Laboratory Diagnosis of Covid 19 – A Review

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Abstract

The novel 2019 coronavirus disease (COVID-19) with its early origin in China has grown into a global pandemic.The infection occurs as a mild flu to severe acute respiratory infection and spreads from person to person through droplet infection.Diagnostic testing has been and will continue to be a big part of the 2019 (COVID-19) coronavirus pandemic. Early diagnosis is the key to timely case management and control of the spread of the virus. This article provides a clear understanding of the COVID19 biosafety steps, specimen collection, storage and transportation guidelines.It also addresses steps that can be taken by cytology laboratories to work during the pandemic and reduce the risk to their employees, trainees and pathologists.

Key Words: *COVID 19, pandemic, biosafety, laboratory, samples*

Introduction

Coronavirus disease 2019 (COVID-19) is a serious acute respiratory infection caused by the novel coronavirus severe acute respiratory syndrome coronavirus 2 (SARSCoV-2), which began initially as a cluster of cases from Wuhan[1].

The World Health Organization declared COVID-19 as a global pandemic on march 11,2020. The disease primarily spreads via close contact of respiratory droplets generated by infected individuals[1]. At the global level, sufficient testing capacity for COVID-19 is not available as it should be and therefore preventing individuals from accessing care[1]. During the initial outbreak period, different countries have followed and implemented various testing strategies, depending on the availability of diagnostics and consumables[1]. However, strict steps taken by the WHO has made the diagnostic available with the mission to "detect, protect and treat" to break the chain of transmission of SARS-CoV-2 (WHO 2020d)[1]. Therefore, early diagnosis and prompt treatment can substantially reduce the number of prospective cases. Hence, laboratory diagnosis of SARS-CoV-2 holds the key in containing and restricting the COVID-19 pandemic[1].This article gives information on sample collection, packing and transport to laboratory for COVID-19 testing[2].

As a part of the preventive strategies, many public health care measures, especially social distancing, have been recommended to the masses[3]. Health care facilities have recommended minimizing the nonessential hospital visits and prioritizing the elective inpatient and outpatient procedures[3]. The basic aim of these recommendations is to limit the spread of the disease among the general public as well as among the Health Care Workers(HCW)[3]. If consistently followed, these measures can lead to less chance of exposure to potentially infectious samples, reduced workload on the lab facilities of a hospital, thereby allowing for convenient reallocation of the laboratory personnel in teams[3].

The World Health Organization (WHO) and the Center for Disease Control and Prevention (CDC) have recommended basic interim guidelines to be adopted for the working of laboratories during the current pandemic[3].

CLINICIAN'S RESPONSIBILITY:

· To identify the appropriate patient for COVID-19 testing[2].

· To identify the appropriate specimen to be collected[2].

LAB TECHNICIAN:

Should have received appropriate training for sample

collection from suspected SARS/COVID-19 cases

· To collect appropriate samples as decided by the treating doctor[2].

· To wear proper PPE as prescribed by NIV Pune[2].

· To strictly follow biosafety measures during collection and packing of samples and to follow the biomedical waste management guidelines for safe disposal of generated bio-waste[2].

All Institutional Biosafety Committee's (IBSCs) must assess the available facilities, trained manpower in handling high risk group (RG3 and above) hazardous microorganisms, personal protection equipment (PPE) and waste disposal mechanism to meet all the requirements prior to initiation of work involving COVID-19[4].

SELECTION OF PATIENT:

Any person who presents with Severe Acute Respiratory Illness (SARI) AND any one of the following i.e. a history of travel from Wuhan, China in 14 days prior to symptoms onset; disease in healthcare worker working in an environment of SARI patients; unusual or unexpected clinical course, especially sudden deterioration despite appropriate treatment; should be urgently investigated[5].

TYPES OF CASES:

· People who are in close contact with suspicious exposure have been advised under a 14-day health observation period that should be started from the last day of contact with infected individuals[1].

· Once these individuals show any symptoms including coughing, sneezing, shortness of breath or diarrhoea, they should require immediate medical attention[1].

· Immediate isolation of the suspected individual should be performed with proper guidelines, and they should be closely monitored for clinical symptoms and diagnosis should be performed in hospital-based laboratories as soon as possible[1].

SUSPECTED CASES:

· Patient with acute respiratory illness, with no etiology that fully explains clinical presentation and a history of travel to or residence in a country /area or territory reporting local transmission of COVID19 disease during the 14 days prior to symptom onset[1].

· A patient with any acute respiratory illness and having been in contact with a confirmed or probable COVID 19 case in the last 14 days prior to onset of symptoms[1].

· A patient with severe acute respiratory infection and requiring hospitalization and with no other etiology that fully explains the clinical representation[1].

PROBABLE CASE:

· A suspect case for whom testing for COVID19 is inconclusive[1].

· A suspect case for whom testing could not be performed for any reason[1].

CONFIRMED CASE:

A person with laboratory confirmation of COVID 19 infection, irrespective of clinical signs and symptoms[1].

SAFETY MEASURES FOR INVESTIGATOR:

Samples should be collected by well-trained healthcare personnel, donning proper personal protective equipment (PPE) which includes masks, gloves, goggles, gown, head cover, shoe cover and hand sanitizer, soap and water following adequate infection control measures including hand hygiene and adequate biosafety precautions to protect individual and the environment[1].

The correct sequence of donning the PPE:

• Home clothes, jewellery, watches, rings, bangles, etc. should be removed and hospital scrub suite should be donned[1].

• Proper hand hygiene to be done using alcohol-based hand rub or soap and water prior to donning of the PPE[1].

• Sequence of donning PPE: Shoe cover, clean and

disposable non-permeable gown, N95 respirator with proper fit testing, Eye goggles/face shield, Head cover, Gloves[1].

The correct sequence of doffing the PPE:

• Doffing should be done only in designated areas[1].

• Any soiling in the PPE must be checked before doffing. If any, the area should be disinfected before doffing[1].

• Hand hygiene must be followed after every step[1].

• Sequence of doffing the PPE: Shoe cover, Gloves, Eye goggles/face shield, Head cover, Gown ,N95 respirator[1].

All the PPE must be disinfected and discarded following the local biomedical waste management rules.

STEPS TO BE FOLLOW UP DURING SPECIMEN COLLECTION:

MATERIALS[2]:

- Viral transport medium
- Polyester tipped plastic shaft swab
- Flocked Swab
- Tongue depressor Disposable
- Face mask N95
- Disposable apron
- Disposable Nitrile Gloves
- Hand sanitizer Alcohol based rub
- Biohazard Discard bag
- Thermocol box
- Gel packs
- Head cap Disposable
- Reusable lab goggle
- Brown tape
- Sealing the sample box
- Surgical spirit
- Disinfect sample coollection area
- Cryo label

VTM Precautions:

- Ready to use VTM to be used within the expiry date. Always check for turbidity or any growth in VTM prior to use for sample collection or before dispatch of VTM to surveillance site. If any turbidity or growth is found, please discard the whole batch of VTM[2].
- Use of cotton-tipped or calcium alginate swabs or swabs with wooden shafts is NOT RECOMMENDED[2].

Steps to be followed for specimen collection using aseptic method[2]:

1. Nasal swab and/or throat swab specimens is to be collected as per GOI guidelines.

2. Sample collection area should be kept clean and table or work surface should be properly disinfected with surgical spirit.

3. Collect required details from the patient and fill the sample collection form.

4. Ensure that patient is seated in a comfortable seating position.

5. Clearly explain the sample collection procedure to ensure full cooperation from the patient.

6. Wear appropriate personal protective equipment (PPE) such as gloves, apron, N95 mask, head cap, goggles before starting the procedure.

7. Label the specimen collection vial containing VTM with the unique participant/ sample ID.

8. Specimen should be collected under good illumination.

9. Throat/ oro-pharyngeal swab collection:

a. Gently tilt the patient's head back

b. Steady the chin

c. Ask the patient to open his/her mouth

d. Use a disposable tongue depressor to hold the tongue well

e. Insert a sterile swab

f. Swab both the tonsils and the posterior pharynx vigorously with a rotating motion, till the patient starts to gag

g. Remove the swab without touching the tongue.

h. The swab is then placed in the labelled tube containing VTM

i. The applicator stick is broken off at the indicated mark (if provided) or at below the level of the tube opening

j. Close and tightly screw cap the tube.

10. Nasal swab collection[2]:

a. Take a fresh sterile swab

b. Gently tilt the patient's head Backwards and steady the chin

c. Insert the swab into the nostril parallel (1-2 cm) to the palate until the resistance is met at turbinate

d. Hold the swab in that position for few seconds and then withdraw slowly in a firmly rotating motion (5 times clockwise and 5 times anticlockwise).

11. Appropriate precautions should be taken in collecting specimens since this may expose the lab technician/ sample collector to respiratory secretions from the patient.

12. Specimens from both nostrils are obtained with the same swab vigorously, irrespective of nasal congestion if any.

13. The nasal swab is placed in the same VTM tube containing throat swab. The applicator stick is broken off as done for throat swab and the tube is screw capped tightly.

14. The vial is then placed in the cool box containing tube rack in between the frozen gel packs.

Sample handling at collection site[2]:

1. A unique specimen ID is written/ pasted on each VTM sample by the lab technician

2. VTM containing samples are to be kept in cool box immediately after collection.

3. VTM tube IDs are cross checked with the details in the filled sample collection form.

4. If the specimens cannot be sent to the laboratory within specified time frame they should be stored at or below –70°C in ultra-low freezer.

5. 5. Repeated freezing and thawing must be avoided.

SPECIMEN COLLECTION:

SPECIMEN TO BE COLLECTED[6]:

TYPE OF SAMPLE:

Upper respiratory tract:

Oropharyngeal and Nasopharyngeal swabs, or wash in out patients should be collected[6].

Lower respiratory tract:

Sputum, endotracheal aspirate or bronchoalveolar lavage in patients with respiratory disease[6].

SPECIMEN TO BE COLLECTED[6]:

TEST:	TYPES OF SPECIMEN:	DURATION:
NAAT	Lower respiratory tract - sputum - aspirate - lavage Upper respiratory tract - nasopharyngeal and - oropharyngeal swabs Consider stools, whole blood, urine, and if diseased, material from autopsy	Collect on existing case. Repeated sampling, possibly to monitor clearance. Further study is required to evaluate the efficacy and reliability of repeated sampling.
SEROLOGY	Serum	Paired samples are needed to confirm with the initial sample collected during the first week of disease and the second sample preferably collected 2-4 weeks later
NAAT	Oropharyngeal and nasopharyngeal swabs	Within the time of incubation of last reported contact
SEROLOGY	SERUM	Lowbound serum taken as soon as possible within incubation period of contact and convalescent serum taken 2-4 weeks after last documented contact

In addition to clinical specimens may also be collected as COVID-19 virus has been detected in blood and stool[7]. The active period and frequency of shedding of COVID-19 virus in stool and urine is uncertain[8].

SPECIMEN COLLECTION DETAILS[2,6]:

SPECIMEN TYPE:	**COLLECTION MATERIALS:**	**TRANSPORT TO LABORATORY**	**STORAGE TILL TESTING**	**COMMENTS:**
Oropharyngeal and nasopharyngeal swab	Dacron or polyester flocked swabs*	2-8 °C (4 °C)	≤5 days: 4 °C >5 days: -70 °C	The nasopharyngeal and oropharyngeal swabs should be placed in the same tube to increase the viral load

Cont.... **SPECIMEN COLLECTION DETAILS[2,6]:**

Bronchoalveolar lavage	sterile container*	2-8 °C (4 °C)	≤48 hours: 4 °C >48 hours: –70 °C	There may be some dilution of pathogen, but still a worthwhile specimen
Tracheal aspirate, nasopharyngeal aspirate or nasal wash	sterile container*	2-8 °C (4 °C)	≤48 hours: 4 °C >48 hours: –70 °C	Not applicable
Sputum	sterile container*	2-8 °C (4 °C)	≤48 hours: 4 °C >48 hours: –70 °C	Ensure the material is from the lower respiratory tract
Tissue from biopsy or autopsy including from lung	sterile container with saline	2-8 °C (4 °C)	≤24 hours: 4 °C >24 hours: –70 °C	Autopsy sample collection preferably to be avoided
Serum (2 samples – acute and convalescent)	Serum separator tubes (adults: collect 3-5 ml whole blood)	2-8 °C (4 °C)	≤5 days: 4 °C >5 days: –70 °C	Collect paired samples: • acute – first week of illness • convalescent – 2 to 3 weekslater

SAMPLE STORAGE (GUIDELINES):

· In the ongoing laboratory testing for COVID-19 diagnosis by molecular diagnostic methods, clinical specimens or a subset of the clinical specimens may need to be retained for various purposes such as performing additional tests, for quality control purposes or for use as control materials to assess newer diagnostic tests[7].

· In addition, a laboratory may need to store specimens for projects aimed at studying genomic epidemiology of the SARS CoV2 virus across regions and over time[7].

· All samples being stored for a long-term must be appropriately labelled indicating laboratory identifiers, date of sample collection and must be stored in properly functioning -80ºC deep freezers. A proper inventory (preferably electronic) of stored samples should be essentially maintained[7].

· With the surge in testing across the country, the total number of tested samples has gone up considerably. Keeping this in view, ICMR advises of the following[7].

a) Laboratories that are serving as validation centres for COVID-19 diagnostic kits are advised to preserve adequate numbers of positive and negative samples to prepare appropriate panels for validation etc[7].

b) At a minimum, all samples testing positive for SARS CoV2 must be retained for at least 30 days from the date of testing before being destroyed. Depending on the freezer space availability in a particular laboratory, one or more aliquots of the positive specimen may be retained for the period[7].

c) A government laboratory may decide on the number of positive / negative samples to retain in the long term based on the availability of freezer space as well as perceived research agenda of the laboratory for COVID-19 in the future[7].

d) If the number of samples tested positive at a laboratory is considerably large and the laboratory is unable to retain all positive samples beyond 30 days, a minimum of 10% of all positives detected at the laboratory in a month or 40-50 positives preferably with

equal numbers of high, moderate and low viral load should be stored for a period of 1 year at the least. A single aliquot of a positive sample may be retained taking into account freezer space availability at the laboratory[7].

e) Considering that the number of samples tested negative at each laboratory will vary depending on the sample load and testing capacity of the laboratory, a minimum of 50 samples or 1-2% of all negative tested samples over a month, whichever is smaller should be retained at the testing laboratory for a period of 1 year. A single aliquot of a negative sample may be retained taking into account freezer space availability at the laboratory[7].

PACKAGING AND TRANSPORT:

1. Samples should be transported at 2-8°C within specified time frame to the testing laboratory[2].

2. Samples should be kept in proper standing position in appropriate test tube rack[2].

3. The VTM containing part of the tube should be in direct contact with frozen gel packs[2].

When there is likely to be a delay in specimens reaching the laboratory, the use of viral transport medium is strongly recommended[7].

Specimens may be frozen to - 20°C or ideally -70°C and shipped on dry ice if further delays are expected[7].

It is important to avoid repeated freezing and thawing of specimens[7].

Transport of specimens within national borders should comply with applicable national regulations[7].

International transport of potentially COVID-19 virus containing samples should follow the UN Model Regulations, and any other applicable regulations depending on the mode of transport being used[7].

SAMPLE PROCESSING IN LAB:

Access to the laboratory should be restricted to authorized personnel only

• Types of samples that can be received in the cytology laboratories, during this pandemic, may include sputum, bronchoalveolar lavage (BAL), endotracheal tube aspirates, and other respiratory secretions[3].

• The samples should be collected in appropriately labeled, tightly-capped, sterile tubes/containers and sent to the cytopathology laboratory either in a biohazard zip-lock bags kept inside a leak-proof cryobox with a biohazard label or a triple packaging system can be used wherein the primary receptacle containing the sample is kept inside a second protective watertight and leak-proof receptacle which, in turn, is covered by a third receptacle to prevent any kind of physical damage to the secondary receptacle during transport[3].

• A trained hospital attendant should transport the sample to the laboratory, as soon as possible. Under no circumstance should the material be handed over to the relative of the patient who would be likely in close contact with the patient for transport to the laboratory[3].

• Cytotechnician should wear protective gear in the form of gown, gloves, cap, N95 mask/triple layer surgical mask, and preferably goggles Fresh, unfixed specimens should be transported by hand, and NOT shipped with pneumatic-tube systems[3].

• All fresh cytology samples received in the laboratory should be considered potentially infectious (may contain the SARS-CoV-2 virus even in samples from undiagnosed, asymptomatic patients) and hence, universal precautions need to be followed while handling such samples3.

SAMPLE DISCARDING:

All the residual samples should be discarded in appropriate disinfectants with confirmed virucidal activity against enveloped viruses[3]. These include 0.1% sodium hypochlorite solution, 0.5% hydrogen peroxide, 62–71% ethanol, quaternary ammonium compounds, or phenolic solutions[3].

MANAGEMENT OF SAMPLE SPILLS IN LAB:

The decontamination of laboratory surfaces in the event of sample spillage should be done immediately using appropriate disinfectants with proven virucidal action against enveloped viruses. The most commonly used is a 1% sodium hypochlorite solution[3].

LAB DIAGNOSIS:

LABORATORY FINDINGS:

The common abnormalities seen in COVID 19 patients are[8]:

Complete blood count : Leucopenia

· Creatinine↑, Aspartate aminotransferase/ Alanine aminotransferase /bilirubin ↑

· C-Reactive protein ↑, Lactose Dehydrogenase ↑, ferritin ↑

Chest X-rays : Acute respiratory distress syndrome[8].

Chest X-rays / imaging are frequently anomalous even in early diseases and show bilateral, peripheral and ill-defined interstitial infiltrates with opacification or lobular and subsegmental consolidation[8].

BIOMEDICAL WASTE MANAGEMENT:

Following processing of sample and following FNA performed on a COVID-19 positive/suspected patient, the full PPE is to be discarded into appropriately designated bins labeled as COVID and as per hospital policy[3].

All laboratory-generated biomedical waste from COVID-19 positive/suspected samples must be processed as per local/institutional regulations and as mandated by pollution control policy and guidelines made by the Central Pollution Control Board, India[3].

LABORATORY MEASURES:

· Re - evaluate your procedures and reduce or eliminate steps which may lead to aerosol formation or droplet creation[9].

· Review the situation weekly or biweekly, and make any required changes[9].

· Establish a chain of command, plan for emergencies and plan for contingency[9].

· Implement crowding management measures: review staffing requirements and realign the workload; consider working in shifts to reduced level[9].

· Follow the recommendations for routine handling of specimens by CDC / WHO in compliance with the guidelines[9].

· Process all specimens having measures that could lead to aerosols or droplets[9].

· Keep updated on the latest developments concerning the COVID-19 pandemic and notify the workers of any new scientific development knowledge; do away with myths[9].

· Remain aware of the current hospital policies and procedures and notify staff of any changes[9].

· Maintain open channels of communication with colleagues and staffs[9].

Don't do:

· Should not trigger needless anxieties or panic, but be honest about the risks[9].

· Don't spread or support rumors or information that doesn't come from a credible source[9].

Conclusion

Early diagnosis is the important key for management of COVID-19. Serological and molecular assays together will further strengthen the diagnosis of SARS-CoV-2. The safety of laboratory personnel is of utmost important, therefore clear understanding of the COVID19 biosafety steps, specimen collection, storage and transportation guidelines. It also addresses steps that can be taken by cytology laboratories to work during the pandemic and reduce the risk to their employees, trainees and pathologists.

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Conflict of Interest – Nil

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