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Diagnostic Techniques During the COVID-19 Pandemic in the Virus Research Diagnostic Laboratory (VRDL) of MGM Medical College Jamshedpur

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Abstract:
Over the past 20 years, viruses that spread from animals similar to humans have caused significant problems worldwide, highlighting the need for better methods to detect COVID-19 viruses early. This presents a major challenge for clinical microbiology in healthcare. There are direct tests, such as RT-PCR and TruNAT, which detect the virus itself. Additionally, indirect tests examine the body's response to the virus. The aim is to explain how these tests operate. Fast and accurate tests enable the detection and management of infected individuals. They also assist in tracing contacts and informing public health decisions.

Keywords: COVID-19, RT-PCR, TruNAT, antigen-antibody-based techniques.

INTRODUCTION

COVID-19 is the third time in 20 years that a coronavirus has moved from animals to humans (Wu F et al., 2020). It has affected the world more than the earlier outbreaks in 2003 (SARS-CoV) and 2012-2015 (MERS-CoV). Over 25 million people in more than 200 countries have caught COVID-19, leading to over 8,40,000 deaths (WHO, 2020; Worldometer, 2020). The virus is likely to keep spreading, especially in densely populated areas. Severe cases can cause serious lung problems and have a death rate of about 6% (ranging from 1-14.4%) (WHO, 2020; Worldometer, 2020; Sohrabi C et al., 2020). Finding people with COVID-19 who do not show symptoms is important to stop the virus from spreading. Good testing is needed to tell if someone has COVID-19 or another illness, reducing unnecessary quarantines and controlling the spread. The World Health Organisation (WHO) has suggested preventive measures to regulate the viral infection. [Dinnes J, et al.,2020]. Three main testing methods are recommended: Reverse transcriptase polymerase chain reaction (RT-PCR), Truenat, and Next-generation sequencing (NGS-based,

antigen rapid testing, and antibody rapid testing [Dinnes J et al,2020; Carter LJ et. al,2020; Kyosei Y et.al.,2021]. These methods help reduce and check for errors.

RT-PCR is the best and most current method for diagnosing SARS infection, but it takes at least four hours and needs skilled technicians. Fast and accurate tests are important for surgery and other procedures. Truenat was first approved by the ICMR, India's top medical research agency (ICMR, New Delhi) (Ghoshal, U et al., 2021), and was aimed at comparing the SARS Truenat and SARS-CoV-2 PCR-based tests. Truenat is a chip-based cartridge technique that requires class II biosafety.

During the second wave of the pandemic, Rapid Antigen Test (RAT) became an important testing method because testing labs faced a deficit of consumables due to logistical issues, a lack of staff, and many healthcare workers testing positive. The RAT is the first self-test for COVID-19 that can be used at home, and it provides results within 30 minutes [Kumar KSR et.al., 2020; Rasmi Y, et al,2021; Chew NWS et al,2020]. Mylab's CoviSelf was the first self-test kit approved by ICMR in India. The test is easy to use with a kit insert.

Serological assays, such as ELISA, identify individuals who might donate plasma and are immune to the virus. These tests help to track contacts and check immune status over time (Jones et al., 2020). This study does not focus on these methods, but the information can help update guidelines and testing protocols in clinics and labs. However, there is limited information on these methods, and scientists need to learn more about their accuracy and limitations (Table 1).

Table 1. Different Covid-19 Diagnostic Assays (Kashyap *et al*., 2020)

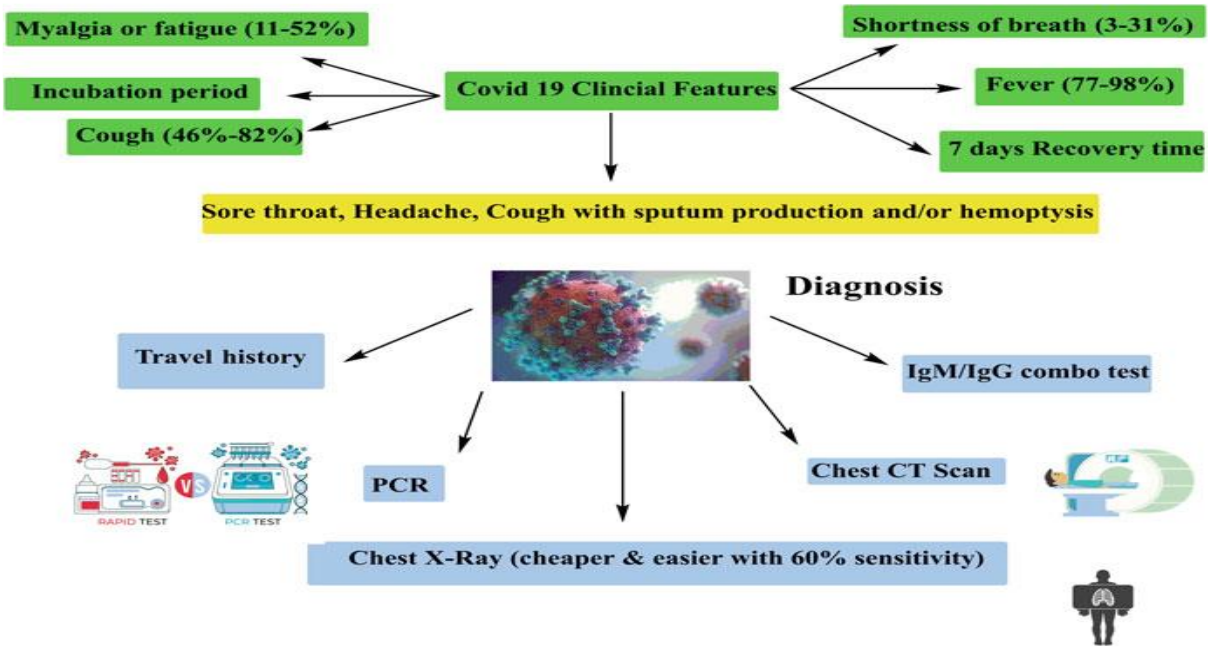
Methodologies	**Duration Required**	**Operating Principles**	**Advantages**	**Limitations**
Real-Time Polymerase Chain Reaction	3-4hrs	Uses a particular primer-probe for detection	Excellent sensitivity, specificity and minimal cross-contamination. Well-established methodology	Many samples are tested simultaneously, which is expensive and time-consuming.
Truenat and (Cartridge-Based) Nucleic Acid Amplification Test)	1hr	uses a particular primer-probe method to detect	Good precision, minimal Biosecurity issues	Lack of reciprocity, very expensive, a restricted quantity of examples
Serology (Classical)	3-4 hrs	IgG\ IgM	Identify exposure	Many samples are tested, with less Sensitivity
Rapid antigen test (RAT)	Within 30 min	Antigen detection	High-speed test, High specificity	Single sample at a time, Limited Sensitivity

Next-generation sequencing (NGS)	1-2 days	Sequencing of the whole genome	The High-sensitivity specificity technique provides detailed information about the specimen.	Highly trained experts, Sophisticated equipment, very expensive.

COVID-19 Diagnosis and Management

COVID-19 Diagnosis and Management COVID-19 is detected using real-time polymerase chain reaction (TaqPath COVID-19 kit) with samples from the nose and throat [Ai, T., Yang, Z. et.al.2020]. There is also a blood test that checks for antibodies [Salehi, S. et.al.2020]. Non-invasive methods such as chest X-rays and CT scans help check the lungs. To prevent COVID-19, a few guidelines have been introduced: maintain a distance of at least 6 feet from others, wash your hands often, avoid large gatherings, and wear face masks. Masks are effective in preventing the virus [Jeremy, H et.al.2021]. Vaccines are available to help the body fight the virus [Anderson, R. M., et.al.,2020]. Many patients have also been treated with drugs like vasodilators, antiviral drugs, and corticosteroids.

Figure 1 signifies COVID-19 clinical features, symptoms, and various techniques for the detection of COVID-19.



Ethical Issue:

The Institutional Ethics Committee granted permission to carry out the test.

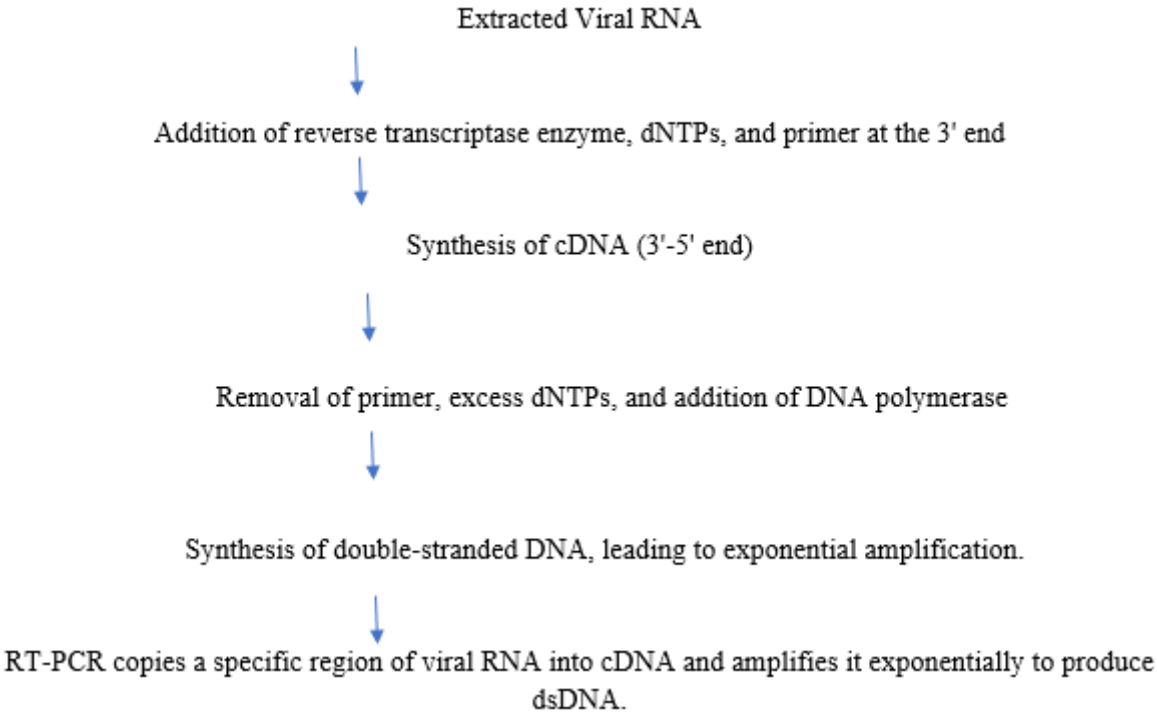
Methodology:

Molecular Assays

The COVID-19 virus is tested using nucleic acid amplification testing (NAAT).

Real-time reverse transcription polymerase chain reaction (rRT-PCR) is employed in NAAT to identify

specific viral RNA sequences, and nucleic acid sequencing is used for confirmation when necessary (Premraj A et al., 2020). This method underpins routine confirmation of COVID-19 cases. Biosafety cabinets (BSL-2) should be used for RNA extraction. Heating samples before RNA extraction is not recommended.



1. Real-time PCR

RT-PCR utilises the 5'-nuclease activity of Taq DNA polymerase. It employs TaqMan probes to detect specific PCR products during cycles. A unique alignment was created for coronaviruses in the Sarbecovirus subgenus, using bat SARS-like coronaviruses and 2019-nCoV. Sequence alignment verifies if confirmatory tests match the Wuhan virus. After testing the E gene in suspected samples, the N and RdRp genes should be checked as additional confirmation. The ORF and RdRp gene tests further verify results. RT-PCR is highly specific, though its sensitivity can range from 65% to 96%, which may produce false negatives, especially early on in the disease. Test sensitivity fluctuates over time after exposure to SARS-CoV-2. For example, false negatives are 100% in the first 24 hours after exposure, decrease to 38% when symptoms appear, and drop to 20% by the third day of symptoms. A review of five studies with 957 suspected and confirmed COVID-19 cases indicated that false negative rates for RT-PCR ranged from 2% to 29%, emphasising the challenge of false negatives (Starr, T.N. et al., 2020).

RNA extracted from respiratory samples (nasopharyngeal or oropharyngeal swabs, sputum, lower respiratory aspirates, bronchoalveolar lavage, and nasopharyngeal or nasal aspirates) is reverse-transcribed into cDNA and then amplified via qPCR [Centres for Disease Control and Prevention, 2020]. This method utilises oligonucleotide primers and probes targeted to regions of the viral N gene [Centres for Disease Control and Prevention, 2020]. Upon annealing of the probe to its target sequence, Taq polymerase's 5′ nuclease activity degrades the probe, thereby the reporter dye separates from the quencher dye, resulting in a fluorescent signal [Centres for Disease Control and Prevention, 2020]. Fluorescence intensity increases proportionally with the cleavage of reporter dyes from their probes in each amplification cycle in response to the increased concentration of the amplicon [Corman, V.M. et al.,2020]. Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), diagnostic

accuracy and 95% Confidence Intervals were calculated. Some drawbacks of RT-qPCR include false-positive or false-negative results that can occur when a specimen is contaminated during improper collection [Centres for Disease Control and Prevention, 2020]. Validate and perform the run, and verify the integrity of the RNA extraction reagents. A specimen is confirmed positive for 2019-CoV reaction when the growth curves of all controls reach the threshold line in 35 cycles or fewer. For the E gene, both RdRp & ORF. Ultimately, RT-qPCR has become widely accepted as a gold standard for nucleic acid detection from various sources due to its accuracy, sensitivity, and decreased risk of contamination [Mackay, I.M. et al,2002]

There are commercially available one-step and two-step RT-PCR assays. One-tube and buffer are used for both RT-PCR processes in a single-step test, which combines reverse transcription and PCR amplification into a single reaction. RT-PCR in a single step can produce quick results, which is suitable for high-throughput diagnosis, and, by limiting sample management, may reduce the probability of human error and cross-contamination (Carter LJ et al., 2021). However, two-step RT-PCR takes longer, has lower detection limits, and offers better sensitivity (Al-Shanti N et.al.,2009).

2. Truenat

The small, lightweight, battery-operated TrueFacet real-time PCR test is chip-based. The gold standard test for diagnosis is polymerase chain reaction (PCR); however, in distant locations with inadequate infrastructure, it must be performed in a well-equipped biosafety laboratory at level II. (UdayGhoshal et.al.,2020)

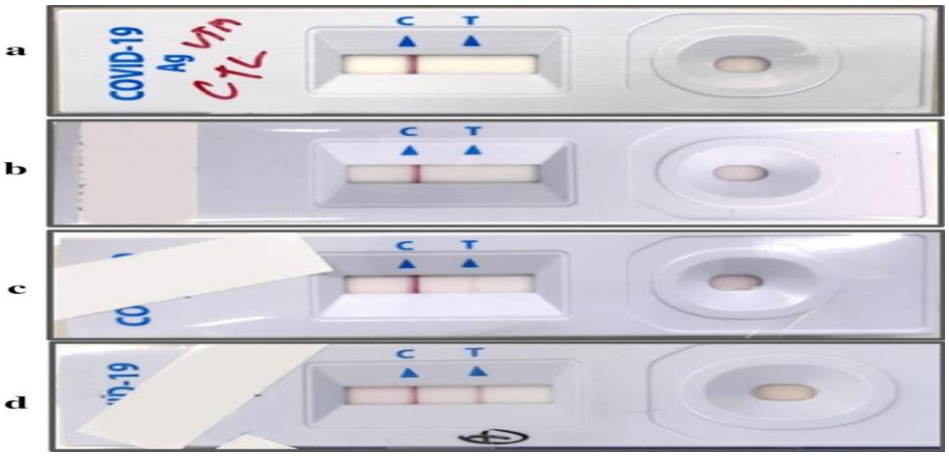
Principle:

TruenatTM SARS-CoV-2 is the working principle of Real-Time Reverse Transcription Polymerase Chain Reaction (RT PCR) based on Taqman chemistry. First, the RNA from the patient's sample is extracted using the Trueprep AUTO/AUTO v2 Universal Cartridge-based Sample Prep Device and Trueprep AUTO/AUTO v2.

If the sample tests positive for beta-CoV, 6 µL of the extracted RNA is used. The RT enzyme changes this RNA into complementary DNA (cDNA) after more heat cycles. In the TruNAT SARS-CoV-2 test, a special probe fluoresces when the test is positive, and a curve appears on the screen during the test. The Cycle threshold (Ct) is the number of cycles needed for the light signal to be stronger than the background. If there is more target nucleic acid in the sample, the Ct level is lower. After the test, the internal positive control (IPC) checks if the test is valid. The IPC goes through all steps, confirming the test from start to finish. If a sample is negative, the test is invalid if the IPC Ct is missing or not in the valid range. Even if the IPC does not amplify in some positive cases with a high target load, the test is still valid. Results can be downloaded to a computer and then printed. The analyser can store up to 20,000 results for future use. In a small study, this test showed 100% accuracy compared to the standard RT-PCR test. TruNAT may not process as many samples as traditional PCR, but it is portable, affordable, easy to use, and simple to understand. This makes it a better choice for checking and confirming COVID-19 in poorer countries. The TruNAT testing program has helped in spreading and controlling COVID-19 in the communities. TruNAT gives results in one hour, while RT-PCR takes 10–12 hours. It also costs less per sample. The sample is collected in a solution that breaks down the virus, making it safe, and no special safety equipment is used. This makes it a great test for the lab's limited facilities. This study has some limitations, such as TruNAT uses a throat swab, while RT-PCR uses a medium with both throat and nose swabs. Both samples were taken at the same time; there would be a 12 to 24-hour delay between them.

3. Rapid test for SARS-CoV-2 antigen detection

The Standard COVID-19 Ag test is a rapid test to find the SARS-CoV-2 N antigen in respiratory samples. The Mylab CoviSelf test kit uses a simple method called lateral flow. It has five parts: a nasal swab, a tube with liquid, a test card with a QR code, a biohazard bag, and instructions. The test card has two lines: a test (T) line and a control (C) line. The control line is coated with a special mouse antibody, and the test line is coated with a mouse antibody against the SARS-CoV-2 N antigen. The test detects the SARS-CoV-2 N antigen using these antibodies with colour particles. The test line shows colour based on the quantity of antigen present in the sample. For data, they showed mean, standard deviation, median, and range for continuous data. They also possess a 95% confidence interval and percentages for categorical data. They calculated positive predictive value, negative predictive value, sensitivity, and specificity using an online tool. Here, a rapid kit is used for SARS-CoV-2 antigen in respiratory samples. 200µL of nasopharyngeal and throat swab samples were added to the extraction buffer. A filter nozzle is used to close the tube, and three drops are added to a test device. Results were read in 15 to 30 minutes. If positive, two-colored lines appeared: test (T) and control (C).



4. Serological Immunoassay

Here, the ELISafe 19™ kit is an Indirect ELISA manufactured for the qualitative measurement of anti-COVID-19 IgG in human serum/plasma. A human serum/plasma sample (1:100 dilution) is added to the microwell plate pre-coated with the S1 recombinant protein of SARS-CoV-2. After incubation, the unbound protein matrix is removed by washing. A horseradish peroxidase (HRP) coupled with anti-human IgG is added to each well. After the incubation period, an immunocomplex of "COVID-19 recombinant S1 protein–human anti-COVID-19 IgG antibody-HRP labelled anti-human IgG tracer antibody" is formed if there is specific SARS-CoV-2 IgG antibody present in the test sample.

The unbound antibody is removed by washing. Then, the HRP-labelled conjugate bound to the microwell. Finally, a chromogenic substrate solution is added and incubated for 20 minutes, followed by the addition of the stop solution. The plate is then read at 450nm. The kit has been validated in patients reported positive for Covid-19 by real-time PCR against the appropriate negative serum/plasma samples. The Sensitivity of the assay is 99% and the Specificity of the assay is 100%. Hence, Serological testing has many advantages over PCR due to its high throughput, reduced workload, and rapid detection [Amanat, F.et.al.,2020]. Helps healthcare personnel identify those who are already immune and can return to work. This will minimise the risk of spreading the virus to colleagues and other patients. Helps in detecting the IgG immune status of COVID-19 recovered patients, and hence their plasma might be used for further therapeutic purposes.

Limitations of the assay: A False-negative result is also possible if Blood samples are collected in the early phase of the onset of infection (within one week after infection onset), and a False-positive result if the Suspect/patient is previously infected with SARS or other strains of Coronavirus or has sticky serum/plasma or autoimmune disease.

Discussion

The COVID-19 pandemic is constantly affecting the day-to-day life of the healthcare systems. It is important to diagnose quickly and accurately. There is a high demand for the viral test to check the body's immune response. This includes testing people who show symptoms and those who do not, as well as their close contacts.

At VRDL, MGM Medical College in Jamshedpur, approximately 7,00,000 samples were tested using RT-PCR, 1,000 samples with Rapid Antigen tests, and 130 COVID-19 Antibodies were tested. Around 300,000 tests were done using the Truenat method at MGM Medical Hospital, Jamshedpur. This Truenat increases the testing capacity and can reduce the lab workload.

Real-time PCR is more efficient in labs, but TruNAT is useful in situations where immediate results are needed. This includes emergency surgeries, maternity wards, and screening patients before procedures like dialysis or chemotherapy.

Truenat also helps confirm suspected cases alongside genetic testing. These tests provide information about how the immune system responds to viruses, and the duration of immunity is valuable for the diseases and those in vaccine trials. These test results can support public health decisions regarding routine life.

Summary and Conclusion:

In January 2020, the WHO declared the pandemic a global emergency. By March 2021, there were over 110 million COVID-19 cases and more than 2.5 million deaths, making it one of the deadliest pandemics in history. This paper discusses laboratory techniques that are more affordable and efficient. It reviews serological, immunological, and molecular tests designed to detect COVID-19. Other nucleic acid tests are under development, but RT-PCR remains the primary method for diagnosing viral RNA. While many tests are still being researched, the number of COVID-19 tests authorised under the FDA EUA is increasing. New testing kits aim to deliver faster results, reduce reliance on expensive laboratory equipment, and minimise times. Despite various challenges, global efforts continue to support these advancements in diagnostic testing. The COVID-19 testing market is expected to expand as more data, resources, and expertise become available.

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