

# Proposal for safe oral pathology laboratory practices in COVID-19 pandemic

Dear Colleagues,

Coronavirus disease (COVID-19) has been declared a pandemic by the World Health Organization (WHO) as it has affected an enormous segment of humanity globally. Oral pathology departments deal with biological tissues for cytopathology and histopathological analyses and blood samples for hematological investigations or research purposes. Such samples contaminated with viable COVID-19 viruses may be potentially infectious to pathologists and laboratory personnel. We propose the following practical guidelines to minimize this risk.

## GENERAL GUIDELINES

The WHO recommends that all the samples collected for laboratory investigations are to be considered potentially infectious and stringent precautions should be taken while handling them.<sup>[1]</sup> The WHO and Centers for Disease Control and Prevention (CDC) have laid down recommendations for infection control in laboratories, [Table 1] which are applicable to Oral pathology laboratories as well [Table 1].<sup>[2-4]</sup> Further, various professional bodies have also given their recommendations regarding prioritization and deferral of pathology work during COVID-19.<sup>[5]</sup> They have recommended that all tests that are not essential for the management of patients should be stopped. They have also recommended that repeat biopsy may only be acquired if an initial sample does not give sufficient information regarding clinical management.<sup>[5]</sup>

- Staff must follow the “standard precautions” including principles of social distancing, hand hygiene (frequent hand washing for 20 s), respiratory hygiene, wearing PPE (masks and gown/laboratory coat and eye protection) and refrain from touching mucosal surfaces (eyes, nose and mouth) even during routine days (with and without handling COVID19 samples) during this period of COVID-19<sup>[6]</sup>
- Entry to laboratory should be restricted to staff, and records should be maintained for entry of visitors or patients
- Laboratory staff should be adequately trained to handle infectious specimens from time to time and

should be trained in good microbiological practices and procedures (according to the WHO and CDC guidelines)

- Donning and doffing (according to CDC recommendations) should be performed in a specifically designated area
- Convenient reallocation of the laboratory personnel in teams should be done to minimize crowding in laboratory, and if one team is exposed, the others are still available to manage the laboratory.

## PATIENT EXAMINATION

- Examination of patients should be deferred until and unless there is an emergency
- Crowding should be avoided, and social distancing should be maintained in the examination and outpatient department areas
- All the patients in waiting areas should be wearing masks at all times. No visitors should be allowed in the examination room
- The use of antimicrobial mouth rinse could reduce the number of microbes in the oral cavity; thus, it is recommended to be used by patients before the oral examination<sup>[7]</sup>
- Personal protective equipment (PPE) must be used before patients' examination, i.e., Goggles/face shield, full-sleeved water-resistant gowns, N95 masks, gloves and shoe covers). The room should be disinfected after the procedure.

## HISTOPATHOLOGY PRACTICES

Histopathology laboratories are fortunate that routine histopathology processing inactivates many viruses such as Ebola, and studies have shown similar results for SARS-CoV-2 as well.<sup>[8]</sup> However, caution must be exercised during each stage of sample processing, in accordance with the guidelines stated as under.

### Specimen receiving

- Only a trained hospital attendant should transport the sample to the laboratory. He/she should be acquainted with guidelines pertaining to safe handling practices and should be wearing masks and gloves

- Specimens should be transported by hand, and not shipped with pneumatic-tube systems
- All specimens should be labeled properly with patient details and status of COVID-19 (negative, suspected, positive or Not Tested). Before taking a biopsy of a COVID suspect or positive patients, communication to oral pathology laboratory is recommended for adequate arrangements to be made for receiving such samples
- Specimens should always be transported in double sealed leak-proof specimen containers, following WHO and CDC guidelines. Specimens from confirmed COVID-19 patients should have a biohazard symbol on the outer container
- Technical staff receiving the specimens should wear double gloves, N95 or surgical masks and shoe covers. On receiving, the exterior of the specimen container should be disinfected.

### Laboratory request forms

- Online request forms should be preferred and made accessible to staff and clinicians to promote digital workflow. The COVID status of the sample should be mentioned on requisition forms (preferably color-coded)
- If electronic requisition forms are not practicable, a clean requisition form not touched by contaminated hands, gloves or any other infective material should be sent. Hence, it is preferable to be filled by the person who has not come in contact with the patient or patient's attendants
- Once received in the laboratory, these forms can preferably be kept under an ultra violet chamber for 60 min to inactivate coronavirus if any.<sup>[9]</sup>

### Grossing of specimens

- Grossing of specimens should be done after the requisite time of fixation and preferably specimens should be grossed by one resident or faculty wearing appropriate PPE. Formalin fixation with 10% neutral-buffered formalin (NBF) of small-sized specimens should be for a minimum 24 h. The volume of NBF should be almost 10–15 times the volume of the tissue specimen. Large size specimens should be subjected to longer formaldehyde fixation (at least 48–72 h)
- Slicing of specimens for fixation should be done under formalin. Care should be taken while handling and sectioning specimens, and discarding and changing solutions to generate minimum aerosols
- One area of grossing should be decided for COVID-19 suspected/positive samples, where air conditioning systems should be switched off with provision

for cross ventilation facility and exhaust system. The items used in the grossing area should be not mixed with other items in the laboratory, to prevent cross-contamination. The grossing table used for such specimens should not be used for other specimens until it is properly disinfected. Staff processing these samples should always wear masks and gloves and discard the processing solutions after processing

- Any aerosol-generating procedure such as bone cutting should be avoided. It should be performed only when absolutely required for staging, management or diagnosis of disease. Hand saw should be preferred over electric bone saw in those conditions
- The designated grossing area and all equipment and items in that area should be disinfected every day after the grossing.

### Storage and discarding of specimens

All specimens marked as COVID-19 suspected or positive should be separately stored in a different shelf, clearly labeled as COVID-19 specimens. They should be separately discarded following institutional recommendations.

### Histological processing of specimens

According to Duan *et al.*, coronaviruses are found to be inactivated at 56°C for 90 min, 67°C for 60 and 75°C for 30 min.<sup>[9]</sup> As routine histopathology processing involves infiltration with paraffin wax at a temperature of 60°C–65°C for 2 h or more, thus it can be considered that formalin-fixed and paraffin-embedded tissue has a low degree of infectivity. Lab staff should, however, wear laboratory coat/gown, gloves and masks while handling specimens, slides and blocks.

### Frozen sections for intraoperative margin assessment and for direct immunofluorescence

As the frozen section involves the use of unfixed specimens and is considered an aerosol-generating procedure, it should be deferred during this period of COVID-19 pandemic. Main deterrents are the persistence of SARS-CoV-2 on inanimate surfaces such as cryostats, which can survive temperatures of –20°C, the temperature used for sectioning fresh-frozen tissues.<sup>[10]</sup> Second, it takes a long time to disinfect cryostat after the procedure. However, if sectioning of fresh-frozen specimens is absolutely necessary, the same protections for technical and medical staff must be applied as for cytopathology (described below).

### Reporting

Pathologists must follow adequate hand hygiene before and after reporting.

- They should use gloves while handling and interpreting slides, as it involves handling by multiple hands before it reaches to the pathologist. Alternatively, immersing slides in 95% alcohol for a few minutes will also inactivate the virus<sup>[11,12]</sup>
- Frequent contact surfaces of microscopes should be sanitized by 70% alcohol solution before initiating the reporting. Pathologists either may report independently either on their microscope or prefer digital reporting wherever feasible. If they are reporting at a multi-header microscope, no more than three members are recommended at a given time, maintaining distance among themselves<sup>[13]</sup>
- One staff and one pathologist team should be dedicated at a time for handling and reporting the specimens to avoid exposure to others. Further, the slides should be kept in closed boxes to avoid exposure.

## CYTOPATHOLOGY PRACTICES

Cytology specimens are highly infective, as it uses fresh samples with no prior fixation for analysis. Further, it requires smear preparation, sometimes centrifugation and cell block preparation, which are aerosol-generating procedures. Oral cytology is still considered a screening tool and in most of cases, requires biopsy for further confirmation. Hence, during this pandemic, it can be avoided or should be limited to emergency procedures with the use of proper precautions as mentioned below.

### Performing cytological procedures

- Cytology involves the scraping of oral mucosa by sample collection tool (spatula/cytobrush, etc.) or fine needle aspiration cytology (FNAC) from the patient's mouth by clinicians or pathologists. Rapid on-site evaluation further exposes pathologists, trainees and technicians. Such procedures should preferably be performed in a well-ventilated room without air conditioning, or air-conditioned with HEPA filters. The use of PPE, including double gloves, full-sleeved water-resistant gown, hood, shoe covers and face shield/goggles, is recommended for these procedures<sup>[13]</sup>
- If FNAC is to be done from lymph node/extraoral swelling (not involving oral cavity), patient should wear a mask throughout the procedure. However, while sampling from oral cavity, patient's head positioning should be in a way to avoid his/her direct breathing toward pathologists. Patients should be counseled not to cough during the procedure. If the patient is uncooperative, sampling should be abandoned and the referring clinician informed<sup>[13]</sup>
- Clinician/pathologist should fix cytology specimens

into alcohol immediately after sampling

- Patient area with separate air circulation should be near to the FNA laboratory.

### Processing of specimens

- Fresh, unfixed specimens should be transported by hand and not shipped with pneumatic-tube systems
- Procedures should be performed wearing an N95 mask, gloves, full-sleeved gown, cap and goggles/face shield<sup>[14]</sup> during the entire procedure
- Droplets or aerosols are generated from various steps in cytology including expelling aspirates from the needle or syringe, smearing the aspirated material, opening containers, removing tube caps, vigorous shaking, vortexing, pipetting, aliquoting, diluting, centrifugation, cytopsin, discarding supernatant and air/heat drying of smears. Precautions should be taken to minimize exposure to aerosol during the processing of samples
- Thus lab staff should use adequate PPE and perform these steps in Class II Biosafety cabinets is to be considered (BSCs). However, if not available, air conditioning systems in the laboratory should be switched off and cross ventilation is to be preferred
- Centrifugation should be done using screw-capped tubes. Centrifuged specimens should be allowed to stand for 5 min, after which the cap should be opened gently
- Aspirated material should be expelled very gently from the needle, and personnel should keep his/her face as distant from the slide as possible<sup>[13]</sup>
- Preparation of air-dried smears should be avoided. If unavoidable, lab staff should refrain from agitating the smears or using handheld fans for drying of smears. Further, drying smears in enclosed coupling jars is recommended to reduce aerosols
- Residual samples should be discarded in appropriate disinfectants with virucidal activity, as described in the disinfection section. If any spillage of samples occurs on tables, it should be disinfected properly
- Used needles should be discarded in puncture-proof containers, as incinerating them can create aerosols.<sup>[14]</sup> The syringe hub should be cut, and the entire syringe should be disinfected (mentioned in disinfection procedures) and discarded in specifically marked biohazard waste bags.<sup>[13]</sup>

## HEMATOLOGICAL INVESTIGATIONS

- Health-care workers collecting the sample (blood, serum and plasma) should follow universal precautions. They should wear appropriate PPE (eye/face protection, mask, gloves, long-sleeved gown)

- Cases where aerosol-generating procedure is performed, for example, Vortexing, loading or unloading samples in rotor and cups of centrifuge should be carried out in biological safety cabinet (BSC). Similarly, sealed centrifugation should be preferred
- If any procedure is performed outside BSC, precautions should be taken to minimize sample release
- One patient should be allowed in the room at a single time to reduce the risk of spreading transmission
- Sample transportation and disinfection protocols to be followed as recommended for COVID-19.

## SURFACE DISINFECTION PROCEDURES AND BIOMEDICAL WASTE MANAGEMENT

Since viruses can remain viable on surfaces (plastic and stainless steel) for up to 72 h, surface disinfection procedures are essential for the inactivation of the virus. Hence, all workstations along with the laboratory furniture and equipment must be appropriately disinfected before and after the procedures. Various disinfecting agents that can be used are sodium hypochlorite (bleach; 1000 parts per million (ppm) (0.1%) for general surface disinfection and 10,000 ppm (1%) for disinfection of blood spills), 62%–71% ethanol, 0.5% hydrogen peroxide, quaternary ammonium compounds and phenolic compounds, to be used according to the manufacturer's recommendations. Particular attention should be paid not only to the selection of the disinfectant but also the manufacturer's recommendation for contact time, dilution (concentration of the active ingredient), care in handling and an expiry date after the working solution is prepared.<sup>[6]</sup>

Following the processing of samples after grossing from a COVID-19 positive/suspected patient, the full PPE is to be discarded as per hospital/institutional policies for COVID-19. All laboratory-generated biomedical waste from these samples must be processed as per institutional recommendations.

## SUMMARY

Personal hygiene, social distancing and precautions during all steps in the oral pathology such as sampling, sample receiving, sample processing and reporting, etc., will ensure the safety of all laboratory personnel during this epidemic. Laboratories that are not able to meet biosafety recommendations should consider transferring specimens to national, regional, or international referral laboratories that can meet the biosafety requirements.

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Nil.

## Table 1: Laboratory safe practices according to the World Health Organization and Centers for Disease Control and Prevention recommendations

### Collection of specimen

All health-care workers collecting the specimens should use appropriate PPE. This includes a medical mask (N95/high level respirator or facemask if a respirator is not available), eye protection, a long-sleeved gown and gloves. If the specimen is collected during an aerosol-generating procedure which increases the risk of infection, particulate respirator (N95/high level respirator) should be prioritized for these personnel

Collection can be performed in a normal examination room. No visitors should be allowed in the room

Cleaning and disinfection procedures recommended for healthcare settings should be performed

### Processing of specimen

Laboratory workers should wear appropriate PPE when handling potentially infectious specimens. PPE should include disposable gloves, laboratory coat/gown and eye protection

Any procedure with the potential to generate aerosols or droplets (e.g. vortexing) should be performed in a certified Class II BSC. Appropriate physical containment devices (e.g. centrifuge safety buckets, sealed rotors) should be used for centrifugation. Ideally rotors and buckets should be loaded and unloaded in a Class II Biological Safety Cabinet

Preparation and chemical or heat fixation of smears for microscopy should be done in a certified Class II Biological Safety Cabinet

COVID-19 associated laboratory waste should be disposed following standard procedures associated with other respiratory pathogens (like seasonal influenza and other human coronaviruses)

After specimens are processed, work surfaces and equipment should be decontaminated with appropriate disinfectants

### Transportation of specimens

Personnel transporting the specimens should be trained in safe handling practices and spill decontamination procedures

Specimens should be transported in leak-proof specimen bags (secondary containers) that have a separate sealable pocket for the specimen (a plastic biohazard specimen bag), with the patient's label on the specimen container (the primary container), and a clearly written laboratory request form

Recommended biosafety practices and transport requirements should be followed

All specimens should be delivered by hand and use of pneumatic-tube systems should not be used to transport specimens

Each patient's full name, date of birth and COVID-19 status should be mentioned on the laboratory request form

Laboratory should be notified as soon as the specimen is transported

COVID-19: Coronavirus disease, PPE: Personal protective equipment, BSC: Biological Safety Cabinet

## Conflicts of interest

There are no conflicts of interest.

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