COMMENTARY

Laboratory Diagnosis, Safety and Testing Strategies of Novel SARS COV 2

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Abstract

The COVID 19 Pandemic is the most defining health care crisis of the present times. It has challenged the health care facilities, overwhelmed the health care personnel and baffled the scientists and researchers. There is no quick fix in a pandemic of this proportion. The past four months has seen many new aspects of this disease, and newer evolving strategies to rein in the pandemic. This commentary seeks to deal with the various aspects of laboratory diagnosis, safety and testing strategies adopted by different countries.

Keywords

Laboratory Diagnosis; Safety; Testing Strategies; Novel SARS COV 2

Novel Coronavirus, SARS-COV2 (Severe Acute Respiratory Syndrome- Corona Virus 2) belongs to the beta-coronavirus, the same family as that of MERS-COV (Middle East Respiratory syndrome-Corona Virus) and SARS- COV. It first originated in Wuhan city, Hubei province of China in December 2019. Hence the infection is named as COVID-19 (Corona Virus Disease). The Corona virus first was considered to be transmitted from animal to human from the live animal market, and from then on human to human transmission occurred resulting in spread of the disease globally. On March 11 2020, WHO declared COVID 19 as a pandemic as by then it had 118,000 cases in 114 countries and 4291 people had lost their lives.(1,2) In January 2020, genetic sequencing identified the virus among the initial cluster of cases as novel SARS-COV2.(3)

The aim of this review is to understand the laboratory diagnosis of COVID 19 and also to assess the various strategies of testing adopted by various countries across the world. The review also addresses the safety protocols for processing of samples in laboratories during the COVID 19 pandemic.

Laboratory Diagnosis of COVID 19

The laboratory tests designed in the diagnosis of COVID 19 is direct demonstration of virus or its antigens, detection of specific antibodies against the SARS CoV 2 and detection of the viral nucleic acid by molecular testing.

a. Molecular methods: In the present times, viral nucleic acid detection by real time reverse transcriptase PCR is widely advocated. Corona virus is a positive sense RNA virus. There are multiple genomic sites which code for specific structural and non-structural proteins of the virus. The structural proteins being envelope glycoproteins spike (S),

envelope (E), transmembrane (M), helicase (Hel), and nucleocapsid (N) and non-structural proteins RNA-dependent RNA polymerase (RdRp), hemagglutinin-esterase (HE), and open reading frames ORF1a and ORF1b(4). Different countries or the research bodies have aimed at targeting at least two proteins to increase the sensitivity and specificity of the testing protocol. A flocked swab is used to collect the specimen from the nasopharynx which is transported in Viral Transport medium to the designated testing centres. In a study by Wang et al, Bronchoalveolar lavage fluid specimens showed the highest positive rates (14 of 15; 93%), followed by sputum (72 of 104; 72%), nasal swabs (5 of 8; 63%), fibrobronchoscope brush biopsy (6 of 13; 46%), pharyngeal swabs (126 of 398; 32%), feaces (44 of 153; 29%), and blood (3 of 307; 1%)(5). Recently, Cartridge Based Nucleic Acid amplification tests have been described. These are self-enclosed systems which integrate extraction, amplification and detection into a 45 minute to 1-hour process. The samples in the VTM is transferred in the cartridges and this is then loaded in the instrument for testing.

The limits of detection of the various molecular tests vary from 1 to 10 copies per reaction.

False negative results could occur due to poor sample collection, degradation of the VTM during transport or the very small amounts of virus present in the sample in early disease. False positives due to contamination is rare when using RT PCR and when two assays are performed.

b. Serological tests(6): Specific IgM and IgG detection in infected patients is useful to determine past infection or immunity in individuals. These tests are not very useful in the early detection of the infection. The tests are usually positive by day 7 – 10 after the patient is symptomatic. These tests also help to detect the immune status in asymptomatic individuals. The detection of specific antibodies is very helpful in the infection prevention protocols and to determine the discharge policy of patients admitted in the health care setups. Commercial kits have been approved for use.

c. Virus culture: SARS CoV 2 can be grown on primary monkey cells and cells lines such as Vero and LLC-MK2 (Lilly Laboratory cell- Monkey Kidney 2) and human airway epithelial cell lines(7). This is not used routinely in the diagnosis due to the prolonged time, biosecurity concern, cost and the less number of laboratories rolling out this facility. Viral cultures are important in case of research and development of vaccines and the therapeutic options for treatment.

d. Rapid antigen detection kits: These tests help in the early detection of the viral antigens in the samples. Research is underway for development of monoclonal antibodies production and to understand the disease pathology to determine when the viral titres are high which determines the sensitivity and specificity of the tests. The sensitivity of these tests may vary between 30 – 80% (8).

Safety for handling samples in COVID-19 suspect / confirmed cases

Specimen collection: The samples must be collected by personnel who are trained for collection, packing and transport of specimen appropriately as per WHO guidelines. All personnel collecting nasopharyngeal swab should be wearing PPE which includes, N95 mask, Face shield, Gown, Gloves. All COVID-19 suspicious/confirmed specimens must be labelled clearly. The specimens must be transported in triple layer packing with biohazard label. Risk assessment has to be done at all stages and appropriate measures has to be taken on site.(9)

Laboratory Safety: All samples should be considered potentially infectious (Table 1)

Centrifugation

All manipulations of potentially infectious materials, including those that may cause splashes, droplets, or aerosols of infectious materials (e.g. loading and unloading of sealed centrifuge cups, blending, vigorous shaking, vortexing or mixing) however, should be performed by trained personnel in BSC 2.

Centrifugation of specimens with infectious potential must be performed using sealed centrifuge rotors or sample cups which are loaded and wait for 5 minutes after complete stoppage of the machine for aerosols to settle down. Any breakage in the centrifuge should be cleaned after 30 minutes as aerosols would have settled and the cleaned and disinfected with 70% Isopropyl alcohol.

Cleaning and disinfection: Since it`s an enveloped virus like MERS-CoV, and persistence of Novel Coronavirus on inaminate objects like glass, metal, plastic ranges from 4 to 9 days, the disinfectants approved are sodium hypochlorite (bleach) (e.g. 1,000 ppm (0.1%) for general surface disinfection and 10,000 ppm (1%), 62-71% ethanol, 0.5% hydrogen peroxide, quaternary ammonium

compounds and phenolic compounds, should be used according to manufacturer's recommendations(9).

The rational for appropriate use of PPE, its disposal and proper hand hygiene plays a very key role in containing the transmission of this novel CoV.

Testing Strategies for COVID 19

Testing strategies are needed to address the uncertainty in making decisions like patient treatment, resource allocation and policy making to control the pandemic. The strategy for lab testing varies from country to country and even state to state depending on the rate of transmission.

The WHO has defined four transmission scenarios and outlined critical priority actions for preparedness, readiness and response actions for COVID -19. The document classifies the time line of disease in any geographic area as no cases, sporadic cases, cluster of cases and community transmission. Depending on the stage experienced the testing strategy should be changed to meet the demands of diagnosis, quarantine criteria, infection prevention/ control, discharge policy and to determine the spread of the infection in the population(11). The different testing strategy may have to be followed within the same country depending on the geographic area.(Table 2)

Summary

Diagnostic testing for COVID-19 is critical to tracking the virus, understanding epidemiology, informing case management, and to suppressing transmission. Testing for COVID 19 forms the core of managing the disease, controlling its spread, and addressing containment and mitigation plans. SARS-CoV 2 is known to be transmitted from human to human either through droplet or via contact with infected individuals or fomites containing the virus. It is hence necessary to have well trained staff and proper measures to handle the specimens from COVID-19 cases. Early extensive detection in contacts/ suspects/patients, isolation and treatment seems to be the answer to contain the infection and flatten the curve.

Authors Contribution

All authors have contributed equally.

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Tables

TABLE 1 PRECAUTIONS TO BE TAKEN WHILE PROCESSING LABORATORY SPECIMENS DURING EPIDEMIC

Type of specimen	Procedure	PPE/ Precautions
Respiratory specimens, tissue, pus, Blood & body fluids, urine, stool	Microbiology and cytology investigations	Biological Safety Cabinet 2. Apron, Gloves, Surgical mask. Procedures such as virus cultivation and handling high virus load should be performed only by trained staff in BSL-3 containment and complete PPE gear (Gown, gloves, goggles, face shield, N95 mask, shoe covers)(9)
Plasma/ Serum	Haematology, Biochemistry and serology	Long sleeve apron, and Gloves. Peripheral smear/QBC for malaria must be processed in Biosafety cabinet.
Fresh Frozen samples from suspected/confirmed COVID	Histopathology	Fresh frozen section should not be performed on suspected/ confirmed COVID patients
Specimen received in Formalin	Histopathology	Gloves, apron, Surgical mask. External surfaces of specimen *containers must be decontaminated* using a disinfectant (70% alcohol/ 0.1% Sodium hypochlorite). The fresh or partially fixed specimens must be handled, opened in BSC type II if needed and *transferred to another formalin filled container* for further fixation in order to be grossed as formalin- fixed routine cases. Allow it to fix for 24 hrs, after which grossing and processing can be done. The SARS COV 2 gets fixed in 24 hrs with formaldehyde and 48 hrs in glutaradehyde(10)

TABLE 2 SUMMARY OF STRATEGIES FOLLOWED BY DIFFERENT COUNTRIES TO DETECT THE INFECTION.

Sl no	Country	Testing strategy	Remarks
1	South Korea	The molecular testing was provided to all the infected and the suspected individuals. Repeat tests were done on contacts and suspects during the quarantine when in doubt.	The suspects who were negative were quarantined for 14 days and monitored for any signs and symptoms of the infection. They kept their borders open but did extensive testing.
2	Germany	The molecular testing was provided to all the infected and the suspected individuals	The quarantine of the positive cases specially the young adults helped to contain the spread. Now the administration is advocating the use of serological tests.
3	United Kingdom	The molecular testing was exclusive for the people with symptoms who visited the health care facilities and investigating specific outbreaks only.	This limited testing resulted in large spread among the contacts and also among the health care workers.
4	Italy	The molecular testing was done for the people with symptoms who visited the health care facilities.	Though the borders were closed the tests were done only in symptomatic. All infected were advised home quarantine which spread the disease among the family members.
5	USA	The molecular testing was exclusive for the people with symptoms who visited the health care facilities and investigating specific outbreaks.	This testing criteria did not encourage increase in the testing facility which limited the detection of the disease in the community
6	India	The molecular testing is done for all symptomatic, travel history, high risk contacts and suspects.	The contact tracing and quarantine and increasing the testing facility should be encouraged to limit the spread of the infection.