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# Coronavirus disinfection in histopathology

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**ABSTRACT**

The 2019 Coronavirus epidemic, provisionally called 2019-nCoV, was first identified in Wuhan, China, in persons exposed to a seafood or wet market. There is an international push to contain the virus and prevent its spread. It is feasible that potentially infectious samples may be received in histopathology laboratories for diagnosis. This technical note presents disinfection procedures and histotechnology processes that should alleviate the risk of infection to laboratory staff. Using data obtained from similar coronaviruses, e.g. severe acute respiratory syndrome (SARS) and Middle East respiratory syndrome (MERS), experts are confident that 70% ethanol and 0.1% sodium hypochlorite should inactivate the virus. Formalin fixation and heating samples to 56oC, as used in routine tissue processing, were found to inactivate several coronaviruses and it is believed that 2019-nCoV would be similarly affected.

**KEYWORDS**

Coronavirus; SARS-CoV-2; histotechnology; biosafety; histopathology laboratory; disinfection

The outbreak of a novel coronavirus disease (provisionally known as 2019-nCoV) in Wuhan, China has spread to 26 countries worldwide. This coronavirus is an acute resolved disease but it can also be deadly, with a 2% case fatality rate. Severe disease onset might result in death due to massive alveolar damage and progressive respiratory failure. As of Feb 15, about 66 580 cases have been confirmed and over 1524 deaths [1]. The World Health Organization (WHO), on 11 February 2020, officially named the virus COVID-19 [2]. The Coronavirus Study Group of the International Committee on Taxonomy of Viruses decided that the virus is a variant of the coronavirus that caused an outbreak of severe acute respiratory syndrome (SARS) in 2002–03. Therefore, this committee named the new pathogen Severe Acute Respiratory Syndrome Corona Virus 2, or SARS-CoV-2 [3].

Since it is possible that infected samples may be submitted to histopathology laboratories for diagnosis, it is important for us to take adequate precautions to protect ourselves and our staff. The World Health Organization [4] recommends that all specimens collected for laboratory investigations should be regarded as potentially infectious. Health-care workers who collect, handle, or transport any clinical specimens should adhere rigorously to the standard precaution measures and biosafety practices listed in Table 1 to minimize the possibility of exposure to pathogens. The Centers for Disease Control and Prevention (CDC) has also released an Interim Laboratory Biosafety Guidelines for Handling and Processing Specimens Associated with Coronavirus Disease 2019 (COVID-19) and these are summarised in Table 2 (https://www.cdc.gov/coronavirus/2019-nCoV/lab/lab-biosafety-guidelines.html).

Unfortunately little is known about the appropriate disinfectants for 2019-nCoV, nor the safety of histological fixation and processing but experts have proposed that effective disinfectants for other coronaviruses (e.g. SARS and MERS) should inactivate 2019-nCoV.

The zoonotic coronavirus, provisionally called 2019-nCoV, was first identified in Wuhan, China, in persons exposed to a seafood or wet market [5]. A zoonosis is an infectious disease caused by bacteria, viruses, and parasites that spread between animals (usually vertebrates) and humans. Major modern diseases such as Ebola virus disease and salmonellosis are zoonoses. Zhu et al. [6] have identified and characterized 2019-nCoV. The viral genome has been sequenced, and these results in conjunction with other reports show that it is 75% to 80% identical to the SARS-CoV and even more closely related to several bat coronaviruses [5,6,].

Coronaviruses are large, enveloped, positive-strand RNA viruses that can be divided into four genera: alpha, beta, delta, and gamma, of which alpha and beta CoV are known to infect humans. Four HCoV (HCoV 229E, NL63, OC43, and HKU1) are endemic globally and account for 10% to 30% of upper respiratory tract infections in adults. Coronaviruses are ecologically diverse with the greatest variety seen in bats, suggesting that they are the reservoirs for many of these viruses [7].

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**Table 1.** World Health Organization standard precautions [4].

| | |
|---|---|
| 1 | Ensure that health-care workers who collect specimens use appropriate personal protective equipment (PPE) i.e. eye protection, a medical mask, a long-sleeved gown, and gloves. If the specimen is collected with an aerosol-generating procedure, personnel should wear a particulate respirator at least as protective as a NIOSH-certified N95, an EU standard FFP2, or the equivalent. |
| 2 | Ensure that all personnel who transport specimens are trained in safe handling practices and spill decontamination procedures. |
| 3 | Place specimens for transport in leak-proof specimen bags i.e. secondary containers, that have a separate sealable pocket for the specimen i.e. a plastic biohazard specimen bag, with the patient's label on the primary specimen container and a clearly written laboratory request form. |
| 4 | Ensure that laboratories in health care facilities adhere to appropriate biosafety practices and transport requirements, according to the type of organism being handled. |
| 5 | Deliver all specimens by hand whenever possible. DO NOT use pneumatic-tube systems to transport specimens. |
| 6 | Document clearly each patient's full name, date of birth and suspected 2019-nCoV of potential concern on the laboratory request form. Notify the laboratory as soon as possible that the specimen is being transported. |

**Table 2.** Summary of the Interim Laboratory Biosafety Guidelines from the CDC.

| | |
|---|---|
| 1 | Laboratory workers should wear appropriate personal protective equipment (PPE) which includes disposable gloves, laboratory coat/gown and eye protection when handling potentially infectious specimens. |
| 2 | Any procedure with the potential to generate aerosols or droplets (e.g. vortexing) should be performed in a certified Class II Biological Safety Cabinet (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. |
| 3 | After specimens are processed, decontaminate work surfaces and equipment with appropriate disinfectants as used with other respiratory pathogens, such as seasonal influenza and other human coronaviruses |
| 4 | For COVID-19 laboratory waste, follow standard procedures associated with other respiratory pathogens, such as seasonal influenza and other human coronaviruses. |
| 5 | Preparation and chemical- or heat-fixing of smears for microscopic analysis should be done in a certified Class II Biological Safety Cabinet |

Human-to-human transmissions of the novel coronavirus (2019-nCoV) have been described with incubation times between 2 and 10 days, facilitating its spread via droplets, contaminated hands or surfaces. There may be additional forms of transmission that are yet not determined. There are a wide range of disinfectants available that can be used to disinfect surfaces [8]. Kampf et al. [9] have noted that other coronaviruses, e.g. SARS and MERS, can persist on inanimate surfaces like metal, glass, or plastic for up to 9 days, but can be efficiently inactivated by surface disinfection procedures with 62–71% ethanol, 0.5% hydrogen peroxide, or 0.1% sodium hypochlorite within 1 min. Other biocidal agents such as 0.05% to 0.2% benzalkonium chloride or 0.02% chlorhexidine digluconate were less effective. Duan et al. [10] found that irradiation with ultraviolet light for 60 min on several coronaviruses in culture medium resulted in undetectable levels of viral infectivity.

Histopathology laboratories are often fortunate in that routine histotechnology processes often inactivate many viruses, e.g. Ebola [11]. The question then arises as to whether there is any evidence that these processes will have any effect on the activity of the coronavirus. Darnell et al. [12] determined that formalin and glutaraldehyde inactivated SARS-CoV in a temperature- and time-dependent manner. While incubation at 4°C inhibited the effect of these chemicals, at 37°C or room temperature, formalin significantly decreased the infectivity of the virus on day 1, while glutaraldehyde inactivated SARS-CoV after incubations of 1–2 days. Xu et al. (1) presented the autopsy findings of a 2019-nCoV patient. In this case study, core biopsies were taken from lung, liver and heart. Based on the quality of the accompanying photomicrographs, it seems that the core biopsies were fixed in formalin, processed through to paraffin and sections stained with H&E.

Duan et al. [10] found that several coronaviruses were made non-infectious after the following exposure times and temperatures: 90 min at 56°C, 60 min at 67°C, and 30 min at 75°C. Paraffin infiltration in most histopathology laboratories uses a temperature of 60–65°C for 2 h or more. It is, therefore, appropriate to consider that the formalin-fixed paraffin-embedded tissue block would have a low risk of coronavirus infectivity.

Based on the previous discussion, it appears prudent to refrain from performing frozen sections on possible cases of 2019-nCoV unless the laboratory is confident in containing aerosols in the cryostat. The same consideration should be applied to the grossing of partially fixed specimens. It appears, based on the limited autopsy study by Xu et al. [1], that only lung tissues exhibit microscopic evidence of 2019-nCoV infection whereas no viral change was noted in liver and cardiac muscle.

In conclusion, it is recommended that appropriate safety precautions be taken (see Tables 1 and 2) and we can be assured that formalin fixation and paraffin embedding should inactivate 2019-nCoV.

## Disclosure statement

No potential conflict of interest was reported by the author.

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