1. Objective

This SOP is designed in the context of the SARS-CoV-2 pandemic to provide assurance that personnel collecting or handling clinical human biological samples as whole blood, serum, biopsies or other type of samples, use the proper precautions to minimize the risk associated with these activities. Adherence by staff to both these policies and general guidelines for safe practices, in each laboratory, will minimize the risks, though no guarantee can be given that these guidelines will ensure absolute protection.

1.1 Synonyms and Abbreviations

BSC – Biological Safety Cabinet
CEDOC – Chronic Diseases Research Centre
EBV – Epstein-Barr Virus
ELISA – Enzyme-linked immunosorbent assay
HBV – Hepatitis B Virus
HCV – Hepatitis C Virus
HIV – Human Immunodeficiency Virus
HPV – Human papilloma Virus
ILO – International Labour Organization
NMS – NOVA Medical School
PEP – Post-exposure prophylaxis
SARS-CoV-2 – Severe acute respiratory syndrome coronavirus-2 (COVID 19)
SV-40 – Simian virus 40
WHO – World Health Organization

2. Scope

These Biosafety recommendations apply to any activity involving the handling of human biological samples and should be applied to all personnel working in NMS / CEDOC Laboratories.

3. Framework

All human biological specimens should be considered as hazardous. This requires the use of specific precautions with all clinical specimens (whole blood, serum, body fluids, biopsies, or other potentially infectious material) - Universal or Standard Precautions.

Standard precautions (which include “universal precautions”) are designed to reduce the risk of transmission of microorganisms from both recognized and unrecognized sources of infection.

4. Flowchart

n.a.

5. Development

5.1 General Guidelines

All work with human samples needs to be risk assessed before it begins. If necessary, contact the Biosafety Committee for advice on this matter.

The work with biological samples should be conducted at **Containment level 2 laboratories (BSL2)** following **Standard Laboratory precautions.** Thus, all activities with biological samples must be processed in a **Class II BSC until they have been properly inactivated** (by any type of process - physical or chemical).

Such activities include:

- Preparation of specimens for molecular testing (for example, respiratory virus PCR);
- Opening of tubes and other activities that may cause splashes, droplets or aerosols;
- Any other manipulation of biological samples with risk of aerosol formation, including the disintegration of biopsy tissues;
- Aliquoting or diluting samples;
- All operations of the Flow Cytometry protocols before the samples are lysed if lysis with fixative is used (ex: BD FACS Lysing) or until the samples are fixed (ex: BD Cell Fix);
- All ELISA serum manipulations before sample plates are washed;

1. It is mandatory to wear personal protective equipment including at least lab coat, disposable gloves and surgical mask.

2. All materials to be placed inside the BSC must be decontaminated before being introduced and before being removed from the BSC with one of the usual disinfectants (eg. 70% ethanol), including the operator's gloves.

3. All centrifugations must be carried out in a centrifuge with suitable containment systems (that is, a hermetically sealed rotor or cups with lids), to limit the risk of contamination if there is a break during centrifugation.

4. The tubes must be centrifuged closed and should be opened inside the BSC. Whenever possible, it is recommended that the centrifuge cup itself or the rotor, if they can be removed, should be opened only inside the cabinet.

5. After processing the samples, and before removing the materials from the BSC, all surfaces, including work surfaces and equipment, must be decontaminated with one of the usual disinfectants (0,5 % bleach or 70% ethanol).

6. For cell culture applications, laboratory workers should **NEVER** work on their own cells.

Staff should be aware that if their own cells are transformed in culture, their immune response may not provide protection in the event of re-exposure through a needle stick or other kind of accident. Autologous cells or tissues should always be considered potentially hazardous as a result of self-inoculation.

5.2 Standard Laboratory Precautions

1. Its mandatory to wear the adequate personnel protective equipment, according to the dispositions of each laboratory (Biosafety level).

2. Hair should be tied up, nails cut short and bulky ornaments (jewelry) should be avoided.

3. Any open wounds, cuts or other kind of lesions should be covered with a waterproof dressing.

4. All necessary precautions should be taken to prevent puncture wounds and cuts.
   For example: snub-nosed scissors should be used instead of pointed scissors;
   chain-mail gloves should be used for high risk cutting procedures;
   safe disposal containers should be used to dispose of needles and any sharp materials.

5. Activities such as eating, drinking, smoking and/or applying cosmetics are prohibited in the laboratory.

6. Hand-to-mouth or eyes contact should be avoided. Materials such as pens, pencils or gum must not be put in the mouth while inside the laboratory.

7. Lab coats and gowns should be removed when leaving the laboratory (to go to offices, tearooms, cafeterias, toilets or seminar rooms).

8. Use of the telephone during work should be avoided. If this is not possible, laboratory telephones must be used in hands-free mode, or they must be disinfected after each use with alcoholic solution.

9. In BSL-2 laboratories, the use of a mobile phone is not allowed.

10. Hands should be washed frequently, preferably with warm running water and soap, or sanitized with a proper alcoholic solution:
    - before putting on the gloves,
    - after removing them,
    - after handling potentially infectious materials,
    - before leaving work areas,
    - any time hand contamination is known, or suspected.

11. All samples must be labelled, and stored in the adequate conditions, respecting the necessary containment (see below).

12. All waste should be carefully disposed of, according to their respective waste group. Particular attention should be given to sharps, which should be placed directly into the designate sharp bins (usually rigid, yellow, group IV containers) for ultimate disposal by incineration.

5.3 Collection, labelling and transport of specimens

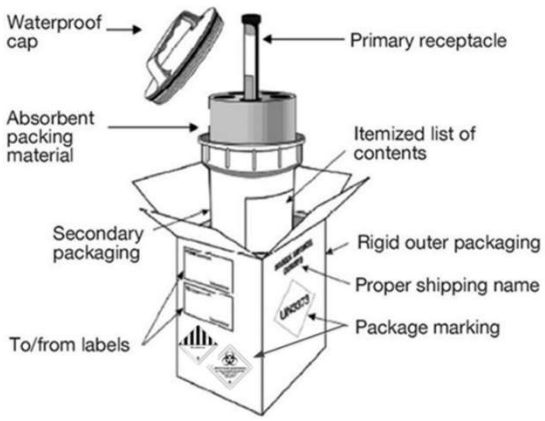
1. Always follow standard precautions and wear gloves for all procedures.

2. Blood samples should be collected from patients only by trained and authorized staff.

3. For phlebotomies, conventional needle and syringe systems should be replaced by single-use safety vacuum devices that allow the collection of blood directly into stoppered transport and/or culture tubes, automatically disabling the needle after use.

4. All materials transported within and between laboratories should be placed in a secondary container to minimize the potential for breakage or a spill and should be identified with the Biological Risk Sign.

5. External transport of biological samples should follow the rules for packaging of infectious substances recommended by the WHO-category B (UN 3373). A triple packaging system should be used with the following characteristics:
    a. Primary container
    b. Secondary container
    c. Outer container



- **Primary container** is the one that contains the sample; must be properly identified; must be liquid and solid-tight and packed in enough absorbent material to absorb the entire contents in case of breakage or spill;

- **Secondary container** is the one that holds the primary containers; should be resistant, waterproof and leakproof to liquids and solids; may contain several primary containers but, in this case, additional absorbent material shall be used to absorb all fluids in case of breakage or leakage.

- **Outer container:** Secondary containers are placed in outer shipping packaging's with suitable cushioning material. Outer containers protect their contents from outside influences, such as physical damage, while in transit.
- Transport procedures should meet the adequate requirements for time and temperature.

5.4 Personal protective equipment (PPE)

1. Always wear protective clothing, including at least lab coat and disposable gloves.

2. For higher risk operations, the PPE must be reinforced (eg. COVID-19 samples) with:
   - normal gown and disposable gown,
   - two pairs of gloves,
   - surgical mask,
   - safety glasses or visor, whenever necessary (risk of splashing / aerosols),
   - FFP2 respirators should also be available for high risk situations.

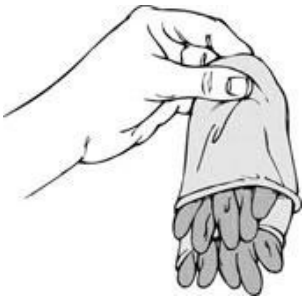
3. Gloves should always be inspected before use to check they are intact.

4. The use of gloves must be structured: they must be periodically cleaned with 70% ethanol and must be discarded at the end of the procedure.

5. For manipulations that require two pairs of gloves, the second (outer) pair must be frequently cleaned and removed before removing the hands from the BSC.

6. Do not leave the laboratory with a disposable gown or gloves in both hands:
    - if it is necessary to use gloves to transport materials / samples between laboratories, only one glove should be used, the hand without a glove being available for opening doors, or handling common surfaces (switches, buttons…).

7. Remove gloves in a way that the skin does not contact with the external surface of the glove.



8. Regularly, the correct procedures should be demonstrated by senior laboratory personnel.

5.5 Laboratory Equipment

1. Plastics should replace glass whenever possible.

2. If glass is necessary, laboratory grade (borosilicate) glass should be used.

3. Discard any article that is chipped or cracked.

4. The use of hypodermic needles and syringes should be limited. They must not be used as substitutes for pipetting devices or for any purpose other than parenteral injection or aspiration of fluids from laboratory animals.

5. Never re-cap, clip or remove needles from disposable syringes.

6. Use sealed tubes for centrifuging to avoid dispersion of droplets and aerosols.

7. Centrifuges used for spinning body fluids should have a sealed rotor and/or sealed buckets with transparent lids which allow staff to see if a breakage has occurred.

8. Equipment should be disinfected at the end of each session, following manufacturer's instructions.

9. Adequate biohazard containers should be available at proper locations for disposal of contaminated materials.

5.6 Decontamination of surfaces and equipment

A diluted bleach solution (1/10) should be prepared daily to be available in the laboratory.

Ethanol should also be available in all laboratories in a 70% solution.

Work Surfaces:

During work, surfaces must be frequently disinfected with 70% ethanol to control infectious risks.

At the end of the day, and whenever there is evidence of contamination, work surfaces should be cleaned with the bleach solution diluted 1/10 and then passed through 70% ethanol.

BSC surfaces should also be cleaned with 70% ethanol and then, at the end of the day or after the work sequence, there must be a 20-minute UV sterilization cycle.

Glassware:

All glassware contaminated with fluids should be discarded into a strong solution of sodium hypochlorite (≈0.5% available chlorine): common use bleach: dilute 1 / 10.

Centrifuges:

Whenever there is evidence of centrifuge contamination, it should be cleaned with 70% ethanol.

In the event of a breakage, place the potentially contaminated material in a disinfectant solution for 24 hours and disinfect the interior of the centrifuge with a non-corrosive agent (*eg*. 70% ethanol). Disinfect the undamaged tubes as well, so that the sample can then be recovered.

Monthly, the interior of the centrifuges and their components must be cleaned and disinfected with 70% ethanol.

Thermal baths:

Whenever there is evidence of water contamination, the bath should be washed, and the water replaced.

Monthly, the thermal baths in use must be maintained:

- Make a thermal shock at 70ºC, for 30 minutes,
- Remove water and wash the bath after thermal shock,
- Refill the bath with ultrapure water,

Record the operation on the Maintenance Map.

Micropipettes:

After each work cycle and whenever there is evidence of contamination, the micropipettes should be disinfected with ethanol at 70%.

5.7 Waste Management

All samples and potentially contaminated materials (tubes, Petri dishes, pipette tips, gloves) must be treated as **group III waste**:

- samples and materials handled in BSC - collected in a double white bag at BSC.
- contaminated liquids - collected in a biological waste container inside the BSC.

Sharp materials and materials / solutions with chemical risk must be treated as **group IV waste**:

- sharps waste (needles, scalpels, broken contaminated glass) - waterproof, watertight, rigid, cut and puncture proof containers
- chemical hazardous waste - collected in a red bag

All bags / waste containers that are inside the BSC must be rinsed with a disinfectant solution before being removed from the BSC, and then placed in the respective container (70L) in the waste room.

5.8 Spills

1. When handling samples and spilled material, the use of PPE is always required.
2. If a body fluid is spilled, it should be cleaned up immediately with 70% ethanol followed by a strong solution of sodium hypochlorite (≈0.5% available chlorine): common use bleach: dilute 1 / 10.
3. Laboratory coats contaminated with body fluid must be placed in a chlorine bath for 30 minutes.
4. If dustpans are used to clear away the broken material, they should be autoclaved or placed in an effective disinfectant. Clothes, paper towels and swabs used for cleaning up should be placed in a contaminated-waste container (Group III container).
5. Sharp or glass fragments should be handled with forceps.

5.9 Emergency Procedures and Protection Against Exposure

Exposure: An exposure that might place health-care or research personnel at risk of **HBV, HCV, or HIV** infection is defined as a percutaneous injury (e.g. a needle stick or cut with a sharp object) or the contact of mucous membrane or non-intact skin (e.g. exposed skin that is chapped, abraded or afflicted with dermatitis) with blood, tissue or other body fluids that are potentially infectious. (ILO/WHO).

Exposure prevention is the primary strategy for reducing occupationally acquired infections. However, there will always remain a risk of occupational exposure from blood-borne pathogens.

- SARS-CoV-2 Transmission:

As for **SARS-CoV-2** transmission, it can occur through direct, indirect, or close contact with infected people through infected secretions such as saliva and respiratory secretions or their respiratory droplets, which are expelled when an infected person coughs, sneezes, talks or sings. Respiratory droplet transmission can occur when a person is in close contact (within 1 meter) with an infected person who has respiratory symptoms (e.g. coughing or sneezing) or who is talking or singing; in these circumstances, respiratory droplets that include virus can reach the mouth, nose or eyes of a susceptible person and can result in infection. Indirect contact transmission involving contact of a susceptible host with a contaminated object or surface (fomite transmission) may also be possible.

Airborne transmission is defined as the spread of an infectious agent caused by the dissemination of droplet nuclei (aerosols) that remain infectious when suspended in air over long distances and time. Airborne transmission of SARS-CoV-2 can occur during medical procedures that generate aerosols ("aerosol generating procedures"). WHO, together with the scientific community, has been actively discussing and evaluating whether SARS-CoV-2 may also spread through aerosols in the absence of aerosol generating procedures, particularly in indoor settings with poor ventilation (source WHO, 2021. https://www.who.int/news-room/commentaries/detail/transmission-of-sars-cov-2-implications-for-infection-prevention-precautions).

Again, exposure prevention is the primary strategy for reducing occupationally acquired infections, for which all staff should respect the NMS and DGS guidelines to avoid SARS-CoV-2 transmission (social distancing, using face masks, proper hand washing). Despite the implementation of these measures, there will always remain a risk of occupational exposure, which may be reduced by proper adherence to the abovementioned recommendations.

5.9.1 Immediate actions

Immediate care to the injured individual should be based on the most current WHO guidelines on post-exposure prophylaxis, which include referral of the designated individual for risk assessment of infection transmission and provision of post-exposure prophylaxis or another needed medical follow-up. This evaluation should be performed immediately in the nearest Hospital or Emergency Department.

General procedures for exposures to a potentially infectious material:

1. Stop work and immediately wash or flush the exposed area with soap and water for 10 minutes.

2. If exposure is to the eyes, flush eyes (holding open) using the eyewash station for 10 minutes.

- ***Puncture wounds, cuts and abrasions***

  The affected individual should remove protective clothing, wash the hands and any affected area(s) with soap and water, apply an appropriate skin disinfectant and seek medical attention as necessary.
  The cause of the wound and the organisms involved should be reported, and appropriate and complete medical records kept.

- ***Ingestion of potentially infectious material***

  Protective clothing should be removed, and medical attention sought. Identification of the material ingested, and circumstances of the incident should be reported, and appropriate and complete medical records kept.

5.9.2 Follow-up actions

Designated individuals should ensure that full reports on the injury and immediate treatment provided are completed in a timely manner. This includes referral of the exposed individual for counselling and testing and another follow-up. An investigation of the exposure incident, including identification of potential actions to prevent similar exposures in the future, should be completed in a timely manner.

5.9.3 Analysis and record keeping

All exposure incidents and respective analysis should be recorded, maintained in the Accident/Incident report form and sent to the Biosafety Committee (biosafety@nms.unl.pt) .

5.10 Occupational Health:

1. All laboratory staff working with human cells and tissues should be enrolled in an occupational medicine program specific for blood-borne pathogens. Laboratory staff working with human cells and tissues should provide a baseline serum sample before starting the work, be offered hepatitis B immunization, and be evaluated by a health care professional following an exposure incident. Similar programs should be considered for work with non-human primates' blood, body fluids, and other tissues.

2. Staff with chronic diseases or immunosuppressed may need some restrictions in their normal laboratory work, and medical advice may be necessary in some cases.
3. Pregnant women, women in puerperium or breastfeeding may also have restrictions in their normal laboratory work. Medical advice may be necessary in these cases.

References:

https://www.who.int/publications/i/item/laboratory-biosafety-guidance-related-to-coronavirus-disease-2019-(covid-19)

https://www.who.int/csr/resources/publications/biosafety/Biosafety7.pdf?ua=1

https://www.cdc.gov/coronavirus/2019-nCoV/lab/lab-biosafety-guidelines.html

https://www.who.int/news-room/commentaries/detail/transmission-of-sars-cov-2-implications-for-infection-prevention-precautions