid, authentic, portable, and promising diagnosis of novel corona virus in the present situation.
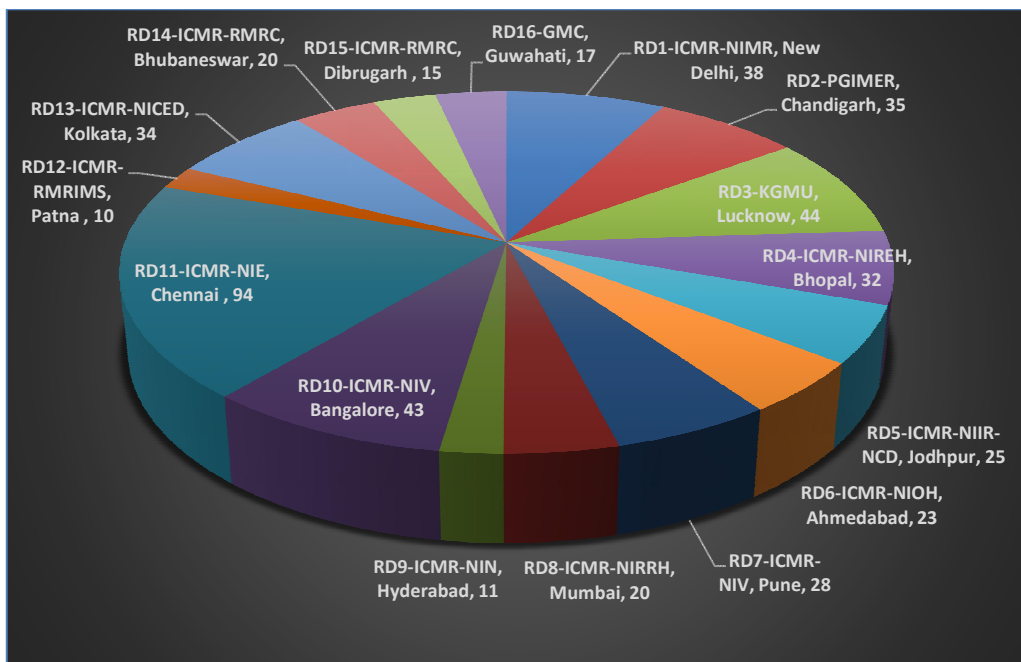

Fig. 9. ICMR regional depots with laboratories for distribution, coordination and validation

5. CONCLUSION

These are the tests used recently to detect the infected person however; it is still challenging to identify the asymptomatic persons in Populist County like India. The ICMR has approved many Government and Private Labs to conduct tests. The U.S. food and drug administration have approved 20 manufacturers and kits for diagnostic testing COVID 19. The test kits like Cobas SARS COV 2 from Roche and Taqpath covid19 combo kit from Thermos fisher are also get validated by ICMR.ICMR is now functions along with four central depots and sixteen regional depots (Fig. 9) with laboratories for distribution, coordination and validation of COVID-19 testing kits and COVID-19 vaccine development. The regional depot 11 (ICMR-NIE, Chennai) is functioning with highest number of laboratories followed by regional depot 3 (KGMU, Lucknow) and regional depot 10 (ICMR-NIV, Bangalore).Apart from this, the efficacy of any test also depends on its perfect handling.

Till now, RT PCR being proved to be the most reliable sensitive method without a false negative case but expensive for use in the larger public interest. In that prospective antigen and antibody test were proved to be quicker and cost-effective. Antibody tests with similar accuracy and specificity as the RT PCR are needed for mass screening. Innovative tests will take interdisciplinary scientific effort and investment. Till then, India can use these existing tests in labs with accreditation and rigorous training to detect each of the COVID cases accurately. Identification of novel biomarker discoveries could also play a crucial role in the early stages of detection of SARS-CoV-2 infection. The TMCOVID-19 test kit is also playing a remarkable achievement for the detection of SARS-CoV-2 within 5 min [42]. There is a need for improvement on smart and rapid testing diagnosis kit like SHERLOCK and Feluda with high sensitivity and specificity for significant detection of SARS-CoV-2.

The rapid spread of a new variant (VUI – 202012/01) of coronavirus has been blamed for the introduction of strict tier four mixing rules for millions of people, harsher restrictions on mixing at Christmas in England, Scotland and Wales, and other countries placing the UK on a travel ban. The government's advisers on new infections now say they have "high" confidence that it is more able to transmit than other variants. All the work is at an early stage, contains huge uncertainties and a long list of unanswered questions. According to BBC News (Dec 30, 2020), first 20 cases of new coronavirus strain found in India as UK returnees test positive. Now, this is an alarming time for all nations to concentrate on significant investment and development in the field of health research.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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