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. The WHO and Centers for Disease Control and Prevention (CDC) have laid down recommendations for infection control in laboratories, which are applicable to Oral pathology laboratories as well. Further, various professional bodies have also given their recommendations regarding prioritization and deferral of pathology work during COVID-19. 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.

- 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.
- 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.
- 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. 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.

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 9 for 30 min. 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. 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\cite{11,12}
• 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\cite{13}
• 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\cite{13}
- 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\cite{13}
- 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\cite{14} 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, cytospin, 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\cite{13}
- 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.\cite{14} 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\cite{13}

**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)
**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.

**Financial support and sponsorship**

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. |

**REFERENCES**

1. Infection Prevention and Control During Health care When Novel Coronavirus (nCoV) Infection is Suspected n.d. Available from: https://www.who.int/publications-detail/infection-prevention-and-control-during-health-care-when-novel-coronavirus-(ncov)-infection-is-suspect
ed-20200125. wLast accessed on 2020 May 22].

2. CDC. Coronavirus Disease 2019 (COVID-19). Centers for Disease Control and Prevention; 2020. Available from: https://www.cdc.gov/coronavirus/2019-ncov/infection-control-recommendations.html. [Last accessed on 2020 May 28].

3. Laboratory Biosafety Guidance Related to Coronavirus Disease (COVID-19) n.d. Available from: https://www.who.int/publications-detail/laboratory-biosafety-guidance-related-to-coronavirus-disease-(covid-19). [Last accessed on 2020 May 28].

4. World Health Organization. Laboratory Testing for Coronavirus Disease 2019 (COVID-19) in Suspected Human cases: Interim Guidance; 02 March, 2020.

5. Guidance for Pathology Testing Prioritisation v1.1 Final. Institute of Biomedical Science n.d. available from: https://www.ibms.org/resources/documents/guidance-for-pathology-testing-prioritisation-v10-final/. [Last accessed on 2020 May 31]

6. Tan SS, Yan B, Saw S, et al. Practical laboratory considerations amidst the COVID-19 outbreak: early experience from Singapore [published online ahead of print, 2020 Mar 20]. J Clin Pathol. 2020;clinpath-2020-206563.

7. Meng L, Hua F, Bian Z. Coronavirus Disease 2019 (COVID-19): Emerging and Future Challenges for Dental and Oral Medicine. J Dent Res. 2020;99(5):481-487.

8. Darnell ME, Subbarao K, Feinstone SM, Taylor DR. Inactivation of the coronavirus that induces severe acute respiratory syndrome, SARS-CoV. J Virol Methods. 2004;121:85-91.

9. Stability of SARS Coronavirus in Human Specimens and Environment and Its Sensitivity to Heating and UV Irradiation n.d. Available from: https://pubmed.ncbi.nlm.nih.gov/14631830/. [Last accessed on 2020 May 22].

10. Compérat E. What Does COVID-19 Mean for the Pathology-Urology Interaction?. Eur Urol. 2020;78:e43-e44.

11. Odega K. Safe Laboratory Practices in the Light of Covid-19 Pandemic: Way Forward in a Resource Limited Setting; 2020.

12. Pambuccian SE. The COVID-19 pandemic: Implications for the cytopathology laboratory. J Am Soc Cytopathol 2020;9:202-11.

13. Srinivasan R, Gupta P, Rekhi B, Deb P, Nijhawan VS, Prasoon D, et al. Indian academy of cytopathologists national guidelines for cytopathology laboratories for handling suspected and positive COVID-19 (SARS-COV-2) patient samples. J Cytol 2020;37:67.

14. SARS Guidance Lab Biosafety for Handling and Processing Specimens CDC; 2019. Available from: https://www.cdc.gov/sars/guidance/lab/app5.html. [Last accessed on 2020 May 28].

Submitted: 29-Jun-2020, Revised: 29-Jun-2020, Accepted:02-Jul-2020, Published: 09-Sep-2020

This is an open access journal, and articles are distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 4.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as appropriate credit is given and the new creations are licensed under the identical terms.

| Access this article online |
|---------------------------|
| Quick Response Code:      | Website: www.jomfp.in |
| DOI: 10.4103/jomfp.JOMFP_278_20 |

How to cite this article: Mishra D, Kaur H, Kakkar A. Proposal for safe oral pathology laboratory practices in COVID-19 pandemic. J Oral Maxillofac Pathol 2020;24:217-21.