

Objectives

- Providing **guidelines** and required biological **Containment Level (CL)** for **handling** of SARS-CoV-2 samples handling.
- Offering **instructions** to **reducing biological risk** to acceptable level and guarantee current in force **law compliance**.

Warnings

- Provided information is based in current knowledge about this and other coronavirus (SARS-CoV and MERS-CoV) and guidelines and recommendations from WHO and other related documents.
- Knowledge about COVID-19 is rapidly evolving, so the current guideline can be reviewed given the new data.
- In case of any discrepancy, regulations and/or normative from each country will prevail over the information showed below.
- Each institution must develop a risk assessment and specific working procedures for its activity. This document cannot be considered as direct application protocol.

Basic control measures

Procedures and basic primary barriers.

- **Biosafety recommendations** from the European "Directive 54/2000/EC" and WHO should be applied.
- Techniques, practices, equipment and Personal Protective Equipment (PPE) must be adapted to the technique performed.
- Hand-washing before entering the lab.
- **Recommended PPE** use in case of aerosol and splashing risk:
 - **Splash-resistant lab coat** (closed at the back + elastics cuffs) or **coverall**, type 4B as lowest protection level.
 - **Gloves for biological hazards**
 - **Face mask** type FFP2 o higher level.
 - **Anti-Splash safety goggles or face shield.**
 - **Disposable head covering/cap.**
 - **Shoe cover.**
- **Non-inactivated biological material** must be used in certified biological safety cabinet (BSC) class II with qualification on effect.
- **Aerosol - barrier micropipettes** (or tips).
- **Screw - capped tubes and bottles.**
- **Material** must be **disinfected** before remove it from BSC.
- All **BSC inner surfaces** must be **disinfected** after work.
- Adequate **programs for cleaning and disinfection and hazardous wastes** management.

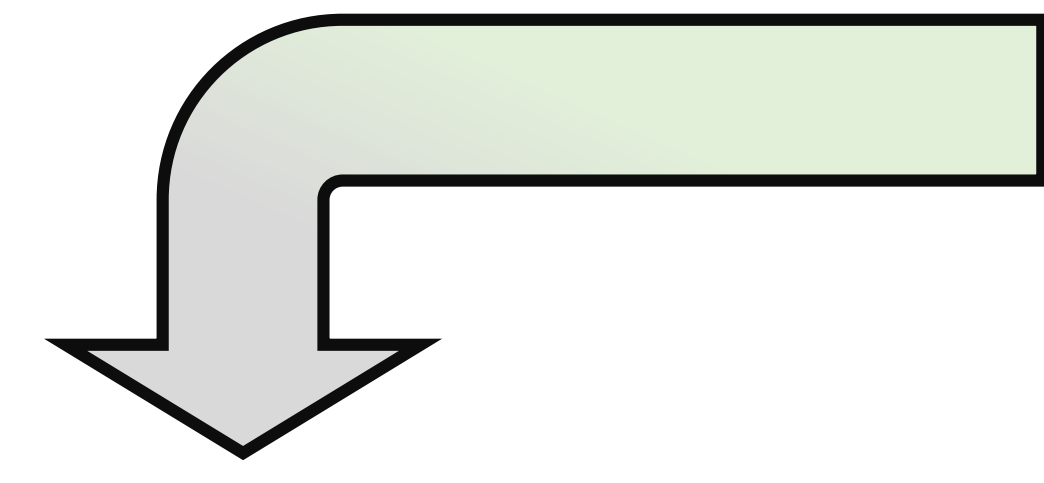
Administrative and organizational.

- **Previous, proactive and ongoing process to identify and assess Biological risk.**
- **Biological Safety Officer** or adviser (**BSO**) should be available.
- **Education and training** on the use of PPE, equipment and emergency and contingency procedures.
- Specific **health and medical surveillance.** It is advised to include diagnostic tests for COVID-19 (initial, periodical and in case of exposure suspicion) and serological monitoring.
- Any **accident or incident** must be **communicated** to the BSO and/or to Occupational Health personnel.

Biosecurity

- **Records** allowing **traceability** for:
 - Entry and transfers to other entities material.
 - Inventory and location.
 - Material usage.
 - Biohazardous destruction and disposal.
- **Safe computer records** on safe location.
- **Access control.**
- Regular inventory **verification.**
- Immediate **notification** on any **deviation** to biosecurity officer.
- Biohazard **signposting.**

Inactivated or fixed by validated methods or with scientific evidence



Out of CL2

Laboratory techniques.

- Manual or automatic **DNA/RNA extraction.**
- **qRT-PCR.**
- **Inactivated biological samples aliquoting.**
- **Microscope staining** and analysis.
- **Staining and examination.**
- **Electron microscopy.**
- **Cytometry.**

Control measures.

- Apply Good Microbiology Practices and Procedures (GMPP).

Transport

External

- **Diagnostic or clinical samples: Category B** (UN3373).
- **Virus cultures: Category A** (UN2814).
- **No private vehicles allowed**
- **Hermetic primary containers.**
- **BCL2:** primary container must be prepared in BSC and secondary container in the lab.
- **BCL3:** Both primary and secondary containers must be prepared in BSC.
- **Secondary container disinfection** before leaving the lab.

Internal.

- **Secondary container** adequate for **ultrafreezers and dry-ice.**
- **Secondary container** must be **hermetic, break-proof and leak-proof** for internal transport.

CL2

Laboratory techniques.

- Diagnostic techniques **not implying virus culture and amplification.**
 - **Preparation** of non-inactivated fresh **samples.**
 - Solid or viscous samples **homogenization** (sputum, bronchoalveolar lavage,...).
 - **Lysis buffer addition.**
 - **Non-inactivated biological samples aliquoting.**
 - **Non-inactivated samples centrifugation.**
 - Fixed samples **cell sorting or Fluorescence-activated cell sorting (FACS)** .
 - **Non-fixed samples cell sorting or FACS** into BSC specifically designed or equipped with Aerosol Management System (**AMS**), certified and qualification on effect.

Facilities.

- Decontaminable **surfaces** for benchtop, floor and walls.
- **Access control**, preferably electronic.
- **Autoclave**, available at the building.
- Recommended **independent ventilation** system **without air recirculation.**

Control measures.

- **Basic control measures.**
- **Biohazard signposting.**
- **Restricted access** only to trained and authorized personnel.
- **Training** on biosafety, equipment and PPE procedures (approved by BSO).
- Use of appropriate **disinfectants.**
- Use of **sealed centrifuge rotors or bucket cups.** Loading and unloading of rotors or buckets within a BSC.
- **Doors and windows closed** at any moment.

Disinfectants

Disinfectants	Concentration	Contact time	Application
Ethanol	70%	1 min	Surfaces
Sodium hypochlorite (bleach)	0,1% (1.000 ppm)	10 min	Surfaces
Sodium hypochlorite (bleach)	1% (10.000 ppm)	10 min	Spills or liquids with organic load
Quaternary ammonium	Manufacturer instructions	Manufacturer instructions	Surfaces and spills or liquids with organic load
Hydrogen peroxide – Peracetic acid	Manufacturer instructions	Manufacturer instructions	
Virkon™	1% (10gr, 1 l)	10 min	
Perasafe™	1,62% (16,2 gr, 1l)	10 min	

CL3

Laboratory techniques.

- **Virus culture, isolation, purification or characterization.**
- Viable cells **cell-sorting or FACS** with **non-inactivated virus or confirmed samples.**

Facilities.

- All from **CL2.**
- Decontaminable **surfaces** also for ceilings.
- **Anteroom** at the access to containment zone.
- Non-opening and sealed **windows.**
- **Independent ventilation** system **without air recirculation.**
- Continuous **Inward Directional Airflow.**
- On site differential **pressure visual control** with alarm.
- **HEPA (H14) filtration on exhaust.**
- Recommended HEPA (H13) filtration on inlet.
- **Material exchange systems** (SAS, airlock, dunk tunk, and **double door autoclave** located on containment barrier).
- **Changing room** for clothes and PPE donning and doffing and decontamination shower at the exit.
- General **effluent treatment** system must be provided.
- **Neutralization of biologicals** generated in the laboratory before removal or discharging on general sewage system (general or local), preferably before leaving BSC.

Control measures.

- All from **CL2**
- **Education and training** on CL3 conditions (approved by BSO).
- **Personal Protective equipment:**
 - Type 4B or higher coverall and boot-covers.
 - FFP3 face-mask, and/or ventilated hood.
 - Use of double glove certified "virus".
- **PPE decontamination** before leaving the working area.
- Standard Operating **Procedures for PPE doffing.**
- **Hand washing, expectoration, nasal mucous cleaning and nails brushing** before leaving BCL3 area.
- **Validated decontamination procedures** for surfaces and rooms.
- All the stocks of biological materials need to be recorded.

References

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3. Chin A et al. Stability of SARS-CoV-2 in different environmental conditions. Lancet Microbe. 2020. + Supplementary annex.
4. G. Kampf et al. Persistence of coronaviruses on inanimate surfaces and their inactivation with biocidal agents. Journal of Hospital Infection. 2020.
5. ISAC. New SARS-COV-2 sorting protocols released (web). 26/3/2020.
6. INSST. Guía Técnica Evaluación y Prevención Riesgos con agentes biológicos. 2014.
7. WHO. Laboratory biosafety guidance related to coronavirus disease (COVID-19). 19/3/2020.
8. Directive 2000/54/EC sobre la protección de los trabajadores contra el riesgo biológico en el trabajo.