Updated January 8, 2025

Requirements for SARS-CoV-2 research

Table of Contents

Containment levels at a glance 1
Institutional Biosafety Committee review and approval 2
Background on COVID-19 and SARS-CoV-2 2
General considerations for COVID-19/SARS-CoV-2 research 3
Research involving live SARS-CoV-2 4
Research involving pandemic human samples 7
Research involving inactivated COVID-19 samples 8
Research involving nucleic acids from SARS-CoV-2 8
Research involving proteins from SARS-CoV-2 9

Containment levels at a glance

Research type	Containment level
Live SARS-CoV-2	BSL2/ABSL2 with enhancements
COVID-19 patient samples	BSL2 with enhancements
Samples from infected animals	BSL2 with enhancements
Pandemic human samples	BSL2 with enhancements (respiratory/GI samples only)
Inactivated COVID-19 samples	BSL1
Nucleic acids from SARS-CoV-2	BSL1
Proteins from SARS-CoV-2	BSL1

Institutional Biosafety Committee review and approval:

As an institution that receives federal funding, UW-Madison is required to comply with the *NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules (NIH Guidelines)*. Experiments that fall under sections III-A through III-D of the *NIH Guidelines* require approval from the UW-Madison Institutional Biosafety Committee (IBC) prior to initiation, even if low risk. Experiments that fall under section III-E of the *NIH Guidelines* may commence simultaneously with IBC notification (i.e., assignment of a biosafety protocol or amendment to an IBC meeting), but still require IBC review and approval.

Experiments that fall under section III-F of the *NIH Guidelines* (i.e., exempt experiments) or that are not subject to the *NIH Guidelines* because they do not involve recombinant or synthetic nucleic acids may still require a biosafety protocol and be subject to IBC review and approval. The IBC is charged by the Chancellor with responsibility for oversight of biological materials that entail a potential risk to humans, animals, plants, or the environment. The IBC is authorized to approve, require modifications to secure approval, or disapprove work with biohazardous materials. The IBC is also authorized to suspend or revoke authorization of work that is not conducted in accordance with their requirements. To determine if your planned research requires a biosafety protocol, please refer to the Bio-ARROW webpage or contact OBS at 608-263-2037 or biosafety@fpm.wisc.edu.

The IBC meets the first and third Wednesday of every month unless otherwise posted; please see the IBC webpage or contact OBS at 608-263-2037 or biosafety@fpm.wisc.edu for upcoming meeting information. Protocols and amendments are pre-reviewed by OBS staff prior to assignment to an IBC meeting. To allow time to complete the pre-review process it is recommended that new protocols and amendments are submitted at least one month prior to the IBC meeting date.

Background on COVID-19 and SARS-CoV-2:

COVID-19 is a respiratory disease that is caused by infection with the coronavirus SARS-CoV-2 (initially called "2019 novel coronavirus" or 2019-nCoV). SARS-CoV-2 is a betacoronavirus in the *Coronaviridae* family. It is closely related to SARS-CoV, the coronavirus that causes severe acute respiratory syndrome (SARS). Like SARS-CoV, SARS-CoV-2 is believed to have a zoonotic origin, perhaps originating in bats. SARS-CoV-2 is a positive-sense single-stranded RNA virus. It has four structural proteins: S (spike), E (envelope), M (membrane), and N (nucleocapsid). S, E, and M along with the lipid bilayer form the viral envelope, and N interacts with the RNA genome to form the helical nucleocapsid. The virus is believed to enter human cells by binding of the spike protein to the receptor angiotensin converting enzyme 2 (ACE2). SARS-CoV-2 produces at least three virulence factors that promote shedding and inhibit the host immune response.

Infection with SARS-CoV-2 results in a range of symptoms from none to mild to severe. Symptoms may include fever, chills, cough, shortness of breath or difficulty breathing, nasal congestion, muscle pain, headache, fatigue, sore throat, loss of taste or smell, and diarrhea. In most individuals, COVID-19 produces only mild flu-like symptoms. Older adults and people of any age who have serious underlying medical conditions are at higher risk for severe illness. Severe illness can lead to hospitalization, need for ventilation, and in the most severe cases, death. Medical conditions known to increase COVID-19 risk include chronic lung disease, asthma, heart conditions, diabetes, chronic kidney disease, liver disease, obesity, and compromised immunity. Many things can cause a person to be immunocompromised, including pregnancy, cancer treatment, smoking, bone marrow or organ

transplantation, immune deficiencies, AIDS, and prolonged use of corticosteroids or certain other medications. Employees are not required to disclose pregnancies, medical conditions, or their immune status, and if they have concerns, they should consult their physician. Treatments are available that can reduce the chances of hospitalization or death in infected individuals who are at higher risk for severe disease. Medications to treat COVID-19 require a prescription and should be started as soon as possible after diagnosis for maximum effectiveness. More information can be found on the CDC's Types of COVID-19 Treatment webpage.

Long COVID is a chronic condition that occurs after SARS-CoV-2 infection. Long COVID symptoms can last weeks, months, or perhaps years and can include fatigue, fever, difficulty breathing, coughing, chest pain, heart palpitations, difficulty thinking or concentrating, headaches, sleep problems, lightheadedness, pins-and-needles sensations, changes in smell or taste, diarrhea, stomach pain, constipation, joint or muscle pain, rash, menstrual changes, depression and anxiety. Anyone who gets COVID may develop Long COVID. Some groups are at higher risk of Long COVID, including women, people experienced more severe illness, people with underlying health conditions, older adults, and individuals that have not received a COVID vaccine. More information can be found on the CDC's Long COVID webpage.

SARS-CoV-2 spreads through respiratory droplets produced when an infected person coughs or sneezes. Other possible means of transmission include aerosols (e.g., an infected person exhaling), transfer of virus through touching the mouth, nose, or eyes after touching a contaminated surface, and fecal-oral transmission. Symptoms may appear 2-14 days after exposure, and some infected individuals never exhibit symptoms. Infected individuals may transmit the virus even if pre-symptomatic or asymptomatic.

COVID-19 vaccines are available. Although studies indicate these vaccines are safe and highly effective at preventing severe illness, they do not guarantee protection from infection. Immunity wanes over time after vaccination, and vaccination may not prevent transmission of the virus. More information on COVID-19 vaccines can be found on the CDC's COVID-19 Vaccines webpage.

General considerations for COVID-19/SARS-CoV-2 research:

Due to the high morbidity and mortality in COVID-19 patients, SARS-CoV-2 was initially classified as a risk group 3 (RG3) virus. As of December 20, 2024, after reviewing the current pathogen characteristics and population impact the National Institutes of Health downgraded SARS-CoV-2 to RG2. When planning any COVID-19 research project, the following should be considered:

- In the event of an exposure to research materials, is there a risk of a lab-acquired COVID-19 infection (i.e., will you be working with live SARS-CoV-2 or materials that contain or potentially contain live SARS-CoV-2, such as clinical samples)?
- If exposure to live virus is possible, then:
    - What is the level of exposure risk? (e.g., Will the virus be isolated or cultured? Will samples come from known COVID-19 patients or are they unknown diagnostic specimens? What type of tissue samples or bodily fluids will be used?)
    - What is the laboratory's experience with coronaviruses or other respiratory pathogens?
    - Who will train personnel to work with the virus/potentially infectious materials?
    - Has the laboratory developed all the necessary SOPs for working with the virus/potentially infectious materials (e.g., disinfection, spill protocols)?

    - Does the laboratory have a SARS-CoV-2 occupational health plan?
    - Have laboratory staff been offered COVID-19 vaccine? For information on vaccine availability, please ask your medical provider or contact Occupational Medicine at occupationalmedicine@fpm.wisc.edu.
- Does the laboratory have the facilities and containment equipment appropriate for the level of risk (as outlined in the sections below)?
- What other hazards are associated with this research (e.g., bloodborne pathogens, viral vectors, sharps), and how will their risk be mitigated?
- If you will be using human subjects or samples collected from human subjects, do you have Institutional Review Board (IRB) approval for this research?
- If you will be using animal models, do you have Institutional Animal Care and Use Committee (IACUC) approval for this research?

The sections below outline the typical requirements for different types of SARS-CoV-2 research. As many factors affect risk (e.g., genetic modifications, equipment, assays and procedures), OBS and the IBC will work with you to determine the appropriate containment level, location, practices, and PPE needed for your specific research. For questions, please contact OBS at 608-263-2037 or biosafety@fpm.wisc.edu.

Research involving live SARS-CoV-2:

Research involving the isolation, enrichment, culture, manipulation, or concentrated stocks of live SARS-CoV-2 requires a minimum of BSL2 containment and must be approved by the IBC prior to initiation. The IBC may require higher containment or additional enhanced practices for work with genetically modified or large volumes of SARS-CoV-2.

Please note that if your laboratory is currently approved to work with SARS-CoV-2 at BSL3/ABSL3, you must amend your biosafety protocol to move this work to BSL2/ABSL2. The amendment must provide a description of how viral stocks and other materials will be transferred from the BSL3/ABSL3 laboratory to lower containment for review by the IBC.

Facility requirements

Air should be single-pass (100% exhausted) and not re-entrained or recirculated into any part of the building. Please note that other aspects of the laboratory layout and workflow should be considered as part of the laboratory's risk assessment.

Administrative controls and practices

Good microbiological practices must be followed when working with any microorganism, though this is especially true when working with a respiratory virus. Work with live SARS-CoV-2 additionally requires the following:

- The laboratory must have established processes to address occupational health considerations for personnel involved in the research. The occupational health plan must include:
    - Hazard communication that includes the risk and symptoms of COVID-19
    - Signed informed consent for those working with SARS-CoV-2

  OBS has developed an occupational health plan template that may be used for this purpose. It can be found under Forms on the EH&S webpage.

- The laboratory must have pathogen-specific emergency response procedures in the event of known potential exposures and development of symptoms with no known exposures.
- Access to the laboratory must be controlled.
- All aerosol-generating activities (e.g., pipetting, homogenization) should be performed inside a certified class II biosafety cabinet (BSC) or other approved containment device.
- EPA-registered disinfectants effective against SARS-CoV-2 should be used for decontamination of work surfaces and equipment.
- Required PPE includes a lab coat or disposable gown, eye protection, and disposable gloves. Additional PPE such as an N95/N100 respirator or PAPR, dedicated lab shoes or disposable shoe covers, and face shield may be required if activities with SARS-CoV-2 will be performed outside of containment.
- When concentrating or purifying virus, or preparing viral stocks:
    - Access to the laboratory must be restricted to those performing these activities
    - Signage should be posted at the laboratory entry regarding the restricted access
    - Respiratory protection (i.e., fit-tested N95/N100 or PAPR) must be worn by all those present in the room during these activities

  Restricted access and respiratory protection are optional during subsequent activities with aliquots of previously prepared viral stocks that are performed in a BSC or other approved containment device (e.g., infecting cell cultures).

COVID-19 patient samples

Since SARS-CoV-2 is a respiratory pathogen, nasal swabs, nasal washes, nasal secretions, sputum, saliva, and lung or respiratory tract tissue present the highest risk of exposure. Work with cells, tissues, and bodily fluids and secretions collected from patients known or suspected to have COVID-19 requires a minimum of BSL2 containment with the following enhancements:

- The laboratory must have established processes to address occupational health considerations for personnel involved in the research. The occupational health plan must include:
    - Hazard communication that includes the risk and symptoms of COVID-19
    - Signed informed consent for those working with SARS-CoV-2

  OBS has developed an occupational health plan template that may be used for this purpose. It can be found under Forms on the EH&S webpage.
- The laboratory must have pathogen-specific emergency response procedures in the event of known potential exposures and development of symptoms with no known exposures.
- Access to the laboratory must be controlled.
- All aerosol-generating activities (e.g., pipetting, homogenization) should be performed inside a certified class II BSC or other approved containment device.
- EPA-registered disinfectants effective against SARS-CoV-2 should be used for decontamination of work surfaces and equipment.
- Additional PPE, enhanced practices, or higher containment levels may be required under the following circumstances:
    - Aerosol-generating activities with COVID-19 samples will be performed outside of containment
    - Cells from COVID-19 patients will be isolated and cultured
    - Cells from COVID-19 patients will be administered to animals

Exceptions for low-risk samples may be considered on a case-by-case basis.

Animal models of COVID-19
Research animals that are infected with SARS-CoV-2 require ABSL2 containment. Where possible, animals infected with SARS-CoV-2 must be housed in microisolator cages. For species that do not have microisolator caging available, animal cages or pens must be housed within an approved HEPA-filtered containment device or within an approved isolation room. Additional PPE, enhanced practices, or higher containment levels may be required for animals housed in open cages or animal procedures performed outside of containment based on risk assessment.

Please note that not all UW-Madison laboratories are considered suitable for housing animals, and locations for animal use must be approved by both the IBC and IACUC.

Samples from naturally or experimentally infected animals
Samples from animals infected with wild type SARS-CoV-2 may be handled similarly to samples from human COVID-19 patients. For the purposes of this guidance, samples collected from animals with a natural infection are equivalent to samples from animals experimentally infected with SARS-CoV-2. It should be noted, however, that experimentally infected animals may have received a higher titer of the virus and carry higher viral loads than those of naturally infected animals.

Only samples from animals known or suspected to be infected with SARS-CoV-2 require additional precautions; samples collected during the pandemic from a laboratory colony, wild or patient population of animals not known or suspected to have SARS-CoV-2 infection (i.e., "pandemic" animal samples) do not require special handling even if derived from a susceptible species. Precautions should be employed that address other zoonotic risks associated with the species as applicable.

If you will be receiving samples from animals infected with wild type or mutant SARS-CoV-2 from a collaborator or commercial source, please provide information about the source in the "Organs, tissues, or biological specimens other information" text box. Samples harvested from animals listed on your biosafety protocol do not need to be listed on the "Cells, Organs, Tissues or Biological Specimens" page.

Chimeric SARS-CoV-2 viruses
Under an Interim Final Rule issued by the CDC's Division of Select Agents and Toxins recombinant SARS-CoV-2 viruses containing nucleotides encoding virulence factors from SARS-CoV have been added to the federal list of select agents and toxins. The creation of chimeric SARS-CoV/SARS-CoV-2 viruses are restricted experiments and require approval from the CDC in addition to the IBC prior to initiation. Experiments using these chimeric viruses must be conducted in select agent registered space by approved personnel. If interested in conducting experiments involving chimeric SARS-CoV/SARS-CoV-2 viruses please contact the Select Agent Responsible Official Christina Pier at 608-712-2359 or pier@wisc.edu.

The creation and use of other chimeric SARS-CoV-2 viruses will be evaluated by the IBC on a case-by-case basis. The IBC may require higher containment or additional enhanced practices for work with chimeric virus, depending on the activities and risk profile of the chimera.

Research involving pandemic human samples:

For the purposes of this guidance, pandemic human samples are defined as mucosal swabs or washes, sputum, blood, serum, plasma, feces, urine, other tissues or bodily fluids collected from **November 1, 2019 through May 11, 2023**. Pandemic human respiratory and gastrointestinal samples should be treated as potentially infectious for COVID-19 unless collected from individuals that have tested negative for COVID-19 at the time of sample collection or the samples are confirmed to be negative for SARS-CoV-2 using a validated method.

Facility requirements (all sample types)

Air should be single-pass (100% exhausted) and not re-entrained or recirculated into any part of the building. Please note that other aspects of the laboratory layout and workflow should be considered as part of the laboratory's risk assessment.

Pandemic respiratory and gastrointestinal tract samples

Work with potentially infectious nasal swabs, nasal washes, nasal secretions, sputum, saliva, lung or respiratory tract tissue, feces or gastrointestinal tract tissue collected during the pandemic from the general population or a patient population not known to have COVID-19 requires BSL2 containment with the following enhancements:

- The laboratory must have established processes to address occupational health considerations for personnel involved in the research. The occupational health plan must include:
  - Hazard communication that includes the risk and symptoms of COVID-19
  - Signed informed consent for those working with SARS-CoV-2

  OBS has developed an occupational health plan template that may be used for this purpose. It can be found under Forms on the EH&S webpage.
- The laboratory must have pathogen-specific emergency response procedures in the event of known potential exposures and development of symptoms with no known exposures.
- Access to the laboratory must be controlled.
- All aerosol-generating activities (e.g., pipetting, homogenization) should be performed inside a certified class II BSC or other approved containment device.
- EPA-registered disinfectants effective against SARS-CoV-2 should be used for decontamination of work surfaces and equipment.
- Additional PPE such as a disposable lab coat, gown, or coveralls, an N95/N100 respirator or PAPR, dedicated lab shoes or disposable shoe covers, and face shield may be required if activities with pandemic respiratory/gastrointestinal samples will be performed outside of containment.

Respiratory and gastrointestinal samples collected before November 1, 2019 or after May 11, 2023 from the general population or a patient population not known to have COVID-19 may be handled using standard precautions. However, it should be noted that these samples may unknowingly contain pathogens including coronaviruses, influenza viruses, rhinoviruses, mycobacteria, and others. It is therefore still recommended that these samples be handled at BSL2 and aerosol-generating activities be performed inside a BSC or other containment device.

Other non-respiratory/gastrointestinal pandemic human samples
Work with blood, plasma, serum, organs, and tissues that are not part of the respiratory or gastrointestinal tracts, and non-respiratory/enteric bodily fluids and secretions (e.g., cerebrospinal fluid) obtained during the pandemic from the general population or a patient population not known to have COVID-19 may be handled using standard precautions. Please note that blood, blood products, and other potentially infectious materials as defined as under the Bloodborne Pathogen Exposure Program require BSL2 containment. All human tissues and bodily fluids may unknowingly contain pathogens, even those not considered to be potentially infectious for bloodborne pathogens (e.g., urine). It is therefore recommended that all human-derived samples be handled at BSL2 and aerosol-generating activities be performed inside a BSC or other containment device.

Research involving inactivated COVID-19 samples:
Although it is typically not required to list inactivated materials (e.g., fixed tissues) on a biosafety protocol, since SARS-CoV-2 is a pandemic pathogen, investigators are asked to list inactivated COVID-19 samples. Relevant samples include cells, tissues, or fluids from COVID-19 patients or animals with a known SARS-CoV-2 infection, and cultured cells or tissues infected with SARS-CoV-2. Work with inactivated COVID-19 samples may be performed at BSL1 containment using standard precautions.

Inactivation of COVID-19 samples should be performed using a method with demonstrated efficacy against SARS-CoV-2, related coronaviruses (SARS-CoV or MERS-CoV), or other highly pathogenic viruses with a lipid envelope. The lab should provide a published reference or data demonstrating viral inactivation, including inactivation time and concentration of reagents. If the laboratory is receiving inactivated COVID-19 samples from a collaborator or commercial source, they should request and retain documentation verifying inactivation of the samples using a validated method.

If you will be working with only inactivated COVID-19 samples, please do not list SARS-CoV-2 on the Microbes page in Bio-ARROW. Rather, please list the inactivated materials in the "Organs, tissues or biological specimens" section and provide a description of the samples, their source, and the inactivation method used in the "Organs, tissues, or biological specimens other information" text box.

Research involving nucleic acids from SARS-CoV-2:
Research involving the subcloning, expression, or analysis of individual genes from SARS-CoV-2 *in vitro*, in microbes, or in cultured cells may be performed at BSL1 if no other agents used in the experiments require a higher level of containment. Recombinant or synthetic nucleic acid constructs and recombinantly modified microbes and cells must be listed on your biosafety protocol and may require concomitant notification of the IBC or IBC approval prior to initiation.

Research involving nucleic acids extracted from COVID-19 samples require BSL1 containment using standard precautions. Relevant samples include cells, tissues, or fluids from COVID-19 patients or animals infected with SARS-CoV-2, and cultured cells or tissues infected with SARS-CoV-2.

If you will be working with only extracted SARS-CoV-2 RNA or cDNA (e.g., deep sequencing experiments), please do not list SARS-CoV-2 on the Microbes page in Bio-ARROW. Rather, please list the nucleic acids in the "Organs, tissues or biological specimens" section and provide a description of the material and its source in the "Organs, tissues, or biological specimens other information" text box.

Research involving proteins from SARS-CoV-2:

Research involving only isolated SARS-CoV-2 proteins does not require a biosafety protocol or OBS/IBC review if all the following are true:

- The proteins are being produced in cells or RG1 microbes through expression of recombinant protein and no SARS-CoV-2 is involved in any step of their production
- Your laboratory is not doing any of the recombinant work but is just receiving the purified protein from a collaborator or commercial source*
- The research does not involve any other recombinant or biohazardous materials

By themselves, SARS-CoV-2 proteins do not cause disease. Therefore, there are no enhanced biosafety requirements for experiments involving only SARS-CoV-2 proteins in the absence of its RNA genome.

*If you will be expressing recombinant SARS-CoV-2 proteins in cells or microbes in your laboratory, please refer to the "Research involving nucleic acids from SARS-CoV-2" section for requirements.