

Biosafety in surgical pathology in the era of SARS-Cov2 pandemia. A statement of the Italian Society of Surgical Pathology and Cytology

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Introduction

Surgical pathology units face chemical and biological risks. While chemical risks have been intensely evaluated since the formalin ban, less attention has been drawn to biological risks. The actual epidemiologic situation due to the SARS-CoV-2 pandemia has raised a series of questions, which need to be addressed as soon as possible. We have to pursue two lines of action: on one hand we must immediately adopt urgent measures to reduce the risk of SARS-CoV-2 infection of laboratory personnel, and on the other hand, we must address crucial technical and organizational aspects of biological risk reduction, preserving as much as possible the quality of tissue and cell samples.

The evaluation of biological risk is an analytical process which involves different steps: a) characterization of the hazard (also known as risk assessment) and b) definition of a risk reduction strategy (also known as risk mitigation) ¹.

Risk assessment implies a) the identification of the intrinsic biologic characteristics of the infectious agent, and b) the identification of the laboratory procedures related to the agent.

The intrinsic biologic characteristics of infectious agents are classified in 4 risk groups (RG) by the laboratory biosafety manual of the WHO ². The RG range from level 1 (RG1) which includes microorganisms that are unlikely to cause human or animal disease, to level 4 (RG4) which includes pathogens which cause serious diseases, that can be readily transmitted from one individual to another, and for which effective treat-

ment and preventive measures are not usually available.

Risk mitigation includes the definition of the appropriate a) level of biosafety of the laboratory, b) type of personal protection equipment (PPE), c) type of infrastructure and equipment, and d) education of involved personnel.

Laboratory biosafety is graded in 4 levels (BSL-1 to BSL-4) as exhaustively described in the laboratory biosafety manual of the WHO ², and these levels are usually also defined by law (in Italy by the D. Lgs. 81/2008). BSL are a series of protections, which include individual safeguards designed to protect laboratory personnel, as well as the surrounding environment and community. The biosafety level required in laboratories derives from the characterization of the risk, and is not automatically derived from the risk group to which the pathogenic agent belongs. It is obvious that the biosafety level for a laboratory which cultivates a RG3 agent, will be higher than the level needed for a laboratory which performs diagnostic tests on inactivated biomaterials on the same agent. Specific checklists, derived from the WHO laboratory biosafety manual, which in Italy are also defined by the National Institute of Labor Safety Insurance (Istituto Nazionale Assicurazione Infortuni sul Lavoro) in its 6th Fascicle published in 2010 ³ are necessary to verify the compliance of a given laboratory with the required biosafety level.

Biosafety in pathology and the actual epidemiological situation

According to the international consensus, **SARS-CoV-2 has to be classified as a Risk Group 3 (RG3)** human pathogen ⁴⁻⁷.

SARS-Cov2 can be transmitted through inhalation of aerosol droplets or through contaminated surfaces, where the virus persists viable up to 72 hours on stainless steel and plastic. In our laboratories we can produce aerosol droplets during centrifugation and vortexing of fluids, while surface contamination can occur in a variety of situations, like leakage of fluids during dissection of fresh or inadequately fixed specimens.

SARS-Cov2 virus has been mostly identified in tissue and biomaterials of pulmonary origin, but it can also be identified in other biomaterials, like blood and stools, while the presence of viral material in urine has not been detected or is at most questionable ⁸⁻¹⁰. The possibility of oral-fecal transmission is controversial, but has to be taken into account. Therefore, given the extensive and partially unknown prevalence of the infection in the general population, we should consid-

ered at high risk of contamination all lung tissues and fluids, but also other specimens, including gastrointestinal specimens ¹¹.

The biomaterials identified as possible sources of contamination in our laboratories are:

- 1 tissue samples derived from autopsies;
- 2 surgical and cytological specimens such as:
 - unfixed surgical and cytological specimens, including tissues for frozen sections and specimens collected with formalin-free vacuum technology,
 - inadequately fixed surgical specimens (e.g.: lung specimens floating in formalin jars, any surgical specimen in a jar with insufficient formalin or which has been kept immersed in formalin for a short period of time),
 - fine needle aspiration specimens for which rapid on-site evaluation is performed,
 - cytological samples collected in transporting mediums which do not guarantee viral inactivation,
 - samples for flow cytometry.

All the above described materials should be considered as potentially infective and our laboratories must strictly adhere to biosafety regulation when manipulating these samples. Conversely, all properly fixed tissues and cytological samples are not at risk and can be safely manipulated using standard procedures ¹². In surgical specimens, SARS virus are inactivated by fixation in 10% buffered, neutral formalin at room temperature for 1 day and by alcoholic fixation in 70% ethanol ¹³. It is prudent, in the actual epidemiological situation, to perform gross sectioning only after samples have been adequately fixed, avoiding as much as possible manipulation of unfixed/inadequately fixed specimens. We also suggest to suspend the use of formalin-free vacuum collection of surgical specimens if the safety level of the laboratory does not guarantee adequate protection.

Manipulation of cytological samples needs more rigid changes in procedure and attention than those required for histological samples, mostly based on the evidence that liquid-based preparations utilize low alcohol concentrations for conservative rather than for fixative purpose.

The recent Laboratory biosafety guidance related to coronavirus disease 2019 released by the World Health Organization, states that “*non-propagative diagnostic laboratory work (e.s.: sequencing, nucleic acid extraction) should be conducted in a facility using procedures equivalent to biosafety level 2(BSL-2)*” ¹⁴. This document does not refer directly to surgical pathology activities, but our activities can be clearly considered as “non-propagative”. The Centers for Disease Control and Prevention states that all surgical

pathology activities, including molecular analyses, performed on formalin fixed or inactivated samples pertaining to patients affected by COVID-19 should be performed in a BSL-2 laboratory¹. Among the requirements for a BSL-2 laboratory are: (a) daily decontamination of all work surfaces when work is complete, (b) use of personal protective equipment (PPE), (c) appropriate disposal of contaminated material, (d) prevention of injuries such as cuts and other breaches of the skin and mucous membrane exposures. All procedures that can cause infection from aerosols or splashes are to be performed within a biological safety cabinet (BSC).

What to do in the actual epidemiological SARS-CoV-2 situation

As it has been reported that formalin and ethanol fixation inactivate the virus, we will pragmatically focus here only on some aspects concerning the manipulations of unfixed/inadequately fixed surgical and cytological samples. We will not consider the management of properly fixed samples and the activities related to autopsy practice, which have been addressed by several agencies including The Royal College of Pathologists⁵, the European Centre for Disease Prevention and Control¹⁵ and the Italian Society of Surgical Pathology (SIAPEC)¹⁶.

- Clinical information.** All samples shall be accompanied by adequate clinical information regarding SARS-CoV-2 status. In particular, the case must be identified if: a) positive for SARS-CoV-2, b) suspicious for SARS-CoV-2. Samples of positive and suspicious cases shall be transferred to the laboratory in a secondary disposable biohazard ziplock bag whenever possible.
- Paperwork.** As the virus can persist on paper at least for 24 h, paperless electronic request transmission should be preferred.
- Frozen sections.** Frozen sections from patients which are positive/suspicious for SARS-CoV-2 infection are strongly discouraged⁶. This recommendation is particularly important when dealing with materials from the upper and lower airways. Should this kind of activity be absolutely needed, personnel who manipulate the samples shall adhere to strict biosafety criteria: frozen sections should be preferentially done in cryomicrotomes which allow aerosol containment [if not available the cryomicrotomes shall be cleaned and disinfected using alcohol 100°C (to avoid ice formation) after each procedure]. Personnel shall be appropriately instructed and shall always wear appropriate PPE.
- Biosafety cabinets (BSC) and fume hoods (FH).** All activities implying the use of potentially infectious unfixed/inadequately fixed material should be performed in BSC. BSC protect workers by a) containing vapors, dusts, gases, and fumes moving them as air flows into the hood and then out of the laboratory via the exhaust system, and b) shielding the worker with a clear sliding window that contains aerosols and prevents injury from splashes that may occur inside the hood. There are three classes of biosafety cabinets: Class I, Class II, and Class III. Class I biosafety cabinets provide personnel and environmental protection but no product protection. Class II and Class III cabinets provide personnel, environmental, and product protection. BSC Class II biosafety cabinets are widely used in biological research laboratories and are differentiated into 4 types, labeled as A1, A2, B1, or B2. Any procedure with the potential to generate aerosols or droplets (e.g., vortexing, centrifuging, pipetting) should be performed in a certified Class II BSC. If no certified Class II BSC is available, or if instruments (e.g., centrifuges, analyzers, automated extraction equipment) cannot be used inside a BSC, extra precautions can be used to provide a barrier between the specimen and personnel. Examples of these precautions include using additional personal protective equipment (PPE) (e.g., mask, respirator, face shield) or other physical barriers (e.g., splash shield, centrifuge safety cups, sealed centrifuge rotors) to reduce the risk of exposure to laboratory personnel. BSC class I with appropriate air flow toward external, which are frequently used in surgical pathology laboratories in Italy, are similar to chemical fume hoods, substantially differing from H because of the use of high efficiency particulate air (HEPA) filters: these BSC are appropriate for risk mitigation. Pure FH, without HEPA filters, and at even more importantly without appropriate air flow toward external, are not appropriate for biosafety.
- Manipulation of unfixed/inadequately fixed surgical specimens.** As stated above we suggest to suspend the use of formalin-free vacuum collection of surgical specimens if the safety level of the laboratory does not guarantee adequate protection. Surgical specimens in formalin jars, may arrive in the grossing room with only partial fixation: these samples have to be manipulated following biosafety procedures. Pragmatically, as fixation is a process which takes time and should be favored by gross sectioning of the organs, we suggest that incompletely fixed samples, especially those of the lungs and gastrointestinal tract, shall be pre-

liminarily grossly sectioned without extracting the samples from the jars followed by direct injection of formalin in the organ/tissue (e.g.: in lung tissue usually floating in the jar), and definitively sampled only after complete fixation. This approach could facilitate formalin fixation (and hence morphological and biomolecular preservation), while minimizing contamination risk.

- **Manipulation of unfixed/inadequately fixed cytological.** Procedures which can produce aerosol shall always performed under high biosafety precautions. Centrifuges should have safety buckets or sealed rotors and should be placed in a BSC. Devices for vortexing tubes should also be placed in a BSC. If the laboratory is not equipped with appropriate BSC, the centrifuges and vortex shall be placed in available chemical fume hoods, possibly with ultraviolet sterilization ¹. It is extremely important to underline that the cytological evaluation of positive/possible SARS-Cov2 samples, especially for those of bronchial/pulmonary origin, should be performed only for extremely necessary cases, possibly limiting and reducing the number of routine samples. The cytological material shall be processed in dedicated BSC under the supervision of specialized technicians wearing adequate protective equipment and whenever possible it is useful to fix the cytological samples which arrive unfixed in the laboratory, using a series of different approaches according to the different cytological samples. All these manipulations shall be done in a BSC. Specifically, for **pulmonary cytological samples** add a 70% (bronchial lavage) to 95% (sputum) alcoholic fixative solution, in a range from 1:1 to 2:1, followed by formalin fixation and paraffin embedding in a cell-block. **Pleural, peritoneal and serous fluids**, after centrifugation and deletion of supernatant, treat with 70% alcoholic fixative solution followed by formalin fixation and paraffin embedding in a cell-block. For **urine**, it is reasonable to add a 95% alcoholic fixation solution in a proportion 2:1. For **liquor**, the possibility of alcohol treatment it is not ideal mostly due to the scant amount of cellular material in the sample. While, this approach may slightly alter the quality of the sample, especially when compared to those usually processed with methanol solution, it may be wiser to modify the standard approach to guarantee safety in the laboratory, which is the main issue in this dramatic emergency condition.
- **Manipulation of liquid based cytological samples (LBC).** These cytological samples are usually collected and transferred to the surgical pathology laboratory in liquid mediums, however the alcohol-

ic concentration may not be high enough for viral inactivation. Therefore, these samples have to be manipulated with high biosafety precautions. For example, for non-cervical *samples processed with Hologic technology* (Marlborough, Massachusetts, US), we suggest the adoption of completely modified “off-label” method based on the collection of cytological material in a 70% ethyl alcohol solution instead of the Cytolyt[®] (Hologic) solution, followed by centrifugation at 600g for 10 minutes (or 1200g for 5 minutes). The next step is to discharge supernatant and resuspend cell pellet adding 30 ml of Cytolyt[®] solution. This phase can be followed by another centrifugation at 600g for 10 minutes, with discharge of the supernatant. Finally, it is necessary to add the specimen to the PreservCyt[®] solution vial, followed by PreservCyt[®] fixation for 15 minutes; the specimen can now be processed on a ThinPrep processor ¹⁷. Cervical cancer screening was stopped in Italy during the COVID-19 emergency: therefore, cervical LBCs should not be an issue for cytology laboratories. However, since the alcohol concentration in the most widespread LBC technologies may not reach 70%, sporadic pap tests may be preferentially done by non-LBC technique, and immediately fixed in 95% alcohol after preparation. If LBC cervical specimens are sent to our laboratories, they have to be manipulated following all biosafety roles.

- **Personal protection equipment.** Personnel which manipulates surgical and cytological samples (unfixed/inadequately fixed), shall always wear a) FFP3 mask (or, if this kind of mask is not available, a FFP2 mask), b) eye and face protection, c) double gloves and d) waterproof scrub. Personnel shall also be instructed how to wear and dispose PPE and must always wash hands when moving from one area of the laboratory to another.
- **Decontamination.** SARS-CoV-2 virus can persist on a variety of surfaces for different periods of time: it has been shown that it can persist up to 72 hours on steel and plastic, and is usually non-viable after 24 hours on cardboard ¹⁸. Therefore, all surfaces and equipments (e.g.: centrifuges, vortex, BSC, grossing surfaces, cryostats) shall be decontaminated using appropriate products. Recently prepared 0.1%-0.5% Sodium hypochlorite solution, 70% ethanol or 0.5% hydrogen peroxide are all adequate for disinfection. UV disinfection of cabinets is useful, but it has to be pointed out that it may not be efficient when the cabinets contain several objects. A manual spray device can be very useful to reach every angle of cabinets or cryostats.

Conclusion

The present epidemiological situation highlights the importance of biological risk management in surgical pathology. All fixed samples and all paraffin blocks are at extremely low (negligible or absent) risk of coronavirus infectivity as it has been reported that formalin fixation and heat exposure (in the range of the temperature for paraffin embedding of tissues) inactivate most coronaviruses^{12,13,19}. However, we are faced daily with manipulation of unfixed/inadequately fixed samples which requires strict adherence to biosafety rules. The widespread use of formalin-free vacuum technology has greatly increased the number of unfixed samples which are manipulated in our laboratories, increasing the need of appropriate risk management approach. This poses important issues of biosafety which require immediate action by our community.

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