



Review Article

COVID-19 and biosafety: A review of biosafety recommendations for cytopathology and histopathology laboratories

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ABSTRACT

Severe acute respiratory syndrome coronavirus -2 (SARS-CoV-2) is the zoonotic coronavirus responsible for the present pandemic of COVID-19. The novel ways of transmission of this virus have eluded and infected the global population, surpassing the confines of the place of its origin in Wuhan, China. The healthcare workers are one of the most susceptible populations and laboratory safety protocols are being devised throughout the world to protect the laboratory personnel, who are the frontline fighters in this war against the virus. The present narrative review is an attempt to encompass the published literature sharing the experience and guidelines of biosafety for those working in histopathology and cytopathology laboratories.

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1. Introduction

Coronavirus is a large group of virus which can cause a variety of illnesses in animals and humans. In humans, several coronaviruses are known to cause a myriad of respiratory infections ranging from the very mild common cold to more severe life-threatening respiratory diseases.

In recent past, two Corona virus related outbreaks have rocked the world as Severe Acute Respiratory Syndrome (SARS) in 2002-2003 and Middle East Respiratory Syndrome (MERS) in 2012. The novel Coronavirus which has now caused the worldwide pandemic in 2019 has been named as severe acute respiratory syndrome corona virus 2 (SARS-CoV-2) by the International Committee of Taxonomy of Viruses (ICTV).¹ The resulting disease has been named as Coronavirus disease 2019 (COVID-19) and the outbreak that started in Wuhan, Hubei province, China had spread throughout the globe and has been labeled as pandemic on 11th of March, 2020 by the World Health organization (WHO).² Although related to the previous two

causal viruses, the rate of human to human transmission of SARS-CoV 2 is higher but with milder symptoms and lesser incidence of gastrointestinal symptoms and case fatalities.³⁻⁵ The consideration that SARS-CoV 2 is harbored and shed through respiratory secretions even when the patient may still be asymptomatic, makes it a highly transmissible and communicable pathogen. COVID-19 has affected the whole world and has completely changed the way we work and live. At the time of writing the review, there are 2,544,792 confirmed cases and 1,75,694 deaths globally.⁶

India, being the second most populous country of the world, faces the threat of rapid transmission of the virus. On 30th January 2020, India reported its first case of COVID-19 in Kerala⁷ and since then the tally has been rising. Lockdown has been enforced, hygienic practices and social/physical distancing have been encouraged but notwithstanding, the ever-growing count has risen to 16,689 active cases of COVID with 686 registered deaths as of on the day of the review.⁸ As this is a new pathogen with humans having no prior exposure and hence immunity, much is unknown about the behavior of the virus,

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transmission dynamics and optimal therapy. The present review is based on the current knowledge of the virus and recommendations by the concerned authorities and should be read in the light of the evolving information in the coming days.

An idea about the route of transmission is very important for the prevention of spread and for biosafety maintenance inside the diagnostic laboratories. The spread of the virus through human to human transmission is believed to involve close and prolonged (15 minutes or more) interaction with the infected person being facilitated through large respiratory droplets and contact transmission.⁹ The contact transmission can be direct (by handshake) or indirect (touching objects contaminated by cases/carriers carrying viruses also known as transmission via fomites). Respiratory infections can be transmitted from one person to another through droplets of different sizes: when the droplet particles are $>5\text{-}10\ \mu\text{m}$ in diameter they are referred to as respiratory droplets and when they are $<5\ \mu\text{m}$ in diameter, they are referred to as droplet nuclei.¹⁰ In case of COVID -19, the primary transmission is believed to be predominantly by respiratory droplets and contact routes.¹¹ These respiratory droplets can travel $< 1\ \text{meter}$ ³ while airborne transmission (particles $<5\ \mu\text{m}$ in diameter) can remain in air for longer periods and travel over distances $>1\text{m}$. However, in aerosol generating procedures (like intubation, endotracheal suction and in various sample processing procedures in laboratory), these small droplet nuclei $<5\ \mu\text{m}$ are produced and may play an important role in disease transmission.³

Fomites are also very important source of infection as the virus can survive on various plastic/metal surfaces, glass, cardboards, disposable gowns/ gloves, and paper surfaces for variable lengths of time.^{12,13}

The safety measures can be dealt under the headings different levels– society at large, hospital, laboratory and individual level.⁹ In this review, we will discuss the safety precautions at the level of cytopathology and histopathology laboratories, based on the published literature.

2. COVID-19 and diagnostic laboratory

The recommendations to be followed in a diagnostic histopathology or cytopathology laboratory are in addition to the general precautions to be followed in any hospital/laboratory setting and also at individual level.

Each laboratory is advised by WHO to conduct its own risk assessment to ensure that it can carry out the requisite tests adhering to the recommended biosafety practices and risk control measures.

One of the first things to begin with, is proper education of personnel and staff working in the laboratory set-up. With the bombardment of information in social media, some of which are based on rumors, it must be appreciated that all those working with patient samples and dealing

directly with patients and their attendants, are very much apprehensive. The fear of unknown and all apprehensions should be allayed and safe laboratory practices should be communicated.

WHO recommends the use of the WHO laboratory Biosafety manual (3rd edition) to be followed till the next edition is released. The document is freely downloadable from <https://www.who.int/csr/resources/publications/biosafety/Biosafety7.pdf>.

As per the WHO guidelines, while handling and processing specimens, including blood for serological testing, laboratory practices and procedures that are basic to good microbiological practices and procedures (GMPP) should be followed.¹⁴ Both job specific and safety/security training should be given to the staff including management of spills.

The laboratory management should ensure proper facility design with supply of personal protection equipments as per requirement of the designated job, ensure regular disinfection, proper waste management as per protocol and must have an emergency/incident response plan at place.¹⁴ They are encouraged to prepare their workflow and standard operating protocols based on safe laboratory recommendations by Centre for Disease Control (CDC) and WHO.

Within a laboratory it must be appreciated that other than the samples, any metal, glass, plastic or even paper may be contaminated by fomites and disinfection of surfaces remains an important issue. Human coronaviruses in general are known to persist on inanimate surfaces such as metal, glass or plastic for up to 9 days.¹³

SARS-CoV is an enveloped virus and the disinfectants with proven efficacy against enveloped viruses eg 0.1% sodium hypochlorite solution (1% solution is used for decontamination of blood spills), 62% to 71% ethanol, 0.5% hydrogen peroxide solution, quaternary ammonium compounds as well as phenolic compounds can be used for surface decontamination.^{3,13,15} The points to consider are to ensure the proper concentration, exposure time (e.g. 10 minutes) and expiry date. Other disinfectants such as benzalkonium chloride (0.05%-0.2%) and chlorhexidine digluconate (0.02%) are considered less effective.^{13,15} All work surfaces and equipments are better be cleaned and disinfected before and after work with the recommended disinfectants for the recommended duration while some laboratories advocate cleaning every 3 hours, whenever feasible.

The use of paper requisitions and reports are discouraged and facilities with laboratory/Hospital information systems (LIS/HIS) are preferred. Various android based applications like Whatsapp can be utilized for better communication among doctors and staff dealing with suspected or proven COVID patients.

Proper signages and posters highlighting the steps of handwashing, doffing and donning of personal protective equipments (PPE) and safe laboratory practices may be used inside the laboratories.

3. COVID-19 and histopathology laboratory

It must be admitted that the knowledge about SARS-CoV is still being updated and various recommendations have been put forward by various societies around the world with the aim to prevent disease spread in the histopathology laboratories.

As per WHO, all specimens sent for histopathology should be considered potentially infectious.^{15,16} Both cytology and histopathology falls under the non-propagative diagnostic laboratory work and hence are to be conducted at a facility using procedures equivalent to Biosafety level 2 (BSL-2) laboratory.

It is best to transport specimens by hand and avoid pneumatic tube systems. Transport of all specimens of suspected/diagnosed COVID-19 patients should be packed and shipped as UN 3373 Biological substance, Category B.^{15,17} Viral cultures or isolates should be transported as Category A UN2814, infectious substance, affecting humans.¹⁵ The packaging for UN3373 consists of 3 components: 1) a water-tight, leak-proof primary receptacle (properly labeled), 2) a leak-proof secondary packaging with absorbent material to protect the primary receptacle, and 3) an outer packaging of appropriate strength with at least 1 surface having minimum dimensions of 100 mm × 100mm.³

Histopathology labs may be a little fortunate as the routine histotechnology process inactivates most of the viruses e. g Ebola,¹⁸ although such data is lacking in the SARS-CoV.¹⁹

Both 10% formalin used for fixation and alcohol has virucidal effects against SAR-CoV as demonstrated by Kampf et al.¹³ Darnell et al²⁰ studied the inactivation of the related SARS-CoV by formalin and glutaraldehyde in a temperature- and time dependent manner. They found that incubation at 4°C inhibited the effect and incubation at 37°C or room temperature, formalin significantly decreased the infectivity of the virus on day 1, while glutaraldehyde did so after incubation of 1–2 days. Duan et al found that several coronaviruses were made non-infectious after the following exposure times and temperatures: 90 min at 56°C, 60 min at 67°C, and 30 min at 75°C.²¹ Since paraffin infiltration in most histopathology laboratories uses a temperature of 60–65°C for 2 h or more, Henwood AF inferred that the formalin fixed, paraffin-embedded (FFPE) tissue block should have a low risk of coronavirus infectivity.¹⁹ He also inferred that cryostat usage may best be avoided due to risk of aerosol generation and also has discouraged the grossing of partially fixed specimens.¹⁹ In the recent guidelines by Royal College of Pathologists also the performance of

frozen sections have been discouraged.²² If frozen sections are absolutely necessary, the number of operators should be reduced to minimum and dissector should wear appropriate personal protective equipment (PPE) which should include fluid-resistant disposable gloves, fluid-resistant disposable apron, eye protection, fluid-resistant (Type IIR) surgical mask (FRSM). All dissections are needed to be performed in a ventilated/fume cupboard which along with cryostat and other surfaces should be decontaminated as per local standard protocol following completion of the work.²²

Alternatively, a dedicated fume hood for specimen manipulation and a dedicated cryostat can be designated for COVID-19 suspected/proven cases as mentioned by Rossi et al.¹⁶

4. COVID-19 and Cytopathology laboratory

The cytopathology lab again falls under the non propagative laboratory and should ensure all the standard and special precautions as mentioned. However, one should be more careful as the samples processed in cytopathology laboratories are more fresh and without fixation thus carrying more chance of spreading infection. Though during community spread of disease, all samples are considered as potentially infectious, Mark et al has divided the cytopathology samples into the high risk, intermediate risk and low risk groups depending on the evidence of detectable virus particles available at present.³ The low risk specimens (include ascitic fluid/peritoneal washings, CSF, synovial fluid, cell blocks, semen, cervico-vaginal smears etc) where there is no/limited evidence of viral detection, can be handled by good microbiological practices and procedures (GMPP).

The high risk group includes nasopharyngeal and oropharyngeal swabs, sputum, bronchoalveolar lavage or other bronchoscopic samples, blood and blood products, feces or anal swabs, tear drops or conjunctival discharges where there is documented evidence of virus isolates or viral RNA.³ The intermediate group includes urine, pericardial or pleural fluids which have limited evidence of viral detection. Both the high risk and intermediate category samples need to be handled cautiously in a Class II biosafety cabinet (BSC) with appropriate PPE.³

Any cytopreparatory steps of high-risk specimens that may produce aerosol/droplet generation including opening of containers, removing tube caps, centrifugation, vortexing, aliquoting and/or diluting, pipetting, blending specimens must be done by technical staff wearing full PPE and in a Class II biosafety cabinet in accordance to the WHO and CDC guidelines.^{23,24} Splash shields, sealed centrifuge rotors or sample cups are recommended for centrifugation and rotors and cups should always be loaded and unloaded in a Biosafety Cabinet.²³ However, if done manually, samples should be processed in plastic (rather than glass) tubes with caps and centrifuged in sealed rotors in a lid on

position.

Air drying or heat drying of smears should ideally be performed under biosafety cabinet^{9,23} and the College of American Pathologists (CAP) recommends that while staining air-dried modified Giemsa stained slides, the fixation (first) step is adjusted to a length of 1 minute. For alcohol fixation, ethanol of more than 70% concentration should inactivate the virus while some feel that placing slides at 99% alcohol may be a safer alternative although it may hamper the quality of the sample.¹⁶ The Papanicolaou stains may be carried out as per the staining protocol. Whether fixatives using much weaker alcohol solutions like PreservCyt[®] and CytoLyt[®] (Hologic, Inc. Marlborough, MA) and SurePath[®] (Becton, Dickinson and Company, Franklin Lakes, NJ) can adequately inactivate the virus is still not known.¹²

The College of American Pathologists (CAP) recommends batching high-risk specimens for processing by one technician/cytotechnologist, preferably using a separate station, if available.²⁴ At the end of the processing, all work benches, stations and equipments need to be decontaminated as mentioned and all processed specimens be discarded as per CDC and WHO protocols. All work surfaces like computer keyboards, doorknobs, tables, microscopes, phones are also better be disinfected several times a day.²⁴

Fine needle aspirations (FNA) should be avoided unless absolutely necessary for patient management.^{22,24} If performed, the number of operators are kept to the minimum and they should wear PPE (including N95/FFP2 respirators, goggles/face shields, long sleeved fluid resistant gowns, fluid resistant gloves) and take universal precautions. The donning and doffing of PPE should be done in a specified area in the proper sequence and not inside the laboratory.

Appropriate hand hygiene is to be maintained after and before coming in contact with the patient and after doffing the PPE.³ Ideally hand washing should be with soap and running water for at least 20 seconds or by an alcohol based hand sanitizer with at least 62% alcohol.⁹ The patient may also be encouraged to wear masks. Squirting of the specimen on the slide is not recommended rather it should be dropped gently on the slide without letting the specimen aerosolized as per Shidham et al.¹²

Rapid onsite evaluation (ROSE) may be performed only if considered absolutely necessary.⁹ ROSE which is very important for assessing specimen adequacy in endoscopic ultrasound-guided (EUS)-FNA and endobronchial ultrasound-guided transbronchial needle aspiration (EBUS-TBNA), carry a high risk of infection and their performance should be weighed against the benefits. Any step during ROSE which may generate aerosol or droplet should be avoided.²⁴ ROSE during FNA or EUS-FNA/ EBUS-TBNA should be conducted in well-ventilated rooms with appropriate PPE followed by proper disposal of all used materials, decontamination of work surfaces

and proper transfer of specimens as per protocol.³ Very recently, the apex body of cytopathology in India, The Indian Academy of Cytologists (IAC) has published the national guidelines meant for cytopathology laboratories for handling suspected and positive COVID-19 patient samples. In this review, Srinivasan R et al²⁵ have dealt the biosafety of cytopathology laboratories in the most comprehensive and detailed manner from the Indian perspective which can be adapted by all cytopathology laboratories across our country.

5. Conclusion

Safety of patients, health personnel and population at large must be ensured during any laboratory test. During the tumultuous times of COVID-19, laboratories and hospitals are exposed to the highly infectious materials. As more and more national and international professional and academic bodies are coming up with their guidelines which are applicable and cater to the local needs and limitations, WHO and CDC guidelines remain the mainstay for safety of laboratory professionals. The present article is a humble attempt to review the published recommendations as applicable for cytology and histopathology laboratories in the light of current knowledge and understanding of COVID-19.

6. Source of Funding

None.

7. Conflict of Interest

None

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