Handling and processing of Pathology samples of suspected or confirmed COVID-19 patient

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ABSTRACT

The SARS-CoV-2 has emerged as a major public health concern. Human to human transmission has been confirmed via droplets, contaminated surfaces, and hands. All the staffs and personnel working in the laboratory are also at risk of contracting this infection, especially during the handling and processing of samples from suspected or confirmed patients of COVID-19. With no definite treatment and vaccine in sight, the way forward is to break the chain of transmission by eliminating the risk of exposure to laboratory staffs by proper handling and processing of all the samples in an appropriate containment level laboratory, proper use of PPE with special attention on disposal and decontamination of work surfaces.

INTRODUCTION

The novel coronavirus (2019-nCoV), officially named Severe Acute Respiratory Syndrome Corona Virus-2 (SARS-CoV-2) and declared a pandemic by World Health Organization (WHO) on March 11, is an enveloped, positive-sense, single-stranded, RNA virus, of orthocorona virinae subfamily, genus betacoronavirus, subgenus sarbecovirus. It spreads through large droplets and contaminated surfaces, persisting for 5 days in steel and glass, 2-6 days in plastics, 8 hours in surgical gloves (latex), 4 hours in copper, 24 hours in cardboard, and up to 3 hours in aerosols thus posing a risk for a human to human transmission. It is, therefore, imperative to handle the samples sent to the laboratory properly, to prevent spread to the healthcare professionals.
personnel and their community.

**Literature Search**

The PubMed database, google scholar were searched and the focus of the search was aimed towards handling and processing of laboratory samples in COVID-19 patients, sample transport, waste management, disinfection, and decontamination. Additional information was retrieved from WHO, CDC, European guidelines and handbooks, newsletter, original articles, commentary, letters to the editor, and authenticated websites. Obtained data was reviewed and isolated to deliver rationalized information. Details were gathered from March 2020 onwards.

**HANDLING OF PATHOLOGY SPECIMENS**

The WHO recommends that all specimens should be regarded as potentially infectious. Many of these samples are also submitted to pathology laboratories; hence it is important to take adequate precautions while collecting, handling, and transporting clinical specimens to protect ourselves and our staff.

**I. LABORATORY SETUP**

The laboratory staff might not know the infectivity status of the patient’s sample or the clinicians or surgeons might not have thought of the possibility of COVID-19. So, the key in these situations is two-way communication, proper labeling of the samples, and following the standard precaution measures by staffs.

For the purpose of laboratory work, human pathogens have been classified into four risk/hazard groups (1-4) (Table 1). In early 2020, UK’s Advisory Committee on Dangerous Pathogens (ACDP) decided to provisionally classify SARS-CoV-2 as a hazard group 3 (HG3) pathogen.

These hazard groups are used to assign pathogens to appropriate Biosafety Level (BL) (Table 2). But, apart from risk group, professional judgment and several other factors like pathogenicity of agents, the outcome of exposure, route of infections, laboratory activity planned, availability of prophylaxis should also be taken into account, rather than just assigning the laboratory activity level according to pathogenic risk group.

Control of Substance Hazardous to Health Regulation (COSHH), 2002 has mentioned that any aerosol-generating procedure such as centrifugation or any chance of splash or droplets while dealing with HG3 pathogens should be conducted in a Biosafety Cabinet (BSC) at containment level 3 (CL3) except for some laboratory activities which can be conducted at containment level 2 (CL2) such as:

- processing of any non-inactivated specimens that results in aerosol generation- preparing, fixing, and staining smears on slides
- hematological work on whole blood, serum, plasma
- immunological work
- aliquoting or dilution of the respiratory tract and urine sample
- histopathology specimens

Routine blood tests can also be carried out at CL2, but risk assessment of procedures should be done beforehand. Though capping and uncapping of blood samples don’t generate an aerosol, still, care must be given to the type of tube used for collection.

The design and facilities within CL3 which deal with the HG3 pathogens have been mentioned in the WHO laboratory biosafety manual. Such labs are designed to maintain a directional airflow into the laboratory room with high-efficiency particulate air (HEPA) filtration for decontamination of air before it is released into the environment.

The decision on the type of work in maximum CL4 depends on risk assessment or staff who think such a containment level is necessary to minimize the risk of transmission in the healthcare setting. Such laboratories are highly equipped and maintain a negative pressure in the laboratory with controlled ventilation.

**II. PERSONAL PROTECTIVE EQUIPMENT**

WHO recommends the rational use of PPE. Precautions must be taken by all laboratory workers while handling and processing the specimens as there is a chance of spill, splash, droplet, and aerosol generation. In a laboratory setting, lab technicians, who are involved in the manipulation of respiratory samples should wear a medical mask, disposable gown, gloves, goggles, or visor for eye protection and close toe footwear. All the staff should be trained in proper

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<td><strong>Hazard Group</strong></td>
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doffing and donning. Laboratory gowns worn in the CL3 area should have side or back closure, long sleeves, fitting cuffs, and a Velcro fastening and of knee-length. All the staff must remove PPE before leaving the laboratory and proper hand hygiene must be followed. Donning and doffing of PPE should be done in a specially designated area and proper guidelines have to be followed.

III. HISTOPATHOLOGY SAMPLES

a. Transfer of histopathology specimens

Histopathology samples should be properly labeled, and the container should be doubly packed in a sealable plastic bag to minimize the spill. The specimen request forms should not be wrapped around the containers and should be sent in a separate waterproof envelope.

b. Fixative and fixation time

To date, we know little about the effective disinfectants for SARS-CoV-2 and the idea about the safety of histological fixation and processing remains on the dark side. Though various expert has suggested that the disinfectants that have shown to lower the infectivity of other coronaviruses, should inactivate SARS-CoV-2 too.

The common fixative used in histopathology is formalin and we are fortunate that this chemical can decrease the viral infectivity in a time and temperature-dependent manner. Darnell et al have mentioned that formalin can inactivate the SARS-CoV at 37°C or room temperature after 1 day and glutaraldehyde completely inactivated the virus by day 2 at 25°C and by day 1 at 37°C. So, proper fixation of specimens with a contact time of 1-2 days with an adequate amount (1:10 ratio) of formalin can be considered before the grossing of tissue samples. The external surface of the specimen container should be disinfected before touch.

Another similar study showed that coronaviruses lose their infectivity when tissue is kept at 67°C for 60 minutes. Paraffin infiltration requires a temperature of 60-62°C, so it implies that formalin-fixed paraffin-embedded tissue block lowers the viral infectivity and decreases the risk of exposure. With these, it appears justifiable that handling and processing of the frozen section can pose a risk and should be deferred. Though the risk of aerosol production during the frozen section is low however there is a possibility of droplet exposure. If the processing is unavoidable then proper use of PPE and decontamination of cryostat should be done.

IV. LOWER RESPIRATORY TRACT SAMPLES, BODY FLUIDS, AND URINE SAMPLES

The rate of detection of the virus is very high in bronchoalveolar lavage, followed by sputum and bronchial brush samples. It has been mentioned that non-respiratory samples like blood, urine, and body fluids are known to contain the virus. However as per Universal precautions by the Centers for Disease Control (CDC), all body fluids except sweat should be considered infectious and standard precautions should be taken. As per frequency and detection of viral nucleic acids, cytopathology samples are categorized into 3 groups as follows:

1. High risk with the virus: Upper and lower respiratory tract samples, nasopharyngeal swab, oropharyngeal swab, sputum, blood and bloody samples, feces and anal swabs, teardrop and conjunctival discharge

2. Intermediate risk with the virus: Pleural effusion, pericardial effusion, urine

3. Low risk with the virus: Ascites and peritoneal washing, uterine cervical smears, vaginal discharge, CSF, synovial fluid, semen, cell blocks.

Considering the scenario of a resource-limited laboratory setting, the cytology samples can be received in alcohol-
based fixatives such as ethanol (with alcohol concentration more than 70%) or formalin to inactivate the virus. However, if weaker alcohol-based fixatives are used, then additional precautionary measures have to be taken. In our setting also we are collecting body fluids in alcohol-based fixatives (95% ethanol) and samples are then processed during the evening time of the same day.

The processing of all cytology samples can lead to aerosol/droplet formation during the opening of containers, removing tube caps, blending, vigorous shaking or mixing, vortexing, pipetting, aliquoting, diluting or centrifugation of fluids, and discarding supernatant including loading and unloading centrifuge rotors and cups which should be performed in a Class II BSC.

In a resource-limited laboratory setting, when centrifugation is required but BSC is not available, then the use of properly capped tubes, careful removal of centrifuge lid and cap after allowing a full 5 minutes rest following centrifugation along with the use of N95 or equivalent mask instead of a surgical mask and visor for eye protection should be carried out to minimize the risk of exposure. It is best to handle the high-risk specimens for processing by one technician at a time by using a separate station.

**V. FINE NEEDLE ASPIRATION CYTOLGY**

Fine needle aspiration cytology (FNAC) on suspected or known COVID-19 cases should be discouraged whenever possible and should only be done when it is likely to change the patient management and on a case by case basis. All the patients, in whom FNAC is mandatory, should wear a mask and should be counseled not to cough during the procedure.

During FNAC, the practice of expelling the material from the needle hub and syringe, smearing the material, drying the smear by shaking or blowing air leads to aerosol and droplet generation. Hence it is recommended not to remove the material from the needle but if required should be done with caution and the process of air drying or even heat drying of smears should be carried out in BSC class II. When a Class II BSC is not available or when patients with suspected or known positive COVID-19 have to be faced then it is recommended to use an N95 or equivalent mask instead of a surgical mask.

Staining of all propanol fixed slides can be done as per each laboratory protocol while for staining of air-dried smears with Giemsa stain, the initial fixation step can be prolonged for 1 minute with daily disposal of all fixatives. After the procedure, needles should be capped and discarded in a sharp resistant waste container. The entire syringe should be disinfected and discarded in biohazard waste bags. Burning of needles should be prohibited as it can lead to the generation of aerosol. As for the good laboratory practices, in our setting, we have been scheduling the FNAC practices, on every alternate day with subsequent fumigation of the FNAC procedure room by chlorine at the end of the procedure. All the air-dried and alcohol-fixed slides are kept untouched in the FNAC procedure room for one whole day before they are transferred to the cytology laboratory for staining. Such practices can at times minimize the risk of exposure.

**VI. HEMATOLOGY SAMPLES**

All the blood samples should be collected by trained personnel. To avoid accidental leakage or spillage, secondary containers with a rack should be used so that the blood samples remain upright, and such secondary containers should be regularly decontaminated. Specimen request or specification forms should not be wrapped around the blood sample but should be sent in separate waterproof envelopes. Fixing and staining of peripheral blood smear does not kill the viruses, so should be handled carefully with forceps, appropriately stored, and decontaminated and/or autoclaved before disposal. Application of the coverslip to each of the peripheral blood smear and bone marrow aspiration slides can also be considered to prevent direct contact with the smears.

**VII. OCCUPATIONAL EXPOSURE**

a. When intact or damaged skin is contaminated by body fluids, blood, secretions from the patient, remove the contaminants with clean tissue or gauze and apply 0.5% iodophor and let it sit for 3 minutes followed by a flush with running water.

b. When mucous membranes, such as eyes and respiratory tract are exposed then flush with plenty of normal saline.

c. Sharp object injury (piercing of body by sharp objects that were directly exposed to the patient's body fluids, blood, secretions): Flush the wounded area with running water and apply 0.5% iodophor.

d. Direct exposure of respiratory tract (falling off a mask, exposure of mouth or nose to a confirmed patient who is not wearing a mask): Immediately leave the area. Gargle with plenty of normal salines or betadine.

A written record of all incidents and accidents must be maintained in accordance with national regulations. The person should be isolated and observed (except for intact skin exposure) for 14 days for any symptoms.

**VIII. DISPOSAL AND DECONTAMINATION**

All the residual samples including sample tubes, containers, syringe used during the FNA procedure, should be discarded.
in appropriate disinfectants with confirmed virucidal activity against enveloped viruses and should be discarded in separate labeled biohazard waste bags. Single-use tubes are highly recommended. All the medical waste should be kept in a double layer medical waste bag or special plastic box for sharps and should be sealed properly and the outer surface should be sprayed with 1000mg/l chlorine-containing disinfectant. The infection level must be attached and should be disposed of by the medical waste disposal unit provided within health facilities.

Decontamination of work surfaces, including the commonly touched surfaces including computer keyboards, phones, and microscopes should be performed multiple times per day is mandatory. Various biocidal agents have shown to be effective in decreasing the viral load such as sodium hypochlorite, ethanol, hydrogen peroxide, quaternary ammonium compounds, and phenolic compounds.

The most easily available disinfectant in the hospital is sodium hypochlorite (bleach). A 0.1% sodium hypochlorite (1,000 ppm) for the general surface and a 1% sodium hypochlorite (10,000 ppm) for the disinfection of blood spills should be used with a contact time of 10 minutes. For the disinfection of metal surfaces and items, alcohol (ethanol or propanol) at 60-70% concentration should be used, as sodium hypochlorite is corrosive on metal surfaces and items.

CONCLUSIONS

Knowledge about safe handling and processing of all pathology specimens is crucial at this time of outbreak to ensure the safety of healthcare personnel involved in the laboratory work as well as to prevent the hospital and healthcare personnel themselves from being a source of infection.

Conflict of interest: None

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