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# Biosafety Measures in a Cytopathology Lab during the Covid-19 Era: A Review

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## Abstract

COVID- 19, caused by the SARS-CoV-2 virus, has been declared a pandemic by the World Health Organization. The COVID-19 pandemic has had a major impact on diagnostic laboratories, particularly those handling cell and tissue specimens. Cytology laboratories receive fresh and potentially infectious biological samples including those from the respiratory tract, from COVID-19 positive or suspected patients. This change conveys genuine ramifications for the pathology laboratory centers. The laboratory need to strike a balance between the safety practices for the pathologists and maintaining the quality of reporting carried out in the center. This article aims to present a guide to the safe functioning of cytology laborotories during the COVID- era. It discusses the safety measures to be observed starting from transportation to processing, and reporting of samples during the pandemic.

**Keywords:** COVID- 19, SARS CoV-2, Bio- safety, Cytopathology, Laboratory.

## Introduction

December 2019 in the city of wuhan, of the people's republic of china, people started presenting with symptoms of an unusual kind of pneumonia, such as fever, dyspnoea, chest discomfort, coryza and cough.[1] It was later identified to have originated via a zoonotic transmission of a novel coronavirus from the seafood market in the province.[2] This virus was part of the beta coronavirida family and showed great homology with the severe acute respiratory distress syndrome corona virus- 1 (SARS CoV-1). Therefore it was named severe acute respiratory syndrome corona virus- 2 ( SARS CoV-2) by the Coronavirus Study Group of the International Committee on Taxonomy of Viruses.[3,4] The high infectivity, lack of immunity and easy transmissibility owing to modern transport developments , has enabled the virus to spread across the globe. The world health organisation declared COVID-19 caused by SARS CoV- 2 a global pandemic on the 11th of march 2020.[5] The disease has so far affected 14,890,035 213 people across 213 countries and territories, with a death toll of 614,124.[6] The pandemic caused by the novel virus has set the populace in a state of anxiety as many key features such as pathogenesis is still unclear.

The first case of COVID- 19 in India was reported in the state of Kerala on the 11th of march 2020.[7] Since then there has been steady increase in the number of cases in the country. As of 21st July 2020, 1,157,218 confirmed cases with a total of 28,128 deaths have been reported in India.[6] Public health measures such as social distancing, ban on discretionary travel, and national lockdown has been implemented. These measures have had a retarding effect on the spread of the disease, but has not terminated it. The world was faced with a similar situation when the Spanish influenza pandemic struck in 1918. H1N1 virus infected one- fifth to one- third of the world population. During that period similar non- pharmaceutical interventions were implemented worldwide to curtail the infection.[8-10] Though many researches think the disease curve in India is not as steep as the other developed countries it is essential to follow a few bio safety protocols to contain the spread of the disease and prevent the over burdening of the health care system.[11]

Health care workers fall under the high risk category to contract the virus as they come across a variety of patient including, asymptomatic carriers, suspected COVID-19 patients, and patients with non-specific symptoms of COVID-19. Several other factors such as limited availability of personal protective equipment ( PPEs), inadequate training for infectious outbreaks of this scale, the tendency of the patients to conceal their epidemiological history, all attribute to the heightened risk of health care professionals getting exposed to the pathogen.[12,13] Cytopathology lab receives several specimens including, oral scrapes, lavage, and sputum which may contain viable pathogens and are hence infectious. This coupled with the fact that the pathologist also come in close contact with select patients when performing procedures such as fine needle aspiration biopsy and Rapid on site evaluation, and the high transmissibility of the virus makes it essential to have certain protocols in place to ensure the safety of the cytopathologists, cytotechnologists, technicians, and other laboratory personnel.[11]

## Virus Pathogenesis And Host Response

**Viral Structure:** SARS CoV- 2 virus belongs to the large coronavirus group which is responsible for cause respiratory and enteric infections in animals and humans. This viral group is subdivided into 3 serogroups of which, serogroup 3 is strictly an avian strain. Serogroups1 and 2 contain the strains of viruses that are known to cause for 30% or more mild upper respiratory tract illnesses in

humans. SARS CoV-1,MERS CoV, and SARS CoV- 2 known to cause serious infections in humans also belong to this serogroup.[14,15] SARS CoV- 2 virus is a large, spherical, enveloped virus of the size 100-160nm. It contains a single stranded positive sense, enveloped RNA within a caspid of 27-32kb.[16] There are four types of spikes present on the surface of the virus- (i) Long glycoprotein S- spike, (ii) Small Hemagglutinin- esterase spike (HE), (iii) Transmembrane glycoprotein and (iv) Envelop protein spike. The spikes are responsible for the diversity of the coronavirus and host tropisim.[17,18]

**Transmission:** A recent study reported the presence of viral RNA particles in the sputum samples, oral swabs, nasopharyngeal swabs, and bronchoalveolar lavage fluid anal swabs, feces, blood, tears, and conjunctival secretions. This suggests the systemic nature of the disease.[19,20,21] But mere presence of viral RNA does not make the sample infectious. Viable infectious viral particles were isolated from the upper and lower respiratory tract specimens only. Hence respiratory droplets are the major route of viral transmission. Another route is transmission is fomite spread, that is touching items that have been contaminated with the viral particles. Small droplet nuclei of the size greater than 5µm can travel up to a distance of 1 meter, where as those of the size smaller than 5µm can spread to a distance greater than 1 meter when aerosolized.[21]

**Pathogenesis:** Within the host cell the virus replicates in 5 steps: attachment, penetration, biosynthesis, maturation and release.

**Attachment:** Genomic analysis revealed that the functional receptor for SARS CoV- 2 is Angiotensin converting enzyme- 2 (ACE-2) receptor. ACE-2 receptors are abundant epithelial cells of the lungs, heart, ileum, kidney and bladder.[22] This makes it possible for the virus particle to directly affect the heart, but so far has not been reported. Once the virus enters the host, the S1 subunit of the membrane spike attaches to the receptor and the S2 sub unit attaches to the cell membrane.[23]

Penetration- Following attachment, the spike proteins undergo protease cleavage. The SARS CoV-2 virus possesses a furin cleavage site at the S1/S2 site making it very pathogenic.[23] The S1 and S2 sub units are non-covalently bound, with S1 supporting the membrane anchored S2 subunit, after cleavage. The membrane spike is then activated by further cleavage at the S2 site , which brings about irreversible conformational changes in the cell membrane cause fusion of the cell membrane with the viral envelope.[24]

**Biosynthesis:** The viral RNA is released into the cytoplasm where it is translated into pp1a and pp1ab polyproteins.[25] These non- structural proteins form replication- transcription complex in a double- membrane vesicle. This complex continues to replicate and produce sub genomic RNA which is responsible for the production of structural and accessory proteins.[26]

Maturation- Viral buds are formed by assembling the genomic RNA, nucleocaspid protein and envelope glyco-proteins together. Finally the vesicle that contains the viral particle fuses to the plasma membrane and is released into the circulation.[27]

The cytopathic effects seen in an infected individual are influenced by a wide variety of mechanisms including- Interference in the signalling pathway, Derangement of cellular function, enhanced cytokine or chemokine expression, inhibition of transcription and translation of cellular proteins. They sometimes cause cellular fusion otherwise may result in apoptosis of the cell.[28] Studies have shown changes such as lack of beating cilia and bronchial epithelial denudation, 96 hours after viral inoculation on the surface layers of human airway epithelium.[29]

**Host Response:** After exposure to the virus, 95% of the patients develop symptoms within 12.5 days with a maximum of 24 days and a minimum of 5 days. The patients present with a range of symptoms from mild to severe respiratory symptoms. The most common presentation is fever which is seen in 90%-96% of the cases, followed by cough in 70% , dyspnea in 45% and muscle soreness or fatigue in 40% of the cases. The least encountered symptoms are sore throat, headache and diarrhoea which is only seen in 10% of the affected population.[30,31] The WHO reported that of all the identified cases only 3% were critical where as 15% of the cases were severe and 82% were mild.[32] In severe cases the disease may progress to Respiratory, Circulatory, Renal failure and finally death due to multiple organ failure. Computed tomography imaging of the patient's lung reveals signs of pneumonia with a peripheral distribution of fine reticular opacities and ground glass appearance.[33] Early lung injury is mostly noticed in the distal airway, this has been atributed to the fact that ACE2 receptors are present in abundance on the apical side of the lung epithelial cells.[34] Apart from respiratory symptoms the patient also presents with hypercoagulable states such as thrombosis and pulmonary embolism in severe cases as a result of endothelial injury. This also increases the permeability of the microvasculature with permits tissue invasion by the virus.[35,36] A study studying the pathologic changes in individuals affected by the SARS CoV-2 virus revealed that pulmonary changes predominated with pulmonary oedema, prominent proteinaceous exudates, vascular congestion, and intra-alveolar fibrinoid material, reactive type II pneumocyte hyperplasia, atypical enlarged pneumocytes with large nuclei and amphophilic granular cytoplasm.[37,38]

Innate immunity of the respiratory tract is made up of 3 components- epithelial cells, macrophages and dendritic cells. The dentritic cells and the alveolar macrophages phagocytose virus infected epithelial cells and present the viral particles to the T cells present in the draining lymph nodes. Now, the $CD4^+$ cells stimulate the B cells to produce antibodies against the virus. Meanwhile, the $CD8^+$ cells phagocytose the infected epithelial cells.[34,39,40] Immunologic studies revealed that the patient presented with leukocytosis wwith lymphopenia, slightly elevated levels of liver enzymes, muscle enzymes, myoglobin and lactate dehydrogenase. In severe conditions the laboratory picture revealed elevated procalcitonin, severe lymphopenia with a reduction in peripheral T cells in particular, elevated D- dimers and pro- inflammatory cytokines.[41] With the increase in severity of the patient's condition, the IL-6 levels in the body increased. The patient exhibited increased expression of checkpoint receptor $Tm3^+$ $PD\text{-}1^+$ subsets on the $CD4^+$ and $CD8^+$ T cells, along with natural killer group 2 member A on the $CD8^+$ T cells which denoted the exhaustion of T cells. Further worsening of the disease status occurs as a result of T cell exhaustion.[42] The increased express of chemoattractants such as IL-8 and IL-6 in the lungs of these patients results in the site being infiltrated by a large number of innate immue cells and adaptive immune cells. Among these pro- inflammatory cells the majority are neutrophils which act as a double edged sword and cause lung injury. The increased expression of cytotoxic $CD8^+$ T cells and pathologic cytopathic T cell derived from $CD4^+$ T cells act to phagocytose the virus, they also propagate the lung injury. The host immune response is further heightened by the expression of CD $14^+$ and $CD16^+$ monocytes that are rarely seen in healthy subjects, hence worsening the immune mediated lung injury.[43,44]

**Comparision of SARS Cov-2, SARS Cov-1 And MERS:**

A comparison of the COVID-19 outbreak and the 2 previous outbreaks of severe respiratory distress caused by coronaviruses could improve our understand the current infection and better prepare us to face the pandemic. SARS CoV-1 , SARS CoV-2 and MERS CoV are there viruses belonging to the genus beta coronavirus, that produce serious respiratiory infections in human beings. Amongst the 3 viruses SARS CoV-1 could provide us with more relavent information as it shares 80% similarity with SARS CoV-2 share when compared to the 50% similarity with MERS CoV virus.[41] SARS CoV- 1 and 2 viruses attach to the Angiotensin Converting Enzyme- II (ACE-2) receptor whereas the MERS CoV attached to the DPP4/CD26 receptor present on the non- cilliated respiratory epithelium. The receptor ACE-2 shows a wide species distribution this explains the cross species transmissibility seen in both the viruses.[45] The SARS CoV-2 virus showed 96% similarity with the bat corona virus and 99% similarity with the virus found in a pangolin. The MERS CoV virus however originated from the bat similar to the COVID virus but was transmitted from an intermediary host, dromedary camels.[45-47] All the 3 viruses manifest with similar range of symptoms, varying from mild- flu like symptoms to severe pneumonia. MERS exhibited a higher case fatality rate of 30% followed by SARS CoV-1 - 10% and finally SARS CoV-2 exhibits a CFR of 2.3%.[48] However the COVID-19 if more widespread as compared to the other coronavirus outbreaks as it has a R0 value of 2.2- 2.6 whereas the MERS CoV only had a R0 value of 0.45. Though the SARS CoV-1 virus recorded a R0 value of 3 the spread of the disease was lesser compared to COVID-19. This discrepancy has been attributed to the fact that it was calculated in the hospital setting where there was very high transmissibility.[49,50] The pathologic presentation of the three diseases are also very similar as they all present with lung injury in varying stages of exudation and organization, in fatal cases.[51]

**Biosafety Measures In A Laboratory**

Risk assessment and risk mitigation together form the basis of biological risk evaluation.[52] In risk assessment the biological hazard is characterized by identifying the intrensic biological characteristics of the pathogen and the laboratory procedures related to it. The WHO has classified the intrinsic biological characteristics of pathogens into 4 groups in the laboratory biosaftey manual:

- Level 1- Involves infectious agents that are unlikely to infect humans or animals.
- Level 2- Includes pathogens which are rarely associated with serious diseases and for which medication is readily available.
- Level 3- Includes pathogens which cause serious or lethal diseases in humans and for which treatment and preventive measures may be available.
- Level 4- Includes pathogens which cause serious or lethal diseases in humans and for which medications and preventive measures are not available.[53]

Risk mitigation is carried out by defining the suitable, Biosafety Levels (BSL) of the laboratory, Personal Protective Equipment (PPE), Type of infrastructure and equipment and Education of involved personnel. Biosafety levels are protocols designed to protect the health care workers, the surrounding environment and the community. The WHO graded the biosafety in a laboratory into 4 groups, with BSL 1 having the least protective measures and BSL 4 having the highest protective measures.[53] The characteristics of the risk determine the biosafety levels and not the risk group the pathogen is present in.

SARS CoV- 2 virus has been classified under Risk Group-3 human pathogens by the international consensus.(54) The cytology laboratory receives specimens from the , upper and lower respiratory tract including, pleural effusion, bronchoalveolar lavage, bronchoalveolar washing, transbronchial needle aspiration, and sputum samples, which are deemed to be the most potentially infectious in relation to COVID-19. Hence the WHO has released a recent statement regarding the biosafety guidance in laboratories stating that " non- propagative diagnostic laboratory work" involving specimens from suspected or confirmed SARS CoV-2 patients must be carried out in BSL -2 laboratories.(55) The safety guidelines for the cytology laboratory can be broadly classified into three categories-

- Pre- analytical
- Analytical
- Post- analytical.

**Safety Measures In Pre- Analytic Procedures**

**Laboratory Requisition Form**: Digital method of request forms are encouraged as the virus can survive on paper from a minimum of few minutes to a maximum of 5 days. Online request forms are available in the hospital information system and can be easily accessed by the laboratory in the hospitals that posses this facility. In scenarios where this is unavailable, the request form can be forwarded to the various departments via digital means such as, email and whatsapp, which can be filled and returned in the same manner. If maintenance of a digital work flow is not possible, then the clinician must ensure that a properly filled requisition form with all the patient details is sent to the lab with the sample. The request forms must contain clear information on the 'patients COVID status, either by writing or by colour coding the form.(11)

**Fine Needle Aspiration or ROSE:** Though patient contact is very minimal in the field of cytopathology, technicians who perform FNA or ROSE are considered to be in very close contact with patients and hence are at a higher risk of contracting COVID-19. Hence these procedures must be done using a complete set of PPE. The PPE must be worn and removed only in the designated area following the proper guidelines for donning and doffing.(56) Before the patient arrives for the procedure, he must be advised to wear a face mask so as to minimise the risk to the HCWs. During the procedure, the patient is asked to remove the mask and face away from the cytopathologist, in case the procedure is obstructed by the mask. Forceful expulsion of the aspirate from the syringe generates aerosols and hence must be avoided. Under unavoidable circumstances, the aspirate must be expelled very slowly. Smear is prepared by holding the glass slide as far away from the technician as possible.(11) After the procedure the syringe used is disposed by, first cutting the hub of the syringe , followed by disinfection of the needle using solutions such as 0.1–1% hypochlorite solution, ethanol in concentrations ranging from 62 to 71%, and 0.5% hydrogen peroxide and finally disposed in tear resistant containers. The needles should not be incinerated as this produces aerosols.(41,57)

ROSE is performed with the help of radiological guidance such as endoscopic ultrasound-guided (EUS)-FNA and endobronchial ultrasound-guided transbronchial needle aspiration (EBUS-TBNA), for the evaluation of deep seated lesions. These procedures must be performed in a separate room with a sonogram and other equipments required for this procedure so as to minimise the nosocomial spread of the infection. Both FNA and EUS-FNA must be conducted in a well ventilated room with an air flow of 60 litres per second per patient. EBUS-TBNA being an aerosol generating procedure, is a greater threat

for disease transmission and hence must be carried out in a room with an airflow of at least 160 liters per second per patient or in a negative pressure room with at least 12 air changes per hour.[58]

The pathologist must be equipped with laboratory gown, gloves, goggles, face shield, and respirator, which are appropriately discarded following procedure. As per WHO guideline the respirators worn should be National Institute for Occupational Safety and Health-certified N95, European Union filtering face pieces Class 2 (FFP2) equivalent, or a higher level of protection.[58] Following any procedure proper hand hygiene must be followed for more than 20 seconds.[11]

Sample Processing:

All the samples sent to the laboratory must be considered potentially infections. The samples must be collected in an appropriate container and transported by hand at 2°C to -8°C, and stored at the same temperature for a short term or at -70°C if the testing is to be performed after a long period of time.[59,60] The personnel delivering the specimen must be trained in safe handling of biological samples and spill decontamination procedures. Pneumatic tubes must not be used for the transport of specimen. UN3373 Biological Substance Category must be used for the transport of specimens from suspected of COVID- 19 positive patients. According to this category the specimen is packed in 3 layers- 1) a leak-proof primary receptacle, 2) a leak-proof secondary packaging, and 3) an outer packaging of adequate strength with at least 1 surface having a minimum dimension of 100mm × 100mm.[61]

Routine sample processing involves steps such as opening of the sample containers, removing tightly fitted caps of the tubes, diluting, shaking, vortexing, and centrifugation may lead to aerosol generation, and hence must be performed in level II biosafety cabinets. In scenarios where the level II BSC is unavailable or the instruments cannot be used within the BSC adequate measure must be taken to ensure a barrier is present between the cytopathologist and the specimen.[61] In case of centrifugation, it must be performed using capped tubes which must be left to rest for 5 minutes before opening them after the procedure.[11] Procedures such as frozen sections must only be performed if the pathologist is sure the aerosol can be contained within the cryostat.[62]

**Safety Measures In Analytical Procedure:** Cytology slides fixed using alcohol based fixatives of concentrations greater than 70% and parrafin embedded cell blocks lead to the inactivation of the SARS CoV-2 virus and hence are a low for transmission and can be handled with standard good microbiological practices.[55,63,64] However, when using unfixed or partly fixed specimen samples there is a high risk of disease transmission and must be treated as 'Risk category 3'. Hence, PPE is mandatory for all technicians and appropriate BSC is required when handling these specimens. Unfixed specimens from unconfirmed COVID -19 patients can be handled in down draft bench by a pathologist fully equipped with PPE, followed by disinfection of the region, as these specimens are included under 'Risk category 2'.[65] Prolonging fixation certainly reduces the risk of infection, but is likely to significantly impair the quality of DNA, and especially RNA, required for genomic analysis, particularly next-generation sequencing.[66] Frozen section diagnosis of fresh specimens obtained from the surgeon are analysed under a level 3 biosafety cabinet. The pathologists are recommended to use non- sterile gloves to avoid contact, when analysing the fixed slide.[41] The microscope used must be disinfected using 70% alcohol before and after analysing the specimen. A maximum of 3 members at 1 meter distance to each other are permitted at a multi- head

station at a time. Following analysis, proper hand hygiene must be followed.[11]

**Sample Discarding:** Disinfectants with confirmed virucidal properties against enveloped viruses such as as sodium hypochlorite 0.1%, a minimum of 62%–71% ethanol, 0.5% hydrogen peroxide, ammonium or phenolic compounds are used to disinfect the residual specimens, sample tubes and containers, which are later discarded in separate biohazard waste bags labelled as COVID-19.[57]

Surface and Equipment Disinfection:

All working surfaces and equipments in the laboratory need to be disinfected with chemical solutions effective against SARS CoV-2 virus, multiple times a day.[41] The cryostat used in frozen section diagnosis must be decontaminated after the procedure using 100% ethanol with or without ultraviolet light to avoid ice formation.[65] Tan et al. suggested usage of 1% sodium hypochlorite solution in case of a spill accident.[57]

**Safety Measures In Post- Analytical Procedures**

**Reporting:** The pandemic has emphasized the implementation of digital pathology in the health care sector. Reporting the results via electronic means are much desired , so as to avoid unnecessary contact. Digitalization also enables the pathologists to collaborate with colleagues for the diagnosis of difficult or rare cases.[65]

**Storage:** Non- sterile gloves and a face mask is worn as a protective equipment while storing and cataloguing the histological slides and cell blocks after reporting.[11]

**Training Of Laboratory Personnel, Residents And Fellows**

The laboratory staff can be divided into smaller teams , which can be made to work in shifts. All the personnel have to be educated on the precautionary measures taken for each procedure, the proper method of donning and doffing PPE and the importance of regular hand washing. Duties must be reallocated to satisfy the demands of the current scenario.[11] As for the residents and fellows, education must not be suspended in this uncertain situation. All activities involving more than 10 people must be shifted to online forms such as Zoom, Skype or Google meet. Slide scanners can be utilised for educational purposes when available. Innovative methods such as webinars and continuing medical education (CME) must be conducted.[41]

**Conclusion**

Novel coronal virus has caused massive disruption in all aspects of life. The effectiveness of the health care system is essential to curb the global spread of COVID-19. Proper biosafety measures combined with effective administrative strategies help us to better battle the pandemic. Periodic updating of the guidelines given by various organisations such as WHO, CDC and the European Center for Disease Prevention and Control, banishes the anxiety regarding the disease and helps prevent transmission to the health care workers.

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