

Biological Inactivation of COVID-19/SARS-CoV-2 Specimens for Handling under BSL-2 Containment Conditions

The SARS-CoV-2 pandemic has resulted in widespread interest for researchers to acquire and work with clinical specimens from known or strongly suspected COVID-19+ subjects. To address the exposure risk associated with these materials when used for common lab analyses, biocontainment guidelines were established at the institutional level based on national/international standards and guidelines [1,2](#).

The minimum biosafety level that may be used for handling COVID-19/SARS-CoV-2 specimens is BSL-2 provided that the specimens have been biologically inactivated. This document summarizes common inactivation methods that are suitable for COVID-19/SARS-CoV-2 specimens in order to permit these materials to be downgraded from a BSL-2 with enhanced containment practices (BSL-2+) lab to a standard BSL-2 lab setting. Research teams may reference this document when preparing registrations or amendments for submission to the Institutional Biosafety Committee (IBC) for approval of their COVID-19/SARS-CoV-2-related activities.

The methods described here represent those commonly used in research labs and are based on a review of available scientific literature that supports their efficacy for inactivating SARS-CoV-2 or other members of the β -coronavirus family. Other methods may be approved by the IBC on a case-by-case basis provided that scientific data that demonstrate successful inactivation can be provided for materials and methods to be used. However, this document and IBC approval process is not applicable to materials that have entered a BSL-3 laboratory. A validated inactivation protocol is necessary for transferring biomaterials from BSL-3 to a lower biosafety level setting.

Inactivation Methods

Heat inactivation

It is well established in the literature that members of coronavirus family can be inactivated by incubation at temperatures above 56°C [3,4,5](#). Shorter incubation times require higher temperatures, but longer incubation times are needed if the sample has a high protein content, as would be expected in samples of blood and other body fluids [6](#). BEI Resources currently distributes SARS-CoV-2 that has been inactivated by incubation for 30 minutes at 65°C [7](#). As experiments with SARS-CoV show inactivation of virus in serum or high protein simulants in as little as 10 minutes [5,6](#), 30 minutes at 65°C is a conservative standard to adopt to assure inactivation. Twice the incubation time at 58°C gave similar results so 1 hour at 58°C is also acceptable.

Chemical Inactivation for RNA extraction

Samples for RNA extraction are collected in storage buffers designed to inactivate proteins and preserve the RNA. These buffers often contain detergents, such as SDS, that should inactivate an enveloped virus, but there is little data on the effect of detergents on coronavirus [8](#) and no studies that looked at these collection buffers specifically. Because of this, SARS-CoV-2 samples in storage buffers should generally be considered viable.

However, inactivation by two of the most common lysis buffers, Trizol and AVL buffer (used in Qiagen kits), has been evaluated for inactivation of MERS-CoV [9](#). When used according to the manufacturer's instructions, there was no evidence of infectious virus when the results of that lysis were diluted to remove cytotoxic chemicals and added to growing cells. Thus, these buffers are expected to inactivate SARS-CoV-2 in the same manner.

Chemical fixation of cells or tissues

Most of the studies on chemical fixation used a coronavirus infected monolayer of cells in tissue culture as the samples. They tested three commonly used fixatives for histology or microscopy: 10% neutral buffered formalin, 4% paraformaldehyde, and 1:1 methanol/acetone ⁹. A 30-minute incubation in formalin or paraformaldehyde at room temperature was sufficient to inactivate MERS-CoV; a 60-minute incubation was required for methanol/acetone.

Unfortunately, there is a lack of literature regarding inactivation of coronavirus in tissue samples. The previously mentioned fixatives should inactivate coronavirus in tissue samples but there are no published details on the time needed to fully perfuse the whole of the tissue sample. The CDC Interim Guidance on Collection and Submission of Postmortem Specimens from Deceased Persons with Known or Suspected COVID-19 ¹⁰ instructs that a tissue sample of 4-5 mm in thickness should be placed in at least 10 times the volume of the sample of 10% formalin and incubated for 72 hours for optimal fixation. Note: Paraffin embedded samples will be fully heat-inactivated because the paraffin infiltration step places the sample at a temperature of 60-65°C for around 2 hours ¹¹.

Expectations for Use of Inactivation Methods

Inactivation procedures must follow of the requirements of the IBC Policy for work with Potential COVID-19/SARS-CoV-2 Specimens ¹.

Specific points to note:

- All inactivation methods used need to be included in the submitted written standard operating procedures (SOPs).
- Personnel must demonstrate proficiency in carrying out the procedure successfully and as written; this proficiency should be documented by the designated oversight person.
- Any inactivation procedure that requires opening the sample container or has the potential for aerosol creation should take place inside a biosafety cabinet.
- Prior to any changes to inactivation methods a new or updated SOP needs to be submitted to VEHS Biosafety for risk assessment.
- IBC approval of transfer of inactivated materials from a lab is limited to the materials and labs detailed in that lab's COVID-19/SARS-CoV-2 Specimen Intake Form and SOPs. Any changes to the materials being transferred or the recipient labs must be communicated to VEHS Biosafety.
- For each batch of inactivated samples, a log must be kept that details:
 - Type of sample (tissue, serum, etc.)
 - Who inactivated the samples
 - What inactivation method was used (specific SOP followed must be noted)
 - Date of inactivation
 - Who samples were transferred to
- With each batch of materials that is being transferred, a written assurance communication with the details of the inactivation procedure should accompany the materials.
- If you have any reason to suspect a failure of the inactivation method, contact Robin Trundy (robin.trundy@vumc.org) or another member of VEHS Biosafety (biosafety@vumc.org) immediately.

References

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