Laboratory biosafety guidance related to the novel coronavirus (2019-nCoV)

Interim guidance
12 February 2020

World Health Organization

1. Introduction

The purpose of this document is to provide interim guidance on laboratory biosafety related to the testing of clinical specimens of patient that meet the case definition of the novel pathogen identified in Wuhan, China, i.e. 2019 novel coronavirus (2019-nCoV), the disease named COVID-19.

As our understanding of the disease caused by 2019-nCoV is limited but rapidly growing, WHO continues to monitor developments and will revise these recommendations as necessary.

Highlights of 2019-nCoV laboratory biosafety

- All procedures must be performed based on risk assessment and only by personnel with demonstrated capability in strict observance to any relevant protocols at all times.
- Initial processing (before inactivation) of all specimens should take place in a validated biological safety cabinet (BSC) or primary containment device.
- Non-propagative diagnostic laboratory work (e.g. sequencing, NAAT) should be conducted at facilities and procedures equivalent to BSL-2 and propagative work (e.g. virus culture, isolation or neutralization assays) at a containment laboratory with inward directional airflow (BSL-3).
- Appropriate disinfectants with proven activity against enveloped viruses should be used (e.g. hypochlorite (bleach), alcohol, hydrogen peroxide, quaternary ammonium compounds and phenolic compounds).
- Patient specimens from suspected or confirmed cases should be transported as UN3373, "Biological. Substance, Category B". Viral cultures or isolates should be transported as Category A, UN2814, "infectious substance, affecting humans".

2. Laboratory biosafety

Ensure that health laboratories adhere to appropriate biosafety practices. Any testing for the presence of 2019- nCoV or clinical specimens from patient meeting the suspect case definition should be performed in appropriately equipped laboratories by staff trained in the relevant technical and safety procedures. National guidelines on the laboratory biosafety should be followed in all circumstances. General information on laboratory biosafety guidelines, see the WHO Laboratory Biosafety Manual, 3rd edition in the interim before its 4th edition is released. http://www.who.int/csr/resources/publications/biosafety/WHO_CDS_CSR_LYO_2004_11/en/

Key points:

- 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.
- When handling and processing specimens, including blood for serological testing, laboratory practices and procedures that are basic to good microbiological practices and procedures (GMPP) should be followed.
- The handling and processing of specimens from cases with suspected or confirmed 2019-nCoV infection intended for additional laboratory tests such as haematology or blood gas analysis should follow local guidelines for processing potentially infectious material.
- Non-propagative diagnostic laboratory work including, sequencing, nucleic acid amplification test (NAAT) on clinical specimens from patients who are suspected or confirmed to be infected with nCoV, should be conducted adopting practices and procedures of "core requirements[1]" as detailed in **Annex 1** below and an appropriate selection of "heightened control measures[2]" as informed by the local risk assessment. In the interim, Biosafety Level 2 (BSL-2) in the WHO Laboratory Biosafety Manual, 3rd edition remains appropriate until the 4th edition replaces it.
- Handling of material with high concentrations of live virus (such as when performing virus propagation, virus isolation or neutralization assays) or large volumes of infectious materials should be performed **only by properly trained and competent personnel in laboratories capable of meeting additional essential containment requirements and practices**, i.e. BSL-3.

[1] **Core requirements:** A set of minimum requirements defined in the fourth edition of 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 risk that cannot be brought below the risk tolerance level with the core requirements only.

- Initial processing (before inactivation) of all specimens including those for sequencing and NAAT should take place in an appropriately maintained and validated biological safety cabinet (BSC) or primary containment device.
- Appropriate disinfectants with proven activity against enveloped viruses used for the recommended contact time, dilution 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.
- Appropriate personal protective equipment (PPE) as determined by a detailed risk assessment, should be worn by all laboratory personnel handling these specimens.
- Patient specimens from suspected or confirmed cases should be transported as UN3373, "Biological. Substance, Category B". Viral cultures or isolates should be transported as Category A, UN2814, "infectious substance, affecting humans".

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

The additional recommendations provided below address minimal/essential working conditions associated with specific manipulations in laboratory settings:

a. Risk assessment

Risk assessment is a systematic process of gathering information and evaluating the likelihood and consequences of exposure to or release of workplace hazard(s) and determining the appropriate risk control measures to reduce the risk to an acceptable level. It is important to note that hazards alone do not pose a risk to humans or animals. Consideration therefore must also be given to the types of equipment used and the procedure(s) that will be performed with the biological agent.

It is highly recommended to start with performing a local risk assessment by each process step, i.e. starting from sample collection, sample reception, clinical testing, PCR and virus isolation (only when and where applicable). Certain hazards will then be considered for each process step such as aerosol exposure during sample processing, eye splash during sample processing; infectious culture material spill; and leaking sample (in case of sample reception) with assessed grade of risk. For each identified risk, appropriate risk control measures including but not limited to the following recommendations should be selected and implemented in order to mitigate the residual risks to an acceptable level.

A risk assessment template is attached as **Annex 2**, intended to serve as an example and to facilitate the process.

b. 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 who are suspected or confirmed to be infected with novel coronavirus, should be conducted adopting practices and procedures described for conventional clinical and microbiology laboratories as described below as "core requirements".

All manipulations of potentially infectious materials, including those that may cause splashes, droplets, or aerosols of infectious materials (e.g. loading and unloading of sealed centrifuge cups, grinding, blending, vigorous shaking or mixing, sonic disruption, opening of containers of infectious materials whose internal pressure may be different from the ambient pressure), however, should be performed in appropriately maintained and validated BSCs or primary containment device 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" (CR), including good microbiological practice and procedure (GMPP), should be followed at all times, including but not limited to the following. More details are explained and demonstrated in the WHO biosafety video series available from the following link:

https://www.who.int/ihr/publications/biosafety-video-series/en/

c. Appropriate disinfectants

• While little is known about this novel virus, in the light of the comparable genetic characteristics with SARS-CoV and MERS-CoV suggest that 2019-nCoV may likely susceptible to disinfectants with proven activity against enveloped viruses, including sodium hypochlorite (bleach) (e.g. 1,000 ppm (0.1%) for general surface disinfection and 10,000 ppm (1%) for disinfection of blood spills), 62-71% ethanol, 0.5% hydrogen peroxide, quaternary ammonium compounds and phenolic compounds, if used according to manufacturer's recommendations. Other biocidal agents such as 0.05-0.2% benzalkonium chloride or 0.02% chlorhexidine digluconate can be less effective.

• Particular attention should be paid not only to the selection of the disinfectant but also contact time (e.g. 10 minutes), dilution (i.e. concentration of the active ingredient) and expiry date after the working solution is prepared.

• Human coronaviruses in general are known to persist on inanimate surfaces such as metal, glass or plastic for up to 9 days[3].

d. Viral isolation

Unless a country decides otherwise, considering the newly acquired knowledge and effective preventive measures described above, viral isolation on clinical specimens from patients who are suspected or confirmed to be infected with novel coronavirus should be performed only in laboratories capable of meeting the following additional containment requirements:

• A controlled ventilation system maintains inward directional airflow into the laboratory room.

• Exhaust air from the laboratory room is not recirculated to other areas within the building. Air must be HEPA filtered, if reconditioned and recirculated within the laboratory. When exhaust air from the laboratory is discharged to the outdoors, it must be dispersed away from occupied buildings and air intakes. This air should be discharged through HEPA filters.

• All manipulations of infectious or potentially infectious materials must be performed in appropriately maintained and validated BSCs.

• Laboratory workers should wear protective equipment, including 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, eye protection (goggles or face shield). Risk assessment should inform the use of respiratory protection (fit-tested particulate respirator, e.g. EU FFP2, US 6 NIOSH-certified N95 or equivalent, or higher protection).

• A dedicated hand-wash sink should be available in the laboratory.

• Centrifugation of specimens should be performed using sealed centrifuge rotors or sample cups. These rotors or cups should be loaded and unloaded in a BSC.

e. Additional risks associated with virus isolation studies

Certain experimental procedures may carry additional risks of virus mutations with possible increased pathogenicity and/or transmissibility, or viruses with 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:

• Co-infection of cell cultures with different coronaviruses, or any procedures that may result in a co-infection;

• Culture of viruses in the presence of antiviral drugs;

• Deliberate genetic modification of viruses.

f. Work with animals infected with novel coronavirus

The following activities require animal facility — Biosafety Level 3 facilities and work practices, as detailed in the WHO Laboratory biosafety manual, 3rd edition.

• Inoculation of animals for potential recovery of the agent from novel coronavirus specimens

• Any protocol involving animal inoculation for confirmation and/or characterization of putative novel coronavirus agents

g. Referral of specimens to laboratories with appropriate containment measures in place

Laboratories not able to meet the above biosafety recommendations should consider transferring specimens to national, regional or international referral laboratories with 2019-nCoV detection capacity that can meet the biosafety requirements.

4. Packaging and shipment

All materials transported within and between laboratories should be placed in a secondary container to minimize the potential for breakage or a spill. An example includes transfer of materials from the biological safety cabinet to an incubator and vice versa. Specimens leaving the BSC should be surface decontaminated. Detailed guidance is provided in the WHO biosafety video series, in particular "Good Microbiological Practices and Procedures (GMPP) 7: transport": https://www.who.int/ihr/publications/biosafety-video- series/en/

Transport of specimens within national borders should comply with applicable national regulations. For cross-boundary transport of novel coronavirus specimens should follow the UN Model Regulations, Technical Instructions for the Safe Transport of Dangerous Goods by Air (Doc 9284) of the International Civil Aviation Organization (ICAO) 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 2019-2020[4] (Applicable as from 1 January 2019). A summary on

[3] Journal of Hospital Infection, https://doi.org/10.1016/j.jhin.2020.01.022

[4] https://www.who.int/ihr/publications/WHO-WHE-CPI-2019.20/en/

transport of infectious substances can also be found in Toolbox 4 of the Managing epidemics handbook: https://apps.who.int/iris/handle/10665/272442.

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. Viral cultures or isolates should be transported as Category A, UN2814, "infectious substance, affecting humans". All specimens being transported (whether UN3373 or UN2814) should have appropriate packaging, labelling and documentation as described in the documents mentioned above.

Interim List of Household Products and Active Ingredients for Disinfection of the COVID-19 Virus

Revised on 03 April 2020
First released on 04 February 2020

For general precautionary cleaning, detergent and water are adequate. For disinfection of areas that are very likely to be contaminated with COVID-19 virus (e.g. bedroom of a person confirmed to have a COVID-19 virus infection), general household products that contain the appropriate concentrations of active ingredients (A.I.s) can be used.

The suitable active ingredients and their effective concentrations are listed in Table 1. Table 1 provides guidance on the effective contact time (which is different among the various A.I.s) required by the A.I.s to act on a surface in order to be effective against coronaviruses. In addition to the use of cleaning agents, other treatments effective against coronavirus include steam and heat treatment. As the COVID-19 virus is new, no study has been published on the virus. This assessment is thus based on published scientific studies on coronaviruses, a group to which the COVID-19 virus belongs.

Table 2 lists common household products that can be contain the appropriate A.I.s for disinfection[1]. Both tables will be updated as new data emerge and data from more products are gathered.

Important points to note when using disinfectants:

1. **Check the labels and use according to instructions, and be aware of the potential hazard of each product.**
2. Avoid contact with eye and skin when handling cleaning products, and keep them away from children.
3. Do not mix different cleaning products and use in a well-ventilated area.
4. For disinfection of highly contaminated surfaces or material, avoid the use of spray, and allow appropriate time needed for disinfection.
5. This product list should be read in conjunction with the Guidelines and Advisories issued by NEA with instruction and guidelines on how to conduct proper cleaning and disinfection of premises.

[1] *The product either contains the appropriate active ingredients listed in Table 1 or is accompanied with data that shows efficacy against coronaviruses.*

Table 1. Active Ingredients and Their Working Concentrations Effective Against Coronaviruses

S/N	Active Ingredient (A.I.)	Contact Time (min)
1	Accelerated hydrogen peroxide (0.5%)[a]	1
2	Benzalkonium chloride* (0.05%)[b]	10
3	Chloroxylenol (0.12%)[c]	10
4	Ethyl alcohol (70%)[d]	10
5	Iodine in iodophor (50 ppm)[b]	10
6	Isopropanol (50%)[b]	10
7	Povidone-iodine (1% iodine)[d]	1
8	Sodium hypochlorite (0.05 – 0.5%)[d, e]	5
9	Sodium chlorite (0.23%)[b]	10

*Alternative name: alkyl dimethyl benzyl ammonium chloride

[a] *Omisbahakhsh, N., & Sattar, S. A. (2006). Broad-spectrum microbicidal activity, toxicologic assessment, and materials compatibility of a new generation of accelerated hydrogen peroxide-based environmental surface disinfectant. American Journal of Infection Control, 34(5), 251-2571*

[b] *Saknimit M, Inatsuki I, Sugiyama Y, Yagami K. (1988) Virucidal efficacy of physico-chemical treatments against coronaviruses and parvoviruses of laboratory animals. Jikken Dobutsu. 37:341-5; Tested against canine coronavirus*

[c] *Dellanno, C., Vega, Q., & Boesenberg, D. (2009). The Antiviral action of common household disinfectants and antiseptics against murine hepatitis virus, a potential surrogate for SARS coronavirus. American Journal of Infection Control, 37(8), 649-652. doi:10.1016/j.ajic.2009.03.012*

[d] *Sattar SA, Springthorpe VS, Karim Y, Loro P. (1989). Chemical disinfection of non-porous inanimate surfaces experimentally contaminated with four human pathogenic viruses. Epidemiol. Infect. 102:493-505; Tested against coronavirus 229E.*

[e] *Lai, M. Y. Y., Cheng, P. K. C., & Lim, W. W. L. (2005). Survival of Severe Acute Respiratory Syndrome Coronavirus. Clinical Infectious Diseases, 41(7), e67-e71.*