

Laboratory biosafety guidance related to SARS-CoV-2 (COVID-19)

Interim guidance

Updated 11 March 2024



Highlights of laboratory biosafety for activities requiring handling SARS-CoV-2 specimens

- All laboratory procedures must be performed based on risk assessment, and only by personnel with demonstrated capability, working in strict observance of any relevant protocols at all times.
- Initial processing (before inactivation) of clinical specimens should take place in a validated biological safety cabinet (BSC) or primary containment device.
- Non-propagative diagnostic laboratory work (for example, sequencing, nucleic acid amplification test [NAAT]) should be informed by a risk assessment and conducted at a facility using procedures and effective risk control measures corresponding to Biosafety Level 2 (BSL-2).
- Point of care (POC) or near-POC assays can be performed on a bench without employing a biological safety cabinet, when the local risk assessment so dictates and effective risk control measures are in place.
- Propagative work (for example, virus culture, neutralization assays, or animal studies) should only be conducted following a careful local risk assessment and should be conducted using heightened control measures, which correspond to Biosafety Level 2 (BSL-2) or higher (for example, in the case of VOC with unknown biological profile).
- Appropriate disinfectants with proven activity against enveloped viruses (e.g., hypochlorite [bleach], alcohol, hydrogen peroxide, and quaternary ammonium and phenolic compounds) should be used at recommended concentrations and contact times.
- Patient specimens from suspected or confirmed COVID-19 cases infected with variants currently circulating in the population should be transported as UN3373, "Biological Substance Category B". Also, viral cultures or propagated isolates may in principle be classified as Category B. However, certain virus variants may be subject to a sound and responsible judgement in classification based on the definition of Category A and factors considered such as circulation of the virus variant (e.g. WHO classified variants of interest [VOI] or variants of concern [VOC]¹) in the population of the areas the material are to be transported from and to.

Background

The purpose of this document is to provide interim guidance on laboratory biosafety related to the testing of clinical specimens of patients that meet the case definition (1) for coronavirus disease (COVID-19) and research work using SARS-CoV-2, the virus that causes COVID-19.

This version updates the interim guidance with revised recommendations on research work and shipping procedures. It is important to note that vaccines which protect against severe illness are available. This guidance document reflects the current state of scientific knowledge.

Laboratory biosafety

It is essential to ensure that public health and research laboratories adhere to appropriate biosafety practices. Any testing for SARS-CoV-2, handling clinical specimens from patients meeting the suspected case definition (1), or propagating SARS-CoV-2 should be performed in appropriately equipped laboratories by staff trained in the relevant technical and safety procedures. National guidelines for laboratory biosafety should be followed in all circumstances. For general information on laboratory biosafety guidelines, see the WHO *Laboratory biosafety manual: fourth edition* (2).

Essential elements of laboratory biosafety

- Each laboratory should conduct a local (i.e., institutional) risk assessment to ensure it is competent to safely perform the intended testing with appropriate risk control measures in place, as exemplified in Annex of the *LBM4 Risk assessment Monograph* (3).
- When handling and processing specimens, including blood for serological testing as well as propagating SARS-CoV-2, laboratory practices and procedures that are basic to good microbiological practice and procedure (GMPP) should be followed.
- The handling and processing of specimens from cases with suspected or confirmed SARS-CoV-2 infection that are intended for additional laboratory tests, such as haematology or blood gas analysis, should follow standard guidelines without additional measures.
- Non-propagative diagnostic laboratory work, including sequencing and NAAT, on clinical specimens from patients who are suspected or confirmed to be infected with SARS-CoV-2, should be informed by the local risk assessment and conducted following the practices and

procedures of “core requirements”¹, as detailed in 4th edition of the WHO *Laboratory biosafety manual* (2), and “heightened control measures”², which correspond to Biosafety Level 2 (BSL-2).

- Handling high concentrations of live SARS-CoV-2 virus variants that are variants of interest (VOI) or variants of concern (VOC)^{3,1} or emerging strains without a known biological profile (such as when performing virus propagation, virus isolation, neutralization assays, and laboratory animal inoculation experiments) or large volumes of infectious materials should be performed only by adequately trained and competent personnel in laboratories meeting heightened control measures and practices which are based on a thorough risk assessment and correspond to BSL-3.
- Initial processing (before inactivation) of all specimens, including those for sequencing and NAAT, should take place in an appropriately maintained and validated BSC or primary containment device.
- The external lysis buffer of the listed common RNA extraction kits is effective in inactivating SARS-CoV-2 without heat or other additional means (4).
- Appropriate disinfectants with proven activity against enveloped viruses (5) should be used for the recommended contact time and concentration and within the expiry date after the working solution is prepared.
- All technical procedures should be performed in a way that minimizes the generation of aerosols and droplets (6).
- All laboratory personnel handling these specimens should wear appropriate personal protective equipment (PPE), as determined by a detailed risk assessment.
- Patient specimens from suspected or confirmed cases should be transported as UN3373, “Biological Substance Category B”.
- While most cultures, virus isolates and propagated material could in principle be classified as Category B, a sound and responsible judgement may be needed in accordance with the definition of Category A infectious substances, i.e., *capable of causing permanent disability, life-threatening or fatal disease in otherwise healthy humans or animals* (2). Factors that should be considered are, for example, variants that are not circulated at present in the countries of origin, transit, and destination of the planned shipment (7).

Recommendations addressing minimal/essential working conditions associated with specific manipulations in laboratory settings

The additional recommendations provided in this section address the minimal/essential working conditions associated with specific manipulations in laboratory settings.

1. Risk assessment

Risk assessment is a systematic process of gathering information, evaluating the likelihood and impact of exposure to or release of workplace hazard(s), and determining the appropriate risk control measures to reduce the risk to an acceptable level. Hazards alone do not pose a risk to humans or animals. The types of equipment used and the procedure(s) performed with the biological agent also play a role. The risk assessment should be reviewed regularly and amended when necessary; when new information becomes available, new procedures are undertaken, or new equipment will be used.

It is highly recommended to conduct a local risk assessment for each processing step (i.e., sample collection, sample reception, clinical testing, polymerase chain reaction (PCR), or virus isolation, if required). Specific hazards will be identified for each step, such as aerosol exposure or eye splash during sample processing, infectious culture material spill, and leaking sample containers. Each step in the process has its own assessed grade of risk, considering the consequences of an exposure or release as well as the likelihood. For each identified risk, appropriate risk control measures should be selected and implemented to mitigate the residual risks to an acceptable level.

Scientific and clinical information on variants and strains is critical when performing a local risk assessment. Those variants that are well-characterized to pose negligible risks to public health may necessitate only certain heightened control measures for virus isolation and propagation if aerosol generation outside the BSC is not anticipated. Strains no longer circulating or emerging without a known biological profile may necessitate careful consideration and additional heightened control measures.

Particular consideration should be given to risks related to human factors. The likelihood of errors and incidents is higher when staff training is insufficient, and staff members are pressured to produce rapid results.

A risk assessment template is provided in the Annex of the *LBM4 Risk assessment monograph* (7) and is intended to serve as an example and to facilitate the process.

¹ **Core requirements:** A set of minimum requirements defined in the 4th edition of the WHO *Laboratory biosafety manual* to describe a combination of risk control measures that are both the foundation for, and an integral part of, laboratory biosafety. These measures reflect international standards and best practice in biosafety that are necessary to work safely with biological agents, even where the associated risks are minimal.

² **Heightened control measures:** A set of risk control measures that may need to be applied in a laboratory facility because the outcome of a risk

assessment indicates that the biological agents being handled and/or the activities to be performed with them are associated with a relatively high risk that cannot be acceptable solely with the core requirements.

³ **Variant of Interest (VOI) and Variant of Concern (VOC)** in WHO: [Updated working definitions and primary actions for SARS-CoV-2 variants:](#)

2. Routine laboratory procedures, including non-propagative diagnostic work and PCR analysis

Non-culture-based diagnostic laboratory work and PCR analysis on clinical specimens from patients suspected or confirmed to be infected with SARS-CoV-2 should adopt practices and procedures described in the “core requirements” for conventional clinical and microbiology laboratories (2).

However, all manipulations of potentially infectious materials, including those that may cause splashes, droplets, or aerosols of infectious materials (for example, loading and unloading of sealed centrifuge cups, grinding, blending, vigorous shaking or mixing, sonic disruption, the opening of containers of infectious materials whose internal pressure may be different from the ambient pressure) should be performed in appropriately maintained and validated BSCs or primary containment devices, by personnel with demonstrated capability.

Examples of routine laboratory procedures include:

- Diagnostic testing of serum; blood (including haematology and clinical chemistry); respiratory specimens such as nasopharyngeal and oropharyngeal swabs, sputum and/or endotracheal aspirate or bronchoalveolar lavage; stool; or other specimens;
- Routine examination of mycotic and bacterial cultures developed from respiratory tract specimens. When handling and processing specimens, “core requirements” (2), including GMPP, should be followed at all times, including but not limited to those under the following subheadings. More details are explained and demonstrated in the WHO [Biosafety video series](#) (8).

3. Point of care (POC) or near-POC assay

Point-of-care or near-POC assays, including single-use lateral flow tests and cartridge-based, sample-to-answer NAAT platforms (e.g., GeneXpert), are now widely available for COVID-19 testing of nasopharyngeal specimens. Whereas lateral flow test procedures are nearly equivalent, POC molecular platforms use different methods and procedures to process samples, making it difficult to generalise specific safety recommendations. Procedurally, cartridge-based and lateral flow POCs are less hazardous than standard tube-based NAATs, and the level of aerosol generation would be minimal (9). Spills and splashes may occur, especially when staff are not adequately trained and may be under pressure to deliver rapid results.

POCs and near POCs can be performed on a stainless-steel bench without employing a BSC when the local risk assessment dictates, and the following conditions are fully met:

- Work is performed on a diaper or large paper towel in a **well-ventilated area** free of clutter, with no documents, computers, or personal items.
- PPE worn is similar to other manual testing, such as but not limited to a full-length long (elastic) sleeved lab coat, safety goggles or glasses, and suitable disposable gloves.

- Risk assessments are carried out to inform the use of respiratory protection as a supplementary precaution.
- Staff are well trained in GMPP.
- There is no rush or increased pressure for test turnaround.
- A validated infectious waste process that includes the disposal of excess specimens is performed.

In the case where the existing GeneXpert or similar platform of the tuberculosis programme is to be temporarily shared for SARS-CoV-2 testing, the equipment should already be installed in a suitable area with sufficient ventilation (Field (10)). In this case, there is no particular need to relocate it. If the equipment has been in use for non-respiratory disease programmes, such as HIV/AIDS, it is important to ensure that the testing area has proper ventilation before starting the test for COVID-19.

4. Use of appropriate disinfectants

SARS-CoV-2 is susceptible to disinfectants with proven activity against enveloped viruses, including sodium hypochlorite (bleach; for example, 1000 parts per million [ppm] (0.1%) for general surface disinfection and 10 000 ppm (1%) for disinfection of sample spills); 62–71% ethanol; 0.5% hydrogen peroxide; quaternary ammonium compounds; and phenolic compounds, if used according to the manufacturer’s recommendations (5).

Particular attention should be paid not only to the selection of the disinfectant but also to the contact time (for example, 10 minutes), dilution (that is, the concentration of the active ingredient), amount of organic material present, ambient temperature, shelf-life, and an expiry date after the working solution is prepared.

SARS-CoV-2 and other human coronaviruses, in general, are known to persist on inanimate surfaces such as metal, glass, or plastic for several days (11).

5. Viral isolation and propagation

Unless the country decides otherwise, virus isolation from clinical specimens collected from patients suspected or confirmed to be infected with SARS-CoV-2 should be performed based on a local and thorough risk assessment that should be informed by various factors. SARS-CoV-2 viruses that are widely circulating in the community with limited virulence and insignificant public health risks do not meet the definition of VOI or VOC, so require less stringent risk control measures.¹ In any case, these measures should be informed by a risk assessment. All manipulations of infectious or potentially infectious materials should be performed in appropriately maintained and validated BSCs by competent personnel.

Additional measures may be required when working with newly emerging viruses that meet the definition of a VOI or VOC. These measures also include:

- Personal protective equipment such as disposable gloves; solid-front or wrap-around gowns, scrub suits, or coveralls with sleeves that fully cover the forearms; head coverings; shoe covers or dedicated shoes and eye protection (goggles or face shield);

- The use of sealed centrifuge rotors or sample cups for centrifugation of specimens. Rotors or cups should be loaded and unloaded in a BSC;
- Risk assessment to inform the appropriate use of respiratory protection, including powered air purifying respirator or fit-tested particulate respirator, for example, EU FFP2, US 6 NIOSH-certified N95 or equivalent, or higher protection).

6. Waste management

Any material known to be potentially contaminated must be managed in such a way as to control the biological risks. This includes the identification, segregation, decontamination, and disposal processes. Where decontamination cannot be performed in the laboratory or on-site (e.g. autoclaving), the waste must be packed in an approved manner and transferred to the treating facility (12).

Not only does the biological agent in patient samples pose a risk, but certain test reagents might also be hazardous and require specific waste management practices for safe disposal. For example, guanidinium thiocyanate (reagent used in RNA extraction kits, including some POC/near-POC) produces toxic gas in the presence of bleach (sodium hypochlorite), so mixing of these two chemicals must be avoided. Sodium azide, an ingredient of some immunoassays and Ag-RDTs, should not be poured down the drain or autoclaved. It is toxic to aquatic life and chelates to some metals in drainpipes and autoclaves to produce explosive metal azides (13).

7. Additional risks associated with virus isolation studies

Certain experimental procedures may carry risks of virus mutations with possible increased pathogenicity and/or transmissibility, altered antigenicity or drug susceptibility. Specific risk assessments should be conducted, and specific risk-reduction measures adopted before any of the following procedures are conducted:

- Coinfection of cell cultures with different coronaviruses or any procedures that may result in a coinfection and, in turn, recombination;
- Culture of viruses in the presence of antiviral drugs;
- Deliberate genetic modification of viruses.

8. Work with animals infected with SARS-CoV-2

A local risk assessment is also key when handling experimental animals since this activity poses additional risks of injury (e.g., bites, scratches). The first step in a risk assessment is to evaluate the animal characteristics that could pose a risk in a given environment (animal facility, specific experimental set-up). In addition to the animals, it is necessary to assess the procedures used, the characteristics of the SARS-CoV-2 strain used, and what effects an infection of experimental animals could have on humans and the environment in the context of a specific activity. Laboratory personnel should have specific training in animal handling, performing experimental procedures in the animal facility, and adequate knowledge of potential hazards associated with such procedures. Infectious materials should always be handled in BSCs. Animal experiments should only be conducted following a careful risk assessment that will inform the containment measures and biological controls necessary to mitigate the risks associated with experimental animal activities

in the facility. The risk assessment template in the Annex of the *LBM4 Risk assessment monograph (14)* can serve as a template.

Some examples of animal experiments with SARS-CoV-2 requiring a risk assessment include:

- Inoculation of animals for potential recovery of SARS-CoV-2;
- Any protocol involving animal inoculation for confirmation and/or characterization of SARS-CoV-2;
- Infection of animals for preclinical vaccine/anti-viral drug testing
- Host-pathogen interaction studies

9. Referral of specimens to laboratories with appropriate risk control measures in place

Laboratories that are not able to meet the above biosafety recommendations should consider transferring specimens to national, regional, or international referral laboratories with SARS-CoV-2 detection capacity that can meet the biosafety requirements.

Packaging and shipment

All materials transported within and between laboratories should be placed in secondary packaging to minimize the potential for breakage or a spill. Specimens leaving the BSC should be surface decontaminated. Detailed guidance is provided in the WHO [Biosafety video series](#) (8), in particular, *Good Microbiological Practices and Procedures (GMPP)* 7: *transport*.

Transport of specimens within national borders should comply with national regulations. Cross-boundary transport of specimens of the virus responsible for COVID-19 should follow the United Nations model regulations, [Technical instructions for the safe transport of dangerous goods by air \(Doc 9284\)](#) of the International Civil Aviation Organization (15), for airlifted transport, and any other applicable regulations depending on the mode of transport being used. More information may be found in the WHO [Guidance on regulations for the transport of infectious substances 2021-2022](#) (applicable as of 1 January 2021) (16). A summary of the transport of infectious substances can also be found in Tool box 4 of the WHO handbook, [Managing epidemics: key facts about deadly diseases](#) (17).

Patient specimens from suspected or confirmed cases should be transported as UN3373, "Biological Substance Category B", when they are transported for diagnostic or investigational purposes. Also, viral cultures, isolates or propagated materials may be shipped in principle as Category B. However, a sound and responsible judgement may be needed in accordance with the definition of Category A, UN2814, "infectious substance, affecting humans" (7), is defined as "*capable of causing permanent disability, life-threatening or fatal disease in otherwise healthy humans or animals*" with consideration of different factors including the circulation of the virus variant in the population of the areas of the origin, type of transit used and destination of the materials, and the status of the characterization of the variant. All specimens being transported (whether UN3373 or UN2814) should have appropriate packaging, labelling, and documentation, as described in the earlier documents.

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