DERLEME REVIEW

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SARS-CoV-2 and Protection Methods in Histology and Cytology Workflow: Traditional Review

Histoloji ve Sitoloji İş Akışında SARS-CoV-2 ve Korunma Yöntemleri: Geleneksel Derleme

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ABSTRACT This study aimed to discuss the prevention methods and recommendations from severe acute respiratory syndrome-coronavirus2 (SARS-CoV-2) during the course of pathology laboratory procedures, before macroscopic dissection and during cytological processes. In the light of the latest scientific data, possible virus viability was evaluated on all surfaces and biopsy samples in Hitit University Faculty of Medicine pathology laboratory. In order to continue the pathology and cytology workflow, virus persistence probabilities according to the size of the biopsies, "intraluminal formaldehyde fixation" before luminal organs and especially bowel dissection, consideration prevention methods from contamination in the cytological and histological work process were determined and suggested. Cytological preparations should not be transported in the open after the smear process and should be transported in protective closed containers. Technical handling of cytology specimens should be performed in a Class II biosafety cabinet with appropriate personal protection equipment. In order to accelerate the drying of the smears, it should be avoided to dry by shaking them in the air or drying with the help of air blowing devices. Before the intraoperative consultation, as in the normal process, clinical and pathology correlation should be established and it should be learned whether the patient carries a risk or not. Given the evolving pathology landscape and the high-risk nature of SARS-CoV-2, a clear understanding of the new information necessary for protection methods and implementation in biopsy process has become increasingly important to help guide for pathology practice. The need to organize definite rules within 'clinic-laboratory procedures and principles' to prevent unnecessary risks and spreads of the virus and to keep safety in pathology laboratories at the highest level is clear. Healthcare workers must be vaccinated.

Keywords: SARS-CoV-2; histology; cytology; COVID-19; pandemic

ÖZET Bu çalışma, patoloji laboratuvarı işlemleri sırasında makroskobik diseksiyon öncesi ve sitolojik süreçler sırasında şiddetli akut solunum sendromu-koronavirüs-2'nin [severe acute respiratory syndrome-coronavirus-2 (SARS-CoV-2)] önleme yöntemlerini ve önerilerini tartışmayı amaçlamıştır. En son bilimsel veriler ışığında, Hitit Üniversitesi Tıp Fakültesi patoloji laboratuvarında tüm yüzeylerde ve biyopsi örneklerinde olası virüs varlığı değerlendirildi. Patoloji ve sitoloji iş akışına devam etmek için biyopsilerin büyüklüğüne göre virüs kalıcılık olasılıkları, lümenli organların ve özellikle bağırsak diseksiyonu öncesi "lümen içi formaldehit fiksasyonu", sitolojik ve histolojik çalışma sürecinde kontaminasyondan korunma yöntemleri belirlenerek önerildi. Sitolojik preparatlar yayma işleminden sonra açıkta taşınmamalı ve koroyucu kapalı kaplar içinde transfer edilmelidir. Sitoloji örneklerin teknik işlemleri uygun kişisel koruma ekipmanına sahip bir Class II biosafety cabinett'te çalışılmalıdır. Yaymaların kurumasını hızlandırmak için, elde tutup havada sallayarak kurutmaktan veya hava üfleyen cihazlar yardımı ile kurutmaktan kaçınılmalıdır. intraoperatif konsültasyon öncesinde normal süreçte olduğu gibi klinik ve patoloji korelasyonu mutlaka kurulmalı ve hastanın risk taşıyıp taşımadığı öğrenilmelidir. Gelişen patoloji ortamı ve SARS-CoV-2'nin yüksek riskli doğası göz önüne alındığında, biyopsi sürecindeki koruma yöntemleri ve uygulama için gerekli olan yeni bilgilerin net bir şekilde anlaşılması, patoloji uygulamasına rehberlik etmek için giderek daha önemli hâle gelmiştir. Virüsün gereksiz risklerini ve yayılmalarını önlemek ve patoloji laboratuvarlarında güvenliği en üst düzeyde tutmak için "klinik-laboratuvar prosedür ve ilkeleri" içerisinde kesin kurallar düzenleme ihtiyacı açıktır. Sağlık çalışanları mutlaka aşılanmalıdır.

Anahtar Kelimeler: SARS-CoV-2; histoloji; sitoloji; COVID-19; pandemi

The severe acute respiratory syndrome-coronavirus-2 (SARS-CoV-2) pandemic beginning in Wuhan city in China is continuing to cause deaths around the whole world at the time this article was written.[1] SARS-CoV-2 is a virus which requires full understanding of transmission routes to prevent spread of the infection and determine biosecurity protocols. All health centers are important places for secondary transmission of SARS-CoV-2 and nosocomial infection may be categorized as from patient to patient, from patient to health worker, from health worker to patient and from health worker to health worker.[2]

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The case death rate varies among countries (Italy 7%, China 7%, Iran 6%).[3] When writing this article, the death rate was 7.6% in the USA and 2.93% in Turkey from the beginning of the coronavirus disease-2019 (COVID-19) pandemic to date.[4,5]

Pathology laboratories are hospital working environments involving potential contact with the virus due to a variety of factors including personnel with COVID-19 infection but who are asymptomatic, patients with non-specific symptoms and infected samples. As a result, pathologists, technicians, biologists and secretaries are at risk of exposure through potentially infected samples and other transmission routes. These factors do not prevent pathology employees from providing the best service; however, they show the need to develop different protection methods.

ENVIRONMENTAL AND AEROSOL TRANSMISSION

Droplet transmission may be caused by droplet nuclei >5 μm diameter which can move a short distance in the air (generally <1 m).[6] Santarpia et al. found personal belongings, medical devices in nearly constant contact with the patient, room surfaces and toilets in the rooms of patients infected with SARS-CoV-2 were positive for viral RNA in a study about aerosol and surface contamination.[7] Corridors appeared to be areas where the transmissivity increased with the movement of viral aerosol particles.[7] An observational study by Kwok et al. determined that individuals had skin contact mostly through the hands (56%) and then contacted their hands to their mouth (36%), nose (31%) and eyes (31%).[8] Kampf et al. stated that human CoVs (HCoVs) (SARS and Middle East respiratory syndrome) could remain on inanimate surfaces like metal, glass or plastic for up to 9 days at room temperature and that temperatures of 30 □ and above shortened the permanence duration.[9] A study by van Doremalen et al. assessed aerosols containing SARS-CoV-2 [$10^{5.25}$ 50% tissue-culture infectious dose ($TCID_{50}$) per milliliter] and the stability of the virus on a variety of surfaces and identified that the virus was more permanent on plastic and stainless steel compared to copper and cardboard.[10] Additionally, live virus was present on plastic and stainless-steel surfaces for up to 72 hours though the virus titer was significantly reduced. Live SARS-CoV-2 was not identified on cardboard after 24 hours and on copper after 4 hours.[10]

The half-life of SARS-CoV-2 on cardboard was identified to be longer than for SARS-CoV-1. The estimated mean half-life of SARS-CoV-2 on stainless steel is nearly 5-6 hours and on plastic is 6-8 hours.[10] SARS-CoV-2 was observed to survive for 4 and 7 days on the internal and external layers of surgical face masks and for 1 day on cloth and banknotes. Additionally, the virus only survived for 30 minutes on paper and morphology was fully lost after 3 hours.[11] A study by Chin et al. observed that SARS-CoV-2 continued to be infectious for 2 days on glass at room temperature and 65% relative humidity and was completely undetectable 4 days later.[12] The virus may survive for only 2 days in hospital wastewater, domestic wastewater and chlorine-free tap water.[11]

Considering that material like paper and plastic equipment are frequently used in pathology laboratories, it may be stated that the SARS-CoV-2 transmission risk is not low for health personnel working in these areas.

TRANSMISSION FROM ORGAN AND TISSUE SAMPLES

The most common form of transmission of SARS-CoV-2 is large droplets due to frequent close and long interaction (15 minutes or more) between infected people and non-infected people, contaminated hands and surfaces. Additionally, transmission may occur through lower and upper respiratory tract samples (sputum, oral and nasopharyngeal swabs, bronchoalveolar lavage fluid, pleural effusion, transbronchial needle aspiration), anal swabs, feces, blood, urine and wastewater.[1,2,6,13] A study of health workers by Chen et al. stated that though swab samples were negative for SARS-CoV-2 RNA, serologic analysis stated the asymptomatic or subclinical infection rate was 17.1%.[14] A study of 98 asymptomatic health personnel by Stock et al. determined 19 of the samples (19.4%) were SARS-CoV-2 positive.[15] The study by Rivett et al. found that though 31 (3%) out of 1,032 asymptomatic health personnel had no symptoms, they were SARS-CoV-2 positive.[16]

SARS-CoV-2 has been identified in many other organs including the lungs, heart, liver, kidneys, gas-

trointestinal system, spleen, lymph nodes, skin and placenta.[17]

In the upper respiratory tract, HCoV 229E, NL63, OC43 and HKU1 types comprise 10 to 30% of adult infections.[18-20] The capability of SARS-CoV-2 to infect human pulmonary tissues and replication ability was understood to be more effective than SARS-CoV considering the elevated amount and density of viral N antigen expression.[21] Due to cell tropism of SARS-CoV-2, high viral load is isolated from saliva, nasopharynx and lower respiratory tract samples in the early stages when symptoms begin and infectious virus particles more than 3.2 times the amount for SARS-CoV were produced within 48 hours after detection of infection.[1,2,22] The presence of SARS-CoV-2 in the lungs is confirmed using immunohistochemistry or reverse transcription polymerase chain reaction (RT-PCR) analysis for a variety of spike and nucleocapsid proteins.[17] These findings are an indicator of the high person-to-person transmissivity due to the presence of high viral load in the respiratory secretions of COVID-19 patients in the first days of admission or during incubation.

There are studies about transmission of SARS-CoV-2 through feces.[23] The study by Chen et al. found 66.7% SARS-CoV-2 RNA positivity.[24] Additionally, autopsies found the virus in epithelial cells of intestinal mucosa.[13] A study by Ruan et al. identified 15% rates in plasma and fecal samples from patients who were advanced or severe cases.[22]

In cardiac and venous tissue, the virus was shown to be expressed in the angiotensin-converting enzyme 2 receptor of cardiac myocytes and vascular endothelium.[25] The study by Polak et al. identified viral particles in one out of 31 cases with electron microscope investigation of damaged interstitial cells in a study of whether SARS-CoV-2 may cause mild pericardial effusion, and 6 cases were positive with RT-PCR investigation.[17]

SARS-CoV-1 was identified to be transmitted in urine for up to 5 days in the study by Duan et al.[26] Wang et al. showed the same virus could be identified in urine at room temperature for 17 days.[27] A study of autopsies by Gu et al. identified SARS-CoV-1 in distal renal tubules.[28] Polak et al. identified SARS-CoV-2 viral particles in tubular epithelium (basically in the proximal tubule) and/or podocytes and endothelial cells with electron microscope examination. RT-PCR analysis found 6 out of 18 kidney tissues were positive for SARS-CoV-2 nucleocapsid proteins.[17]

Lagana et al. stated they found perimortem liver changes in COVID-19 patients including congestion and ischemia along with hepatic steatosis (75%), mild acute hepatitis (50%) and portal inflammation (50%) findings. The livers of 55% of patients who died from COVID-19 were positive for the virus with PCR.[29] Polak et al. stated that SARS-CoV-2 may cause steatosis, cirrhosis and congestion in the liver and that RT-PCR analysis found 6 out of 15 liver samples were positive for SARS-CoV-2.[17]

In brain tissue Gu et al. found SARS-CoV-1 in the cytoplasm of neurons in their autopsy study.[28] Polak et al. stated that in terms of the effect of SARS-CoV-2 on brain tissue, the studies with large patient series were inadequate apart from case reports.[17]

In placenta tissue, SARS-CoV-2 may infect placental syncytiotrophoblasts and cytotrophoblasts and there may be vertical transmission to infants born of mothers infected with the virus.[30]

The World Health Organization (WHO) recommends accepting that all samples collected for laboratory research may be potentially transmissive.[31]

In terms of mental health, Lai et al. analyzed the disrupted mental health status of 1,257 people among health sector workers and found complaints like depression, anxiety, insomnia and distress at rates of 50.4%, 44.6%, 34.0% and 71.5%, respectively.[32] First-line health workers dealing with the direct diagnosis, treatment and care for COVID-19 patients were associated with higher risk of depression, anxiety, insomnia and distress.[2] Though there is no one-to-one contact with patients, it should not be ignored that these mental disorders may develop among pathology workers.

PROTECTION METHODS IN PATHOLOGY LABORATORIES

Unfortunately, the SARS-CoV-2 pandemic caused some risks in terms of safety of pathology laboratory procedures; however, within this scope, information

has not been confirmed.[31] In a pandemic situation, the viral load of CoVs on inanimate surfaces is unknown, so it is appropriate to reduce viral load by disinfection of working surfaces in pathology laboratories.[31] The WHO recommends consistent and accurate following of environmental cleaning and disinfection procedures, that environmental surfaces be cleaned well with water and detergent, that hospitals apply effective and adequate amounts of disinfectants (like sodium hypochlorite) and stated that these recommendations should be noted.[31]

PATHOLOGY OR CYTOLOGY REQUEST FORMS

A different transmission risk is valid for the "pathology or cytology request forms" containing both clinical and clinicopathological information for patients and generally on A4 paper. SARS-CoV-2 may be transmissive for up to 3 hours on paper and the live virus carries transmission risk for the first 24 hours on cardboard.[10,11] Therefore, it is appropriate to switch to a digital recording system in pathology laboratories where request forms are used.

MACROSCOPIC STUDY ROOMS

It is understood that 62-71% ethanol, 0.5% hydrogen peroxide or 0.1% sodium hypochlorite concentrations are effective on CoVs within 1 min.[9,10] It appears necessary to use this disinfection method in pathology laboratories, especially in macroscopy working areas. Ethanol is a broader spectrum and stronger viricidal agent than propanol. Ethanol at 80% concentration is very effective against enveloped viruses within 30 seconds.[9] The WHO recommends 70% ethanol concentration for disinfection of small surfaces.[33] This appears to be an applicable disinfection method for microscopic working areas, especially, in pathology laboratories.[34]

Incubating samples for 24 hours in formaldehyde solution or for 24-48 hours in glutaraldehyde may inactivate the virus.[1,33] The diffusion rate of formaldehyde is important and determination of this involves a positive correlation between the penetration depths of formaldehyde with the fixation duration.[35] Formaldehyde diffusion is only 2-4 mm within 24 hours. Samples with diameter less than 10 mm may be fixated in a short duration with diffusion, while larger samples require longer durations for fixation. As a result, large samples should be cut into thin slices to shorten the fixation duration.[36] Diffusion of formaldehyde into tissues should be carefully performed for large tissue and organ samples belonging to COVID-19 patients in the macroscopy room. Fixation is affected by many factors like temperature, tissue thickness, ratio of tissue to fixative volume and fixation duration. Before dissection or slicing of large samples, the formaldehyde infusion rate should be noted, and formaldehyde injection should be performed with an injector without spraying and then it is appropriate to leave the sample for at least 24 hours. In light of this knowledge, it is considered the virus will be inactivated in tissue samples with nearly 2-3 cm diameter after being left in formaldehyde for 24 hours linked to the tissue features (solid, cystic, fibrous, fatty, spongy, etc.) and diffusion rate of the formaldehyde solution.

When working with colectomy (partial/total) specimens from COVID-19 patients in the macroscopy room, there is a potential risk of fecal transmission during opening due to luminal content droplets, aerosols or spraying of content.

As the intestinal content is not adequately fixated in colon resection specimens, personal protective equipment should be worn before opening the specimen and then intraluminal formaldehyde injection should be performed in the distal and proximal sections with the aid of an injector (Figure 1a). It appears appropriate to open the specimen for investigation and sampling after waiting for at least 24 hours. As a result, especially for resection of organs with lumens (like colon, stomach and gallbladder), application of intraluminal formaldehyde fixation appears to be an important method to reduce transmission (Figure 1b, Figure 1c).

Histologic samples may be categorized in three groups according to potential transmission risk. The first group does not have risk; the second group has low risk and the third group has high risk (Table 1). This histologic categorization was performed noting formaldehyde fixation rate and tissue features. The

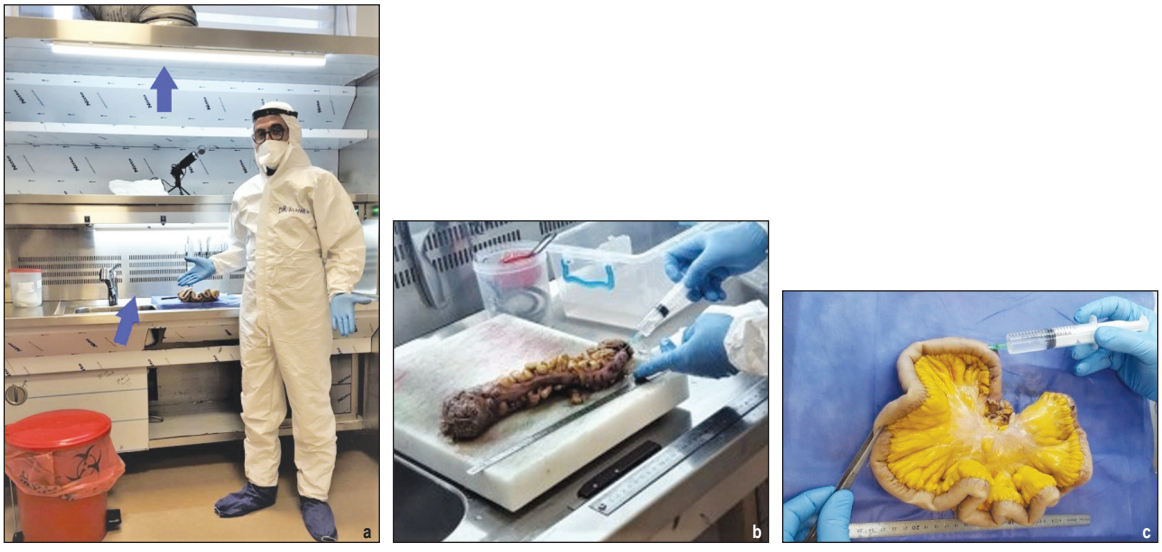

FIGURE 1: a) Mask, face guard, gloves, and protective clothes should be made standard in macroscopic study environments. **b, c)** Before opening specimens with lumens, careful formaldehyde injections should be performed into the lumen.

TABLE 1: Samples belonging to non-risky, low- and high-risk groups.

Biopsy group without risk	Biopsy group with low risk	Biopsy group with high risk
Tissues smaller than 4 cm	Tissues larger than 4 cm with slow fixation rate in formaldehyde	Tissues larger than 4 cm with slow fixation rate in formaldehyde
Endoscopic biopsy	Hemorrhagic curettage/abortus material	Frozen investigation
Punch biopsy	Excision material	Resection of organs with lumens (esophagus, stomach,
Incisional biopsy	Small resection/amputation materials	small intestine, colon, bladder, gallbladder,
Excisional biopsy	(aneurysm, hematoma, vein, hemorrhoid, appendix,	Whipple procedure material, etc.)
Wedge/shave biopsy	pleura, pericardia, peritoneum, ureter,	Placenta and appendages
Tru-cut/needle biopsy	myomectomy, saliva gland resection)	Orchiectomy
TUR/TRUS biopsy		Prostatectomy
Partial resection (breast, liver, soft tissue, etc.)		Extremity amputation/soft tissue resections
		(solid/cystic lesions) for tumor or non-tumor reasons
		Lobectomies (lung, liver, etc.)
		Mastectomy
		Splenectomy
		Laryngectomy specimens
		Hysterectomy, bilateral/unilateral salpingo-oopherectomy
		Fetal or large autopsy materials

TUR: Transurethral resection; TRUS: Transrectal ultrasonography.

group without risk have formaldehyde fixation within 24 hours and tissues smaller than 4 cm; the low-risk group includes tissues larger than 4 cm but with slower formaldehyde fixation rate.

To prevent exposure or release of pathogens, the WHO recommend that guidelines about sample collection, transport and storage for cases suspected of COVID-19 should be noted, as summarized in Table 2.[37]

The US Centers for Disease Control and Prevention reported the need to collect all samples in appropriate containers, storage between 2 °C and 8 °C for 72 hours after collection of samples for routine tests for respiratory pathogens or storage at -70 °C if there is a delay.[4]

In addition to this information, it is recommended that maximum 4 personnel be present within the macroscopy room during procedures.[38]

TABLE 2: To prevent exposure or release of pathogens, the World Health Organization recommend that guidelines about sample collection, transport and storage for cases suspected of coronavirus disease-2019 should be noted.[14]

Specimen type	Collection materials	Transport to laboratory	Storage until testing	Comment
Nasopharyngeal and oropharyngeal swabs	Dacron or polyester flocked swabs	2 °C-8 °C	≤5 d, 2 °C-8 °C >5 d, -70 °C (dry ice)	The nasopharyngeal and oropharyngeal swabs should be placed in the same tube to increase the viral load
Bronchoalveolar lavages	Sterile container	2 °C-8 °C	≤48 h, 2 °C-8 °C >48 h, -70 °C (dry ice)	There may be some dilution of the pathogen, but it is still a worthwhile specimen
(Endo)tracheal aspirates, nasopharyngeal or nasal wash/aspirates	Sterile container	2 °C-8 °C	≤48 h, 2 °C-8 °C	
Sputum	Sterile container	2 °C-8 °C	≤48 h, 2 °C-8 °C >48 h, -70 °C (dry ice)	Ensure that the material is from the lower respiratory tract
Tissue from biopsy or autopsy including from the lung	Sterile container with salin	2 °C-8 °C	≤24 h, 2 °C-8 °C >24 h, -70 °C (dry ice)	
Serum	Serum seperator tubes (adults, collect 3-5 mL whole blood)	2 °C-8 °C	≤5 d, 2 °C-8 °C >5 d, -70 °C (dry ice)	Collect pair samples: Acute: First wk of illness Convalescence: 2-3 wk later
Whole blood	Collection tube	2 °C-8 °C	≤5 d, 2 °C-8 °C >5 d, -70 °C (dry ice)	For antigen detection, particularly in the first wk of illness
Stool	Stool container	2 °C-8 °C	≤5 d, 2 °C-8 °C >5 d, -70 °C (dry ice)	
Urine	Urine collection cantainer	2 °C-8 °C	≤5 d, 2 °C-8 °C >5 d, -70 °C (dry ice)	

HISTOLOGIC TISSUE PROCESSING ROOMS

Routine tissue processing for histologic tissue samples involves exposure to 90-70% alcohol and 56-57 °C paraffin wax heat.[39] Again, during submerging of histologic tissue samples into paraffin wax, the paraffin wax melting temperature is 56-57 °C which creates a suitable environment to inactivate the virus.[39] During staining of histologic sections, 70-96% ethyl alcohol is used in order.[40] Routine histologic procedures involving samples being heated within liquid paraffin inactivating the virus in morbid samples. Additionally, thermal processes at 56 °C for 90 minutes, 67 °C for 60 minutes or 75 °C for 30 minutes inactivate the virus.[26]

Ethanol is used at concentrations of 70% of more for tissue processing protocols and inactivates the virus when applied to surfaces for 1 minute.[33]

Considering SARS-CoV-2 is inactivated in tissues with formaldehyde fixation and submerged in paraffin and in histologic slide preparations, these samples may be assessed within the lowest risk group and security precautions taken.

MICROSCOPIC INVESTIGATION

As the SARS-CoV-2 virus can remain viable for up to 72 hours on plastic surfaces, transmission to personnel can occur via "slide transport trays" (Figure 2). After staining processes for slides, the possibility of transmission to the plastic carrier surface while arranging slides on the transport trays should be remembered and daily disinfection should be performed. After slides containing tissue sections are closed with lamellas, care should be taken to leave them in 70% ethyl alcohol for at least 1 minute before presenting them for microscopic investigation.

Multidisciplinary microscopic studies should not be performed unless necessary; if possible, it is recommended to use digital pathology methods. Before

FIGURE 2: Virus transmission between personnel may occur through "slide transport trays".

and after the use of multi-head microscopes, objectives and all contact surfaces should be disinfected with alcohol. The study duration should be kept as short as possible.

CYTOLOGIC STUDY

As varying rates of viral load were identified in different clinical samples, it is recommended to assess cytology samples in 3 groups as high risk, moderate risk and low risk for COVID-19 infection (Table 3).[6]

Cell blocks are included in the low-risk group as formalin fixation and submerging in paraffin may inactivate SARS-CoV-2.[6]

It is unknown whether fixatives using low-concentration alcohol solutions like PreservCyt and CytoLyt (Hologic, Inc) and SurePath (Becton, Dickinson and Company) sufficiently inactivate SARS-CoV-2, so during processing of fluid-based cytology and other cytological materials, transport and study without personal protective equipment may increase transmission risk.[6] Biosecurity precautions should be taken during smear preparation, ThinPrep processing, cytospins and centrifugation. As a result, after spreading processes for cytological preparations, they should not be transported in the open and should be transferred within closed containers (Figure 3, Figure 4). For manual opening, separation, dilution, vortexing, centrifuging, pipetting, mixing and/or smear staining of these samples, it is necessary to work in a Class II biosafety cabinet with appropriate personal protective equipment.[31] To increase the speed of drying of smears, they should not be held in the hands and waved in the air and drying with airblowing devices should be avoided.[4]

Though valid for liquid biopsies, in line with the Centers for Disease Control and Prevention recommendations, surgical samples taken from patients with suspected or confirmed COVID-19 should be transported as UN3373 *Biological Substance Category B*.[4] UN3373 packaging comprises 3 components: 1) Leak free primary container, 2) Leak free secondary packaging and 3) External packaging with adequate protective features made of glass, metal or plastic with minimum dimensions 100 mmx100 mm.[4]

TABLE 3: It is recommended to assess cytology samples in 3 groups as high risk, moderate risk and low risk for coronavirus disease-2019 infection.[15]

High risk with virus	Intermediate risk with virus	Low risk with virus
Upper and lower respiratory samples	Pleural effusion	Ascites and peritoneal washing
Nasopharyngeal swab, oropharyngeal swab, sputum, and	Pericardial effusion	Uterine cervical smears
all types of bronchoscopic sampling	Urine	Vaginal discharge
Blood and bloody samples		CSF
Feces and anal swabs		Synovial fluid
Tears drops and conjuctival discharge		Semen
		Cell blocks
High risk-frequently detectable viral nucleic acids or viral isolat		Limited or no evidence of viral detectiones
Intermediate risk-limite devidence of viral detection		Disinfected samples
Reccommendall procedures in Class II biosafety cabinets with appropriate personal protection		GMPP should be followed

CSF: Cerebrospinal fluid; GMPP: Good Microbiological Practices and Procedures.

FIGURE 3: Cytologic preparates should not be carried in the open after procedures.

All samples should be delivered by hand instead of with a pneumatic tube system. Personnel transporting samples should be trained about secure use practices and spill decontamination procedures. The health services specialist sending the sample should make a timely report to the laboratory about the transported sample. It is very important that the information on the standard "pathology request form" is completed and accurately barcoded (labeled).[4]

In situations where fine needle aspiration (FNA) or rapid on-site examination (ROSE) are mandatory, it is necessary to perform the procedure by taking advanced biosecurity precautions (e.g. use of N95 masks) to prevent infections.[6] Additionally, the clinical patient-procedure-treatment stages should be discussed well and the decision made about whether ROSE is definitely necessary or not.[4]

STUDY OF FROZEN SECTIONS

While it is uncertain whether cryostat contains aerosols, it is necessary to avoid making frozen sections from samples belonging to possible COVID-19 patients.[1] SARS-CoV-2 may be present in patients with unknown COVID-19 diagnosis, without diagnosis, while presymptomatic, asymptomatic or with mild symptomatic infection and in the healing period while shedding the virus.[13]

As a result, just as in the normal process before intraoperative consultation, clinical and pathological correlation should be ensured and it should be learned whether the patient is at risk or not. If patients positive for SARS-CoV-2 require emergency or unexpected intraoperative consultation, the process may be considered for continuation after personal and surface protective precautions are taken. However, after the procedure, the frozen section room and devices should

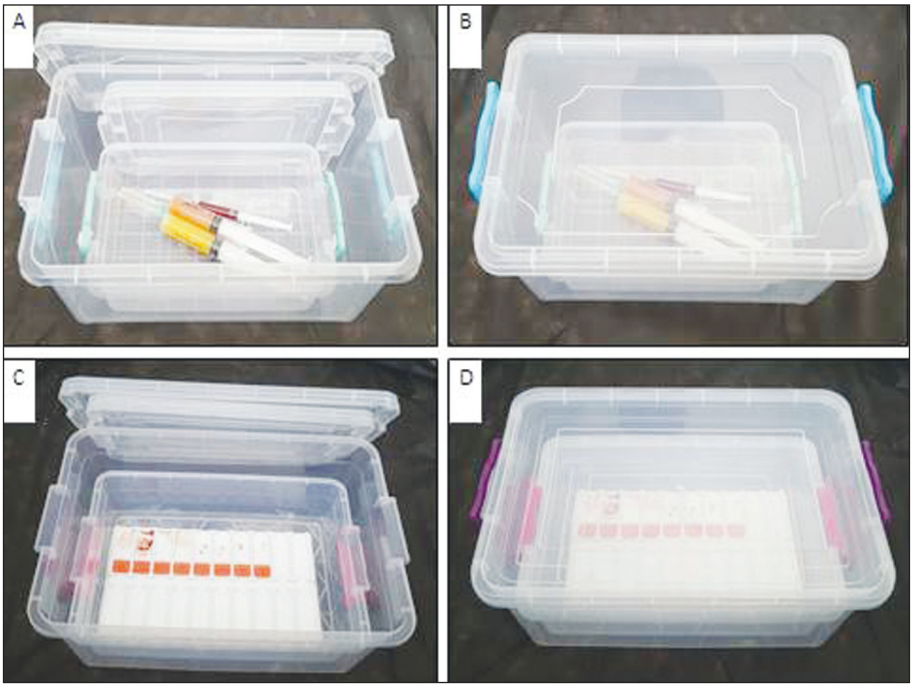

FIGURE 4: Examples of a carrying method in enclosed containers after procedures for cytologic preparates.

undergo frequent disinfection. These processes should also be applied for patients negative for the virus.

ACADEMIC PATHOLOGY

Use of a multi-head microscope at the same time during both assistant training or for several pathologists to discuss a case forms a risk of viral transmission due to close contact. Web-based video conference tools and electronic virtual connections can be used to perform in-pathology tele-conferences to prevent delays in academic pathology practice. This implementation may also be valid for multidisciplinary meetings.

LIMITATIONS

Like all systematic investigations, there are a variety of limitations that need to be discussed for our investigation. Considering the extraordinary rapid spread of COVID-19, rapid provision of new information and new procedures and principles about precautions, only general recommendations can be made about protective precautions for pathology laboratories. COVID-19 is a relatively new disease with limited published information available. Though the number of publications related to the histo-cytopathology of this disease are increasing every day, we cannot ignore the possibility that we could not include the most recent publications.

CONCLUSION

In this current study, we have presented new suggestions for some histological procedures (for example, intraluminal formaldehyde fixation in colon resection). We have mentioned some details about the risk of contamination and prevention methods during both histological and cytological procedures and made recommendations in the light of new literature.

Due to limited service capacity, in addition to the WHO's directives (https://apps.who.int/iris/handle/10665/331329) to reduce transmission risk while dealing with a pandemic like COVID-19 in pathology laboratories, the following steps should be taken; due to limited service capacity, in addition to the WHO's directives (https://apps.who.int/iris/handle/10665/331329) to reduce transmission risk while dealing with a pandemic like COVID-19 in pathology laboratories; pathology staff should be trained and informed at intervals and vaccinated. Automatic contactless control of entry and exit to pathology laboratories should be provided. Infection prevention and control protocols specific to the pathology laboratory for hand hygiene should be determined and implemented, including glove changes during movement between rooms. Personal protective equipment should be provided for personnel to protect against contact and aerosol viral transmission. The floor, door handles, slide transport trays, personal and communal materials and devices should be disinfected frequently. The slides to be examined should be presented to the pathologist/cytologist after waiting for 1 minute in at least 70% ethanol after the staining process is completed. It is appropriate to switch to a digital recording system in pathology laboratories where request forms are used. It will be appropriate to apply the new methods we recommend for samples that require macroscopic examination (as there is SARS-CoV-2 transmission risk from feces in a colon resection, before investigation perform additional "intraluminal fixation" with formaldehyde and leave for 24 hours; for pulmonary lobectomy, spleen and similar size surgical specimens, before investigation perform additional "intraparenchymal fixation" and leave for 24 hours for disinfection). Negative pressure work rooms with appropriate air exchange and negative pressure corridors according to the outdoor environment should be created and frequent ventilation of the work rooms should be ensured. New preventive measures and application methods should be established for frozen section, ROSE, blind in situ biopsies, squash-touch-preparation assessment and FNA procedures applied to patients infected with SARS-CoV-2.

As in other countries, hospitals and other health organizations in Turkey gave first priority to emergency and basic health services and limited clinical services.[4] Surgical procedures were performed based on the clinical urgency of the case. These precautions undoubtedly affected pathology laboratories. Pathology preparations reduced. The workflow of laboratory personnel was re-organized with "flexible

working hours". Shift work, staged breaks and "work with minimal adequate personnel" methods were used. Personnel with any clinical discomfort (diarrhea, subclinical fever, sore throat, fatigue, mild cough) consulted with an infectious disease doctor and were checked with viral tests and if necessary quarantined at home for 2 weeks. Emergency situation plans were created for the possibility of more than one pathology laboratory personnel becoming sick. The need to organize definite rules within "clinic-laboratory procedures and principles" to prevent unnecessary risks and spread of the virus and to keep safety in pathology laboratories at the highest level is clear.

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Conflict of Interest

No conflicts of interest between the authors and/or family members of the scientific and medical committee members or members of the potential conflicts of interest, counseling, expertise, working conditions, share holding and similar situations in any firm.

Authorship Contributions

***Idea/Concept:** Yılmaz Baş; **Design:** Yılmaz Baş, Havva Hande Keser Şahin; **Control/Supervision:** Yılmaz Baş, Havva Hande Keser Şahin; **Data Collection and/or Processing:** Yılmaz Baş, Havva Hande Keser Şahin; **Analysis and/or Interpretation:** Yılmaz Baş, Havva Hande Keser Şahin; **Literature Review:** Yılmaz Baş; **Writing the Article:** Yılmaz Baş; **Critical Review:** Yılmaz Baş, Havva Hande Keser Şahin; **References and Fundings:** Yılmaz Baş, Havva Hande Keser Şahin.*

REFERENCES

1. Henwood AF. Coronavirus disinfection in histopathology. J Histotechnol. 2020;43(2): 102-4. [Crossref] [Pubmed]
2. Xiao J, Fang M, Chen Q, He B. SARS, MERS and COVID-19 among healthcare workers: A narrative review. J Infect Public Health. 2020;13(6):843-8. [Crossref] [Pubmed] [PMC]
3. Porcheddu R, Serra C, Kelvin D, Kelvin N, Rubino S. Similarity in case fatality rates (CFR) of COVID-19/SARS-COV-2 in Italy and China. J Infect Dev Ctries. 2020;14(2):125-8. [Crossref] [Pubmed]
4. Centers for Disease Control and prevention (CDC). Evaluating and Testing persons for Coronavirus Disease 2019 (COVID-19). CDC 2019. Cited: 26 september 2020. available from: [Link]
5. Republic of Turkey Ministry of Health [Internet]. Copyright 2018 [Cited: 28 December 2020]. Minister Assesses the Latest Situation on Coronavirus. Available from: [Link]
6. Chen CC, Chi CY. Biosafety in the preparation and processing of cytology specimens with potential coronavirus (COVID-19) infection: Perspectives from Taiwan. Cancer Cytopathol. 2020;128(5):309-16. [Crossref] [Pubmed] [PMC]
7. Santarpia JL, Rivera DN, Herrera VL, Morwitzer MJ, Creager HM, Santarpia GW, et al. Aerosol and surface contamination of SARS-CoV-2 observed in quarantine and isolation care. Sci Rep. 2020;10(1):12732. Erratum in: Sci Rep. 2020;10(1):13892. [Crossref] [Pubmed] [PMC]
8. Kwok YL, Gralton J, McLaws ML. Face touching: a frequent habit that has implications for hand hygiene. Am J Infect Control. 2015;43(2):112-4. [Crossref] [Pubmed] [PMC]
9. Kampf G, Todt D, Pfaender S, Steinmann E. Persistence of coronaviruses on inanimate surfaces and their inactivation with biocidal agents. J Hosp Infect. 2020;104(3):246-51. Erratum in: J Hosp Infect. 2020. [Crossref] [Pubmed] [PMC]
10. van Doremalen N, Bushmaker T, Morris DH, Holbrook MG, Gamble A, Williamson BN, et al. Aerosol and surface stability of SARS-CoV-2 as compared with SARS-CoV-1. N Engl J Med. 2020;382(16):1564-7. [Crossref] [Pubmed] [PMC]
11. Aboubakr HA, Sharafeldin TA, Goyal SM. Stability of SARS-CoV-2 and other coronaviruses in the environment and on common touch surfaces and the influence of climatic conditions: A review. Transbound Emerg Dis. 2021;68(2):296-312. [Crossref] [Pubmed] [PMC]
12. Chin AWH, Chu JTS, Perera MRA, Hui KPY, Yen HL, Chan MCW, et al. Stability of SARS-CoV-2 in different environmental conditions. Lancet Microbe. 2020;1(1):e10. [Crossref] [Pubmed] [PMC]
13. Pambuccian SE. The COVID-19 pandemic: implications for the cytology laboratory. J Am Soc Cytopathol. 2020;9(3):202-11. [Crossref] [Pubmed] [PMC]
14. Chen Y, Tong X, Wang J, Huang W, Yin S, Huang R, et al. High SARS-CoV-2 antibody prevalence among healthcare workers exposed to COVID-19 patients. J Infect. 2020;81(3):420-6. [Crossref] [Pubmed] [PMC]
15. Stock AD, Bader ER, Cezayirli P, Inocencio J, Chalmers SA, Yassari R, et al. COVID-19 infection among healthcare workers: serological findings supporting routine testing. Front Med (Lausanne). 2020;7:471. [Crossref] [Pubmed] [PMC]
16. Rivett L, Sridhar S, Sparkes D, Routledge M, Jones NK, Forrest S, et al. Screening of healthcare workers for SARS-CoV-2 highlights the role of asymptomatic carriage in COVID-19 transmission. Elife. 2020;9:e58728. [Pubmed] [PMC]
17. Polak SB, Van Gool IC, Cohen D, von der Thüsen JH, van Paassen J. A systematic review of pathological findings in COVID-19: a pathophysiological timeline and possible mechanisms of disease progression. Mod Pathol. 2020;33(11):2128-38. [Crossref] [Pubmed] [PMC]

18. van Boheemen S, de Graaf M, Lauber C, Bestebroer TM, Raj VS, Zaki AM, et al. Genomic characterization of a newly discovered coronavirus associated with acute respiratory distress syndrome in humans. mBio. 2012;3(6):e00473-12. [Crossref] [Pubmed] [PMC]
19. Belouzard S, Millet JK, Licitra BN, Whittaker GR. Mechanisms of coronavirus cell entry mediated by the viral spike protein. Viruses. 2012;4(6):1011-33. [Crossref] [Pubmed] [PMC]
20. Paules CI, Marston HD, Fauci AS. Coronavirus infections-more than just the common cold. JAMA. 2020;323(8):707-8. [Crossref] [Pubmed]
21. Chu H, Chan JF, Wang Y, Yuen TT, Chai Y, Hou Y, et al. Comparative replication and immune activation profiles of SARS-CoV-2 and SARS-CoV in human lungs: an ex vivo study with implications for the pathogenesis of COVID-19. Clin Infect Dis. 2020;71(6):1400-9. [Crossref] [Pubmed] [PMC]
22. Ruan Q, Yang K, Wang W, Jiang L, Song J. Clinical predictors of mortality due to COVID-19 based on an analysis of data of 150 patients from Wuhan, China. Intensive Care Med. 2020;46(5):846-8. Erratum in: Intensive Care Med. 2020. [Crossref] [Pubmed] [PMC]
23. Sahin M. Working plan update for medical biochemistry laboratories during the COVID-19 pandemic. Int J Med Biochem. 2020;3(2):111-2. [Link]
24. Chen Y, Chen L, Deng Q, Zhang G, Wu K, Ni L, et al. The presence of SARS-CoV-2 RNA in the feces of COVID-19 patients. J Med Virol. 2020;92(7):833-40. [Crossref] [Pubmed]
25. Zheng YY, Ma YT, Zhang JY, Xie X. COVID-19 and the cardiovascular system. Nat Rev Cardiol. 2020;17(5):259-60. [Crossref] [Pubmed] [PMC]
26. Duan SM, Zhao XS, Wen RF, Huang JJ, Pi GH, Zhang SX, 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(3):246-55. [Pubmed]
27. Wang XW, Li JS, Jin M, Zhen B, Kong QX, Song N, et al. Study on the resistance of severe acute respiratory syndrome-associated coronavirus. J Virol Methods. 2005;126(1-2):171-7. [Crossref] [Pubmed] [PMC]
28. Gu J, Gong E, Zhang B, Zheng J, Gao Z, Zhong Y, et al. Multiple organ infection and the pathogenesis of SARS. J Exp Med. 2005;202(3):415-24. [Crossref] [Pubmed] [PMC]
29. Lagana SM, Kudose S, Iuga AC, Lee MJ, Fazlollahi L, Remotti HE, et al. Hepatic pathology in patients dying of COVID-19: a series of 40 cases including clinical, histologic, and virologic data. Mod Pathol. 2020;33(11):2147-55. [Crossref] [Pubmed] [PMC]
30. Hecht JL, Quade B, Deshpande V, Mino-Kenudson M, Ting DT, Desai N, et al. SARS-CoV-2 can infect the placenta and is not associated with specific placental histopathology: a series of 19 placentas from COVID-19-positive mothers. Mod Pathol. 2020;33(11):2092-103. [Crossref] [Pubmed] [PMC]
31. World health Organization. Infection prevention and control during health care when novel coronavirus (nCoV) infection is suspected. Interim guidance. 19 March 2020. Cited: 1 December 2020. available from: [Link]
32. Lai J, Ma S, Wang Y, Cai Z, Hu J, Wei N, et al. Factors associated with mental health outcomes among health care workers exposed to coronavirus disease 2019. JAMA Netw Open. 2020;3(3):e203976. [Crossref] [Pubmed] [PMC]
33. Luqman Z, Iqbal N, Ali HM, Zahid MM, Sikandar A, Kausar R. Disinfection of corona virus in histopathology laboratories. Clin Anat. 2020;33(6):975-6. [Crossref] [Pubmed] [PMC]
34. Annex G. Use of disinfectants: alcohol and bleach. World Health Organization. Infection Prevention and Control of Epidemic- and Pandemic-Prone Acute Respiratory Infections in Health Care. Geneva: WHO; 2014. p.65-6. [Link]
35. Henwood AF. Ebola and histotechnologists. Journal of Histotechnology 2018;41(2):71-3. [Crossref]
36. Steicke M, Yang G, Dinh TN, Dunster-Jones M, Sargisson O, Ahmady F, et al. The penetration of methanol into bovine cardiac and hepatic tissues is faster than ethanol and formalin. Eur J Histochem. 2018;62(1):2880. [Crossref] [Pubmed] [PMC]
37. World Health Organization. Laboratory testing for coronavirus disease (COVID-19) in suspected human cases. Interim guidance, 19 March 2020. Cited: 1 December 2020. Available from: [Link]
38. Chesnick IE, Mason JT, O'Leary TJ, Fowler CB. Elevated pressure improves the rate of formalin penetration while preserving tissue morphology. J Cancer. 2010;1:178-83. [Crossref] [Pubmed] [PMC]
39. Lamas NJ, Esteves S, Alves JR, Costa FE, Tente D, Fonseca P, et al. The Anatomic Pathology laboratory adjustments in the era of COVID-19 pandemic: The experience of a laboratory in a Portuguese central hospital. Ann Diagn Pathol. 2020;48:151560. [Crossref] [Pubmed] [PMC]
40. Slaoui M, Bauchet AL, Fiette L. Tissue sampling and processing for histopathology evaluation. Methods Mol Biol. 2017;1641:101-14. [Crossref] [Pubmed]