Cytologic and Histologic Samples From Patients Infected by the Novel Coronavirus 2019 SARS-CoV-2: An Italian Institutional Experience Focusing on Biosafety Procedures

Esther Diana Rossi, MD, PhD, MIAC [1]; Guido Fadda, MD[1,2]; Antonino Mule, MD[1]; Gian Franco Zannoni, MD [1,2]; and Guido Rindi, MD[1,2]

The 2019 coronavirus pandemic, which started in Wuhan, China, spread around the globe with dramatic and lethal effects. From the initial Chinese epicenter, the European diaspora taxed the resources of several countries and especially those of Italy, which was forced into a complete social and economic shutdown. Infection by droplets contaminating hands and surfaces represents the main vehicle of diffusion of the virus. The common and strong efforts to contain the pandemic have relevant effects on the management of samples from histopathology laboratories. The current commentary reports and focuses on the protocols and guidelines in use at a large tertiary Italian hospital that accordingly are proposed for adoption in Italian laboratories as a potential model for national guidelines for the coronavirus emergency. ***Cancer Cytopathol* 2020;128:317-320**.

KEY WORDS: biosafety guidelines; coronavirus; cytology; fine-needle aspiration; histology; personalized medicine.

This commentary provides an overview of laboratory biosafety related to the testing of histological and cytological specimens from Italian patients infected with the novel pathogen first identified in Wuhan, China, at the end of 2019 and known as the novel coronavirus 2019-nCoV or 2019 SARS-CoV-2. The virus, also known as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), quickly spread throughout the world in January 2020, and now involves most countries around the globe.[1-5] The infection presents most often as an acute respiratory illness with varying degrees of severity, and the fatality rate ranges from 1% to 5%.[1,2] During the preparation of this article in mid-March, 2020, the infection accounted for >275,000 cases and 11,000 deaths worldwide, but the number of infected individuals continues to rise rapidly. Fatalities are secondary to severe alveolar damage and progressive respiratory disease, more often occurring in the elderly or in patients with compromised health. 2019 SARS-CoV-2 infections include 2 different classes: 1) patients with pulmonary symptoms (interstitial bilateral pneumonia) severe enough for them to be treated in the hospital and leading in many cases to death; and 2) asymptomatic, 2019 SARS-CoV-2–positive patients, who mostly are untested. These 2 classes of patients indicate distinct rates of transmission in all countries affected,[1] although those patients who are asymptomatic and able to spread the virus represent a key area of interest for scientists.

On February 11, 2020, the World Health Organization (WHO) formally named the virus COVID-19,[3] confirming that it is a variant of the virus that had caused a serious and lethal acute respiratory syndrome

Corresponding Author: Esther Diana Rossi, MD, PhD, MIAC, Anatomic Pathology Section, Department of Life Sciences and Public Health, Catholic University of the Sacred Heart, Agostino Gemelli University policlinic Foundation, IRCCS, Largo Francesco Vito 1, 00168 Rome, Italy (esther.rossi@policlinicogemelli.it).

[1]Unit of Anatomic Pathology, Agostino Gemelli University Policlinic Foundation, IRCCS, Rome, Italy; [2]Anatomic Pathology Section, Department of Life Sciences and Public Health, Catholic University of the Sacred Heart, Rome, Italy

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All the authors contributed equally to this article.

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(SARS) in 2002 through 2003.[3-6] The human-to-human transmission of the virus occurs mostly via droplets contaminating the hands and surfaces, although other means of transmission are possible, and the incubation period typically ranges from 2 to 14 days.[7] A wide variety of efforts to contain the virus are ongoing all over the world, including in Italy, which is facing one of the most dramatic health emergencies of the last century.

Beginning in China, the infection quickly spread to and ravaged northern Italy at the beginning of February 2020.

The entire country of Italy rapidly became the epicenter for COVID-19 among the European countries. Currently, there have been >33,190 COVID-19–positive cases and deaths in Italy, surpassing the numbers in China (3402 vs 3265 deaths as of March 19, 2020). At the end of February 2020, the Italian government enforced a regional lockdown in the north of the country. This was followed 1 week later by a shutdown of the entire country with even more severe restrictions implemented on March 21, 2020, and the adoption of a shutdown model with markedly limited aid for patients. Just as the first line of medical care is represented by specialists involved in respiratory care and emergency medicine, clinical pathology laboratories represent the receiving end for samples from infected patients.

The prospect of COVID-19–positive specimens in our clinical laboratories requires very careful consideration of the measures needed to minimize the spread of the virus and to protect medical pathologists and staff who likely will handle infectious samples on a daily basis. The WHO guidelines recommend that all samples collected for histopathology should be considered to be potentially infectious.[3] Specific regulations were proposed to reduce exposure to the virus, including a stipulation that any testing should be performed by professional staff who are well trained in the relevant technical and safety procedures. As reported by Henwood in his editorial, the US Centers for Disease Control and Prevention also released interim laboratory biosafety guidelines for the handling and processing of coronavirus specimens (these can be found online at https://www.cdc.gov/coronaviruses/2019-nCoV/lab/lab-biosafety-guidelines.html).[7]

Limitations in our knowledge regarding COVID-19 infections have led to several critical unanswered questions concerning which disinfectants are most effective for COVID-19, as well as the appropriate fixation and processing methods for both histological and cytological samples. Despite the fact that many aspects of this novel virus are unknown, the genetic similarities between COVID-19 and Middle East Respiratory Syndrome (MERS) coronavirus suggest that COVID-19 may be susceptible to disinfectants containing sodium hypochlorite (ie, bleach). Currently, as described by Kampf et al, one of the most effective disinfectants used for removing the virus from surfaces is 62% to 71% ethanol, 0.5% hydrogen peroxide, or 0.1% sodium hypochlorite for 1 minute.[8] Historically, histopathology protocols have almost always been effective in inactivating a broad range of viruses, even Ebola, and recent studies demonstrated that formalin and glutaraldehyde were able to inactivate SARS-CoV in a temperature-dependent and time-dependent manner.[7-11]

Based on those data and following guidelines from the WHO, the study institution in Rome, Italy, developed an internal protocol for histological and cytological samples from patients with suspected or proven COVID-19 infection. The aim of the protocol is the safety of laboratory personnel who have been properly trained to ensure that appropriate risk control measures are in place.

The histological protocol for surgical samples includes the following steps: 1) maintaining formalin fixation for 48 hours in a dedicated fume cabinet; 2) grossing of the specimen in a dedicated, certified, class II biological safety hood; 3) an additional 24 hours of formalin fixation in a dedicated fume cabinet; and 4) sampling and additional specimen grossing in a dedicated, certified, class II biological safety cabinet.

The above biosafety procedure is performed by trained technical and medical personnel wearing all appropriate personal protective equipment (PPE). The procedures are performed in a laboratory room with a controlled ventilation system that maintains inward directional airflow, and a dedicated hand wash sink must be available in the laboratory.

The following additional specific precautions were included: 1) separate storage and grossing spaces should be used (ie, a COVID-19–dedicated fume cabinet and a COVID-19–dedicated fume hood); 2) proper sanitation of external and internal components of both the cabinet and hood after use; and 3) waste handling of sharps and other materials according to routine procedures for hospital infective waste.

With regard to frozen sections, these sections should be limited to essential cases unless the laboratory cryostat is equipped to avoid the generation of aerosol droplets. In conjunction with the above, a COVID-19–dedicated fume hood for specimen manipulation and a COVID-19–dedicated cryostat were designated in our frozen section laboratory. Contributing to the limitations in the number of frozen sections performed, all outpatient activities as well as elective surgeries at our institution were postponed.

Similar protocols were needed for the handling and processing of cytological samples. The procedural changes for cytology were more rigorous than for histological samples because the majority of liquid-based cytology preparations use relatively low alcohol concentrations.

In response to the COVID-19 pandemic affecting Italy, the Italian Cytology Committee chaired by Drs. Guido Fadda from Rome and Giovanni Negri from Bozen-Bolzano University in Bolzano, Italy, on behalf of the National Society of Anatomic Pathology and Histology, used the national website ITAPAT to outline specific recommendations for pulmonary and oral cytology samples. Most important, cytological evaluations should be performed only for essential cases, thereby limiting and reducing the number of routine pulmonary and oral cytology samples. For cases deemed to be essential, the cytological specimens should be handled with proper security measures to protect all the technical and medical staff involved in the procedure. The cytological material is processed in a dedicated hood under the supervision of specialized technicians wearing adequate PPE (eg, masks with visors, eye protection [goggles or face shields], disposable medical gloves, a disposable water-repellent gown or coveralls with sleeves that fully cover the forearms, and shoe covers or dedicated shoes). The glass slides are placed into a 70% alcohol fixative solution and, if needed, 99% ethanol is added to the fixation solution, which is considered the safest way to handle cytological samples. Despite the fact that this method of processing ultimately may alter the quality of the cytology sample when compared with samples processed using methanol-based fixatives, the decision to modify the standard approach for cytology sample preparation was made to guarantee safety in the laboratory.

Below is our modified "off-label" method currently adopted at the study institution for all liquid-based cytology specimens processed under the Hologic protocol:

1. Collect the sample in a 70% ethyl alcohol solution
2. Centrifuge at 600 g for 10 minutes or 1200 g for 5 minutes
3. Pour off the supernatant fluid and resuspend the cell pellet
4. Add 30 mL of CytoLyt solution to reduce biological contamination
5. Centrifuge at 600 g for 10 minutes
6. Pour off the supernatant fluid
7. Resuspend the cell pellet
8. Evaluate the cell pellet; if necessary, repeat from step 5
9. Add an appropriate amount of specimen (depending on the size of the cell pellet) to the PreservCyt solution vial
10. Allow to stand in PreservCyt for 15 minutes
11. Run on a ThinPrep 2000 processor or ThinPrep 5000

This specimen processing procedure is performed by technical and medical personnel, all of whom are wearing appropriate PPE. Those laboratories that are not able to meet the biosafety recommendations should consider transferring specimens to regional or national referral laboratories with COVID-19 detection capacity that can implement the recommended biosafety requirements. As an additional step toward improved laboratory safety, the study laboratory stopped preparing cell blocks for cytology specimens. Similarly, rapid on-site evaluation procedures were discouraged.

It is important to emphasize that in this emergency, the diagnostic processing and evaluation of cytological samples should be limited to essential cases for which the final cytological diagnosis is likely to significantly influence patient management. During the COVID-19 pandemic, the adoption of strict protocols and guidelines is critical for establishing and maintaining a safe work environment. Although the modification of original protocols may result in certain unanticipated diagnostic issues, the benefits in terms of laboratory biosafety during this pandemic are considered more important.

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REFERENCES

1. Li R, Pei S, Chen B, et al. Substantial undocumented infection facilitates the rapid dissemination of novel coronavirus (SARS-CoV2).

Science. Published online March 16, 2020. doi:10.1126/science.abb3221
2. Henwood AF. Coronavirus disinfection in histopathology. *J Histotechnol*. 2020:1-3. doi:10.1080/01478885.2020.1734718
3. World Health Organization. Laboratory biosafety guidance related to coronavirus disease 2019 (COVID-19) Interim guidance. February 12, 2020.
4. Zhou P, Yang XL, Wang XG, et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. *Nature*. 2020;579:270-273.
5. Wu F, Zhao S, Yu B, et al. A new coronavirus associated with human respiratory disease in China. *Nature*. 2020;579:265-269.
6. Andersen KG, Rambaut A, Lipkin WI, Holmes EC, Garry RF. The proximal origin of SARS-CoV-2. *Nat Med*. Published online March 17, 2020. doi:10.1038/s41591-020-0820
7. Henwood AF. Ebola and histotechnologists. *J Histotechnol*. 2018;41:71-73.
8. Kampf G, Todt D, Pfaender S, Steinmann E. Persistence of coronaviruses on inanimate surfaces and its inactivation with biocidal agents. *J Hosp Infect*. 2020;104:246-251. doi:10.1016/j.jhin.2020.01.022
9. Duan SM, Zhao XS, Wen RF, et al; SARS Research Team. Stability of SARS coronavirus in human specimens and environment and its sensitivity to heating and UV irradiation. *Biomed Environ Sci*. 2003;16:246-255.
10. 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.
11. Xu Z, Shi L, Wang Y, et al. Pathological findings of COVID-19 associated with acute respiratory distress syndrome. *Lancet Respir Med*. Published online February 18, 2020. doi:10.1016/S2213-2600(20)30076-X