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Current Approaches to Detect COVID -19, Limitations and Challenges

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Nikita Harekrishna Gurav

Asst. Prof. Dr. Shivajirao Kadam College of Pharmacy, Kasabe Digraj, Sangli 416305.

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ABSTRACT

At the end of 2019, a novel virus from corona family SARS-CoV-2 began generating captions all over the world because of the unprecedented speed of its transmission. It has been already witnessed the transmission of SARS-CoV-2 is symptomatic as well as non- symptomatic (silent carrier). In early 2020 within 2-3 months it became epidemic all over the world and leads to thousands of death with 2-5% mortality rate. Early detection of infection following proper preventive measures is the only way to prevent transmission of this SARS-CoV-2 since no proper treatment for COVID-19 is established yet. The aim of this article is to update about COVID-19 infection, existing methods of detection and their mechanism, such as current approved methods of diagnosis of COVID-19 are RT-PCR and serology tests, limitations of current methods including challenges. As well as ongoing developments to overcome the limitations to meet the challenges. The article also shortlisted the preventive measures and management of SARS-CoV-2 epidemiological crisis.

INTRODUCTION

In December 2019, a novel life-threatening virus from coronavirus family erupted from Wuhan city, China. [6] The viruses possess spikes like protein coat seems like king's crown as a result virus named in Latin term Coronavirus. Coronavirus is a larger family of viruses that cause symptoms identical to Severe Acute Respiratory Syndrome (SARS); discovered in China in 2002, and the Middle East Respiratory Syndrome (MERS) was first reported in Saudi Arabia in 2012.[1] Initially, the virus tentatively named as '2019 novel coronavirus' (2019-nCoV). Other names attributed as 'severe acute respiratory syndrome coronavirus 2' (SARS-CoV-2) by the International Committee of Taxonomy (ICTV) and 'COVID-19 virus' by WHO.[2] The infection caused by this is termed as 'coronavirus disease 2019' (COVID-19).[2] Like zoonotic SARS and MERS SARS-CoV-2 also have been caused by spillover of animal coronavirus to human.[2] It is considered coronavirus likely originated from bats.[3] Coronavirus affects both animals and humans. In animal, the virus may turns into a new virus that affects humans that may be more lethal forms of coronavirus since they can lead to life-threatening pneumonia.[1] While in human it causes common cold, severe acute respiratory syndrome.[1, 2, 3]

COVID-19 is more contagious and less sever compared to SARS and MERS,[1,3] Leading cell reservoir for SARS-CoV-2 invasion in the lung tissue are alveolar epithelial cells where angiotensin converting enzyme II (ACE II) is leading host cell receptor for SARS-CoV-2 results in diffuse alveolar damage and hyaline membrane formation, the most frequent complication of COVID-19. CD4 T and CD8 T lymphocytes are more frequently reduced in patient with COVID -19.[3] The risk of environmental contamination from patient with SARS-CoV-19 is considered to be possible.[3] Also, there are 2-5% risk of infection with it among health care personnel.[3]

The specific symptoms of COVID-19:[3,6,7]

- Fever (88%)
- Dry Cough (67%)
- Fatigue (38%)
- Phlegm production (33%)
- Shortness of breath (19%)
- Muscle pain (15%)

- Sore throat (14%)
- Headache (14%)

The symptoms may outbreaks within 2 to 14 days (incubation period) after being infected.[4] According to WHO 60% people do not shown symptoms of COVID -19 even though infected with coronavirus such people termed as 'silent carrier' of coronavirus.[4] It clears that risk of human to human transmission of SARS-CoV-2 occur in both symptomatic and non-symptomatic phases.[1,4] A person with laboratory confirmation of SARS- CoV-2 infection irrespective of sign and symptoms defined confirmed case of COVID-19.[3]

The suspect case of COVID- 19 defined by the World Health Organization (WHO) as a patient with any acute respiratory illness and have been in contact with a confirmed or probable COVID-19 case in the last 14 days prior to onset of symptoms.[3]

COVID-19 threat is higher for infants, children and people over the age of 60 and with diabetes, cancer and cardiovascular disease since they have low immunity as compare with young.

From December 2019 to end of February 2020 contagion with coronavirus became epidemic. Almost 200 countries and territories around the world infected with COVID-19. All updates with regards to the disease monitored by the Centers for Disease Control (CDC) and the WHO.[1]

The epidemiological link may involve[1]

- Travel to an area that experienced an outbreak,
- Close contact with an individual with a confirmed or high risk of infection, or
- Close contact with an individual with respiratory symptoms who had been in a geographic location that witnessed an outbreak of the infection.

Following several generations of spread with a country, local transmission of disease occurs, and patients may present with no history of travel to a location with a known outbreak. But even with widespread community transmission, testing can help track the disease, especially as it penetrates into lesser-hit regions.

The infection of COVID -19 can be detect by laboratory tests fall into two categories. [1,2,9]

1. **Molecular tests** which detect active infection.

2. **Serology tests** which detect antibodies. Serology tests are for surveillance or investigational purposes not for diagnostic purposes.

The first step in any coronavirus test is collecting a sample from upper and lower respiratory specimens such as[1,12]

- **Swab** – In this case, a special swab is used to take a sample from nose or throat.

- **Nasal aspirate** – In this case, a saline solution will be injected into nose and, then a sample is taken with a light suction.

- **Tracheal aspirate** – In this case, a thin tube with a torch, also known as a bronchoscope, is put into your mouth to reach lungs from where a sample is collected.

- **Sputum** – Sputum is thick mucus that gets accumulated in the lungs and comes out with a cough. During this, patient required to cough up sputum in a special cup or a swab is used to take a sample from nose.

- In case of serology test **blood sample** – In this case, a blood sample is taken from a vein in the arm.

- Additional clinical specimens may be collected as COVID-19 virus has been detected in blood and stool,[2]

- Autopsy material including lung tissue in case of patients who are deceased.[2]

The collected swab should be placed into separate sterile tube containing 2-3 ml of viral transport media refrigerated at 2-8 ºC. In case of delayed analysis the center for disease control and prevention CDC recommends that nasopharyngeal and oropharyngeal swab should be kept at 2-8 ºC <4 days or Specimens may be frozen to - 20°C or ideally -70°C and shipped on dry ice if further delays are expected.[1,2]

Specimen rejection criteria[2,3]

- Specimens not kept at 2-8 ºC (<4 days) or frozen at -70 ºC or below
- Incomplete specimen labelling or documentation
- Inappropriate specimen type
- Insufficient specimen volume
- Repeated freezing and thawing of specimens

1. Molecular test[2]

Nucleic Acid Amplification Tests (NAAT) such as real time Reverse Transcription Polymerase Chain Reaction rRT-PCR is the reference technique for the etiological diagnosis of SARS-CoV-19 infection. The test kit, called the Centers for Disease Control and Prevention (CDCP) 2019-Novel Coronavirus (2019-nCov) Real-Time Reverse Transcription (RT)-PCR Diagnostic Panel (CDC 2019-nCoV Real Time RT-PCR).

In this test, technician extracts viral genetic material called RNA from the sample and uses it to produce a complementary strand of DNA that the rRT-PCR technique amplifies, or makes thousands of copies of, to get a measurable result.

The full genome of the novel coronavirus was published on 10th January 2019, just weeks after the disease was first identified in Wuhan, China.[13] A week later, a group of researchers led by German scientists released the first diagnostic protocol for COVID-19 using swabbed samples from a patient's nose and throat; this PCR-based protocol has since been selected by the WHO. The laboratory at Westmead Hospital also does a complete sequencing of every virus sample to look for possible new strains of SARS-CoV-2 and has shared some of those sequences in the international Global Initiative on Sharing All Influenza Data (GISAID) database for other researchers to study. The staff also cultured the virus and imaged it using electron microscopy.[13]

The quick sequencing of the SARS-CoV-2 genome and distribution of the data early on in the COVID-19 outbreak has enabled the development of rRT-PCR to diagnose patients based on snippets of the virus's genetic code.

General steps involved in detection SARS CoV19 infection by rRT-PCR[3]

1. RNA isolation from upper and lower respiratory specimens
2. RNA purification
3. Reverse transcription of RNA to cDNA
4. cDNA amplification with real time PCR instrument
5. Fluorescent signal detection

When the samples are sent to a lab, where researchers apply chemicals to remove everything but the RNA. Enzymes are then added to transcribe the RNA into DNA. Next, this DNA is put into a real-time PCR (RT-PCR) machine along with another set of chemicals. The RT-PCR machine thermal cycle processes that essentially Xeroxes the DNA, making thousands of copies of any genetic material in the samples. Scientists then use sets of DNA fragments that complement fragments found in the coronavirus. If any viral genetic material is present, these fragments will bind to it. The viral genes targeted so far include the N, E, S and RdRP genes.[2,13] Chemical markers attached to the DNA release fluorescence when this DNA binding occurs. It's these flashes of fluorescence that scientists use to determine whether the virus is present in a sample.[10]

The accomplishment of rRT-PCR testing depends on several factors, including the experience and expertise of laboratory staffs, laboratory environment (e.g., avoidance of contamination), and the type and condition of specimens being tested.

Criteria for the patient to be considered as COVID-19 positive or negative by rRT-PCR according to WHO[2,11]

- A positive result for at least two different targets on the COVID-19 virus genome, of which at least one target is preferably specific for COVID-19 virus using a validated assay (as at present no other SARS-like coronaviruses are circulating in the human population it can be debated whether it has to be COVID-19 or SARS-like coronavirus specific); OR

- One positive result for the presence of β-coronavirus, and COVID-19 virus further identified by sequencing partial or whole genome of the virus as long as the sequence target is larger or different from the amplicon probed in the rRT-PCR assay used.

- CDC considers a person under investigation to be negative for active COVID-19 infection following one negative rRT-PCR test on the recommended specimens. Since a single negative result does not completely rule out SARS-CoV-2 infection, in some circumstances additional specimens may be tested.

- CDC considers a patient to be negative for active SARS-CoV-2 infection following two consecutive negative rRT-PCR tests on all specimens.

One or more negative results do not ensure to not have the COVID-19 virus infection.

Number of factors could lead to a negative result in an infected individual, including:

- Poor quality of the specimen, containing little patient material.
- The specimen was collected late or very early in the infection.
- The specimen was not handled and shipped appropriately.
- Technical reasons inherent in the test, e.g. virus mutation or PCR inhibition.

Limitations of rRT-PCR[10]:

1. rRT-PCR tests take few hours to complete.

2. Transporting samples to central labs takes time.

3. People has to be wait a week or more for test results as labs flooded with samples struggle to keep up.

4. rRT-PCR is that it detects only active infections. If someone has previously contracted the coronavirus and has recovered, rRT-PCR won't detect it.

5. If the viral load, (or the number of viral particles falls) below the level of detection for the test a false negative occurs with a PCR.

6. Detection of small sequence of gene resembling other virus gene belonging corona family may leads to false positive result.

7. Virus shedding patterns are not yet well understood and further investigations are needed to better understand the timing, compartmentalization and quantity of viral shedding to inform optimal specimen collection.[2]

Ongoing projects and future prospects in conventional RT-PCR test:

Some pharmaceutical companies and biotechnology institutes are developing modified version of the PCR tests to overcome the limitations of existing test. Few of them are extracted below:

- Development of method which quickly copying the genetic material in a sample so that any viral genes are detectable consists of small size equipment, a set of one-time-use cartridges that contain all the chemicals needed for the procedure. The patient gives a sample, usually via a nasopharyngeal swab, which is inserted into the cartridge. The cartridge goes into the testing device, which heats and cools it to facilitate the proper chemical reactions. The results come back in less than an hour.[10]

- Abbott is developing a machine can run large numbers of tests at a time, in 5 minutes, consisting standard 96-well plates, thus 96 samples can run at once. Thus, a point-of-care test might be able to provide quicker answers to individual patients.[10]

- CRISPR technology (Clustered Regularly Interspaced Short Palindromic Repeats) based diagnostic protocol developed by researchers at the McGovern Institute at MIT uses paper strips to detect the presence of a target virus and claims to take around one hour to deliver the result.[10,13]

- Anglo-French Biotech Company Novacyte is developing real-time PCR diagnostic kit for COVID-19, which it says will deliver results in two hours.[13]

- The US Centers for CDCP, has developed its own assay that looks for three sequences in the *N* gene, which codes for the nucleocapsid phosphoprotein found in the virus's shell, also known as the capsid. The assay also contains primers for the *RNA-dependent RNA polymerase* gene. The principles of testing are the same; just the genetic targets that vary.[13]

- The laboratory at Westmead Hospital also does a complete sequencing of every virus sample, developing a test which can confirmed with whole genome sequencing resulting in no false positive findings.[13]

- The biotechnology companies Roche Diagnostics, LabCorp and Thermo Fisher Scientific producing different commercial coronavirus RT-PCR kits. The primary difference from one kit to another is which coronavirus genes each test targets. CDC-approved kits target regions on a gene that codes for the protein that makes the virus's nucleocapsid, an envelope that houses its RNA.[12]

The major limitation of PCR technology is only it detect only active cases but getting a handle on the pandemic will require tests that can detect anyone who has ever been exposed to SARS-CoV-2 even if they fought it off without showing symptoms. A different diagnostics approach would be to devise blood tests for antibodies against the SARS-CoV-2 virus.

2. Serology test: [8,16]

Serology testing determines former infection in people who may have been exposed to the virus. Our body's immune system produces antibodies immunoglobulin M (IgM) and immunoglobulin G (IgG) to attack and kill viruses, bacteria, and other microbes during infection.[16] The presence of antibodies to SARS-CoV-19 point out that a person had been previously infected with the virus and developed an immune response.

For detection of antibodies to SARS-CoV-2 CDC has 2 phase approach in serology test[11]

1. **Screening test**: 2 tests of Enzyme-Linked Immunosorbent Assay (ELISA) for confirming presence and concentration of specific antibodies.

2. **Microneutralization test:** to confirm the positive result. It is a highly specific confirmatory test used to measure neutralizing antibodies or antibodies that can neutralize virus. However, compared with the ELISA, the microneutralization assay is labor-intensive and time-consuming, requiring at least 5 days before results are available.

Assessment of result:

- If a clinical sample is positive by either ELISA, or positive by microneutralization, the specimen is determined to be confirmed positive.

- If a clinical sample is positive by both ELISAs, and negative by microneutralization, the sample is determined to be indeterminate.

- If a clinical sample is positive by only one ELISA, and negative by microneutralization, the sample is determined to be negative.

- If a clinical sample is negative by both ELISAs, the sample is determined negative.

Rapid Detection Test (RDT)

Several academic laboratories and medical companies are working on RDT.[15] 5 out of 17 antigen detection and 27 out 53 antibodies have been selected for preparation of rapid diagnostic test.[17] SureScreen Diagnostics, for example has developed a testing strip to detect antibodies to the coronavirus in the blood; it works similarly as home pregnancy test, with a paper readout and a colored line to indicate infection.[10]

Such tests are relatively inexpensive and simple, usually using blood from a finger prick. Some can produce results in 10 to 15 minutes. That could make ramping up screening much easier than for diagnostic tests.[15]

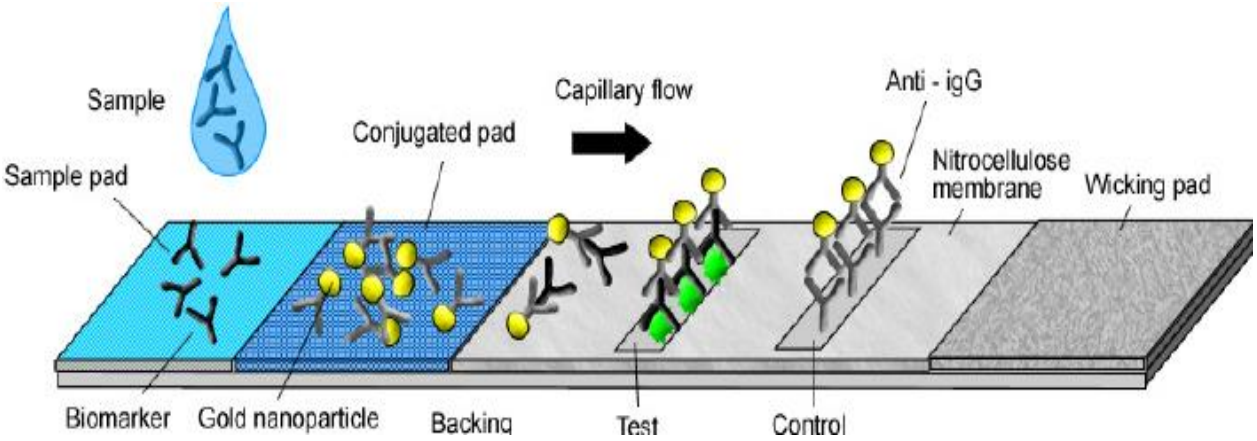

Figure No. : Typical rapid diagnostic test strip

Serology tests are for surveillance or investigational purposes and not for diagnostic purposes—they are tools developed in response to the SARS-CoV-2 outbreak. Information is limited about SARS-CoV-2 and how the virus is spread. As public health scientists learn more about SARS-CoV-2, the approach to conducting these types of laboratory tests might change.

Limitation of serology test:

Rapid antibody testing likely won't help detect cases early, as body takes typically 5-8 days to produce antibodies response to the virus.[10]

Other Laboratory abnormalities predicting severe COVID 19[3]:

Increased:

↑ Neutrophil count

↑ Lactate dehydrogenase (LDH)

↑ Alanine aminotransferase (ALT)

↑ Aspartate aminotransferase (AST)

↑ Total bilirubin

↑ Creatinine

↑ Cardiac biomarker

↑ D- dimer and C- reactive protein (CRP)

↑ Prothrombin time (PT)

↑ Procalcitonin indicate bacterial superinfection in patient.

Decreased:

↓ Lymphocyte count

↓ Albumin

Typical Histological Finding[3]

- Cellular fibromyxiod exudate
- Formation of hyaline membrane

Treatment of COVID-19[1]:

There are no specific treatments for the coronavirus infection, however, the healthcare professional may suggest some steps that'll help you to relieve the symptoms. Some of the steps can follow to ease the symptoms are:

- Drinking a lot of fluids
- Getting plenty of rest
- Taking over-the-counter medicines
- In case condition gets worse or show signs of pneumonia, the patient should get admitted to a hospital. Some of the common symptoms of pneumonia are severe cough, labored breathing, and high fever.
- If the patient diagnosed with coronavirus, one should follow the below steps to prevent any spread of infection, don't leave home unless to get medical help.
- Always wear a face mask in public places or when you're around other people.
- Don't share any personal items such as drinking cups, eating plates, towels, or any other items with anybody.
- Always wash hand thoroughly for around 20 seconds. In case soap water is not available, can use an alcohol-based sanitizer, which at least contains 60% alcohol.
- **Convalescent plasma treatment** may be a future promising treatment, it is based on the simple principle that the antibodies formed can be isolated from the blood of recovered patients and then injected into patients who are infected, and the antibodies will start stimulating the sick patients' immune systems.[10,14]

Prevention or management of epidemiological crisis[3]

- Major investments on laboratory resources
- Reinforcement of regional networks of clinical laboratories
- Installation of mobile laboratories
- Proactive establishment of laboratory emergency plan

CONCLUSION

Zoonotic SARS-CoV-2 is more contagion and human to human transmission of SARS-CoV-2 occur in both symptomatic and non-symptomatic phases. Effective reproductive number is higher and pathogenicity is lower for COVID 19 than SARs and MERS. Early detection of infection is the only way to minimize the spread of COVID-19 and prevent to become epidemic. Many biotechnology and pharmaceutical companies rolling out molecular and serological test for COVID-19 but yet not have reach up to the mark. The existing tests methods has many limitations. The government must invest to encourage the scientist to make much sophisticated test method which will be more trustworthy, simple and in lower cost.

REFERENCES

1. Coronavirus testing – how to test for Coronavirus? – Different types of tests, Narayan Health, March 13, 2020. https://www.narayanahealth.org/blog/coronavirus-testing-how-to-test/
2. Laboratory testing for coronavirus disease 2019 (COVID-19) in suspected human cases. World Health Organization, Interim guidance. 2 March 2020.
3. https://area9lyceum.com/covid19_course/
4. Mould Josephine, 23 Mar 2020People with mild or no symptoms could be spreading COVID-19, https://www.weforum.org/agenda/2020/03/people-with-mild-or-no-symptoms-could-be-spreading-covid-19/
5. Early Release - Severe Acute Respiratory Syndrome Coronavirus 2 from Patient with 2019 Novel Coronav, Centre for Disease Control and Prevention, https://wwwnc.cdc.gov/eid/article/26/6/20-0516
6. Symptoms of Coronavirus, Centre for disease control and prevention, https://www.cdc.gov/coronavirus/2019-ncov/symptoms-testing/symptoms.html
7. Syed Ashikujaman, Coronavirus: A Mini-Review, International Journal of Current Research in Medical Sciences, (2020). 6(1): 8-10.
8. Shipping of CDC 2019 Novel Coronavirus Diagnostic Test Kits Begins https://www.cdc.gov/media/releases/2020/p0206-coronavirus-diagnostic-test-kits.html
9. CDC test for COVID-19, Center for Disease Control and Prevention, https://www.cdc.gov/coronavirus/2019-ncov/about/testing.html
10. Pappas Stephanie, 1 April 2020, Coronavirus testing is ramping up. Here are the new tests and how they work. https://www.livescience.com/coronavirus-tests-available.html
11. CDC Laboratory Testing for Middle East Respiratory Syndrome Coronavirus (MERS-CoV) https://www.cdc.gov/coronavirus/mers/lab/lab-testing.html
12. Daley Jim, 27 March 2020, Here's How Coronavirus Tests Work—and Who Offers Them, PCR-based tests are being rolled out in hospitals nationwide, and the Food and Drug Administration is fast-tracking novel approaches as well. https://www.scientificamerican.com/article/heres-how-coronavirus-tests-work-and-who-offers-them/
13. Nogrady Bianca, 3 March 2020, Current methods to detect infections of the novel coronavirus rely on identifying particular genetic sequences, but new assays are being developed to meet the growing demand for rapid answers, https://www.the-scientist.com/news-opinion/how-sars-cov-2-tests-work-and-whats-next-in-covid-19-diagnostics-67210
14. Fatima Amanat, Thi Nguyen, Veronika Chromikova, Shirin Strohmeier, Daniel Stad Bauer, Andres Javir, Kaijun Jinag, Guha Astagiri, Aeunkumar, Jose Polanco, Maria Bermudez Gonzalez, Daniel Caplivski, Allen Cheng, Jussi Hepojoki, Viviana Simon, Florian Krammer, A Serrological assay to detect SARS-CoV seroconversion in humans, **doi:** https://doi.org/10.1101/2020.03.17.20037713

15. Reuters, March 27, 2020 U.S. Companies, Labs Rush to Produce Blood Test for Coronavirus Immunity, https://www.nytimes.com/reuters/2020/03/27/business/27reuters-health-coronavirus-immune-test-insight.html
16. Joseph Andrew, 27 March 2020, The Next Frontier In Coronavirus Testing: Identifying The Full Scope Of The Pandemic, Not Just Individual Infections, https://www.statnews.com/2020/03/27/serological-tests-reveal-immune-coronavirus/
17. Available from: https://www.finddx.org/covid-19/sarscov2-eval-immuno/