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RULES FOR WORKING SAFELY IN THE BIOSECURITY LABORATORY LEVEL 3 (BL3)

A biosafety level 3 laboratory (BL3) is designed and equipped to ensure its suitability for working with group 3 microorganisms, which are those that pose high risks to laboratory personnel but low risks to the community. Within this laboratory, the use of microorganisms of a higher classification is prohibited, as stated in attachment XLVII of Legislative Decree 81/2008 and subsequent updates.

Access to the laboratory is restricted to personnel who are engaged in specific experimental and diagnostic operations within the Department of Surgery, Oncology and Gastroenterology, specifically in the sections of Oncology and Immunology, UOC IDMO, and USD Basic Oncology, experimental, and translational.

This manual has been prepared in accordance with the guidelines suggested by the World Health Organization (WHO), the Centers for Disease Control and Prevention (CDC), and the Istituto Superiore della Sanità (ISS).

Access authorization will be granted to operators after they have received instruction on proper experimental conduct. To obtain authorization, the following steps must be followed:

1. Conduct an interview with the Laboratory Manager to discuss the procedures to be followed during experimental operations and receive a guided tour of the laboratory.
2. Read the provided manual.
3. Receive practical instruction, which includes observing experimental procedures carried out in the laboratory by a specialized operator and executing the same procedures under the supervision of an authorized operator.
4. Undergo an assessment by the Laboratory Manager to determine suitability for laboratory access.

1. Standard Operating Procedures

In a Biosafety Level 3 laboratory, biological agents belonging to the 3rd risk group are handled, with a particular focus on diagnostic and research activities involving HIV. It is

strictly prohibited to conduct experimental operations with these agents outside the biosafety laboratory. Only authorized personnel involved in research or diagnostic programs are permitted access to these facilities. All personnel entering the Biosafety Level 3 laboratory must be fully informed about the potential risks involved, receive thorough instruction on operating procedures to ensure proper handling, and strictly adhere to the described protocols.

Personnel accessing the BL3 laboratory must always adhere to safety practices and:

1. Be aware of the chemical products and biological materials used, as well as the associated risks involved in their handling, and strictly follow the safety procedures for their use.
2. Understand how to respond in different emergency situations.
3. Never underestimate the risks associated with working in a BL3 laboratory.

Note: The laboratory manager must be notified in case of pregnancy, which would prevent involvement in activities within biological risk (BL2, BL3) laboratories, as well as those involving chemical hazards and the use of radioisotopes. This requirement applies to both staff members and operators from external entities, as well as guests and visitors.

1A. Access procedures:

1. The "two-person" rule must be applied, meaning that no individual should work alone in the laboratory.
2. The biohazard sign must be displayed on the laboratory door, clearly identifying the microorganisms being handled and stating the name of the laboratory manager who oversees access and sets conditions, including vaccination requirements if available (e.g., HBV).
3. Access to the laboratory is restricted to authorized operators who are knowledgeable about the rules. The entrance must always remain closed.
4. Entry and exit should be strictly conducted through the designated passage known as the "filter area."
5. Any guests (e.g., trainee students, personnel from external entities, and maintenance staff) may enter the laboratory but must be accompanied by one of the managers and comply with the prescribed dressing procedures.

6. Guests must be informed of the potential risks associated with laboratory operations and should not enter while experimental procedures are in progress.
7. Access is prohibited for individuals under the age of 18.
8. All laboratory doors, including internal ones, must remain closed during work.
9. Corridors and emergency exit doors should be kept clear of equipment and materials at all times and should never be occupied, even temporarily.

1B. Personal protection procedures

The personal protective equipment (PPE) used in the BL3 is designed to provide protection against contamination from infectious materials. However, it does not offer protection against potential aerosol infections.

The process of dressing with appropriate PPE occurs between the first and second filters, immediately after the first access door.

To enter the BL3 laboratory, it is necessary to wear the following:

A disposable gown, an overcoat (new, water-repellent and reinforced) with rear fastening and elasticated wrists, shoe covers, two pairs of gloves (it is recommended to use vinyl gloves in contact with the skin) to be worn over the gown cuffs. The gown is disposable and should be discarded after use, even if not visibly contaminated.

Protection for the mucous membranes is afforded through the use of an airway protection device (FFP2 mask), protective goggles, or a splash guard visor.

Hair should be tied up and covered with a headgear.

After dressing in a disposable gown, shoe covers, headgear, and the first pair of gloves, individuals can proceed through the yellow door and enter the laboratory. Inside the laboratory, it is necessary to put on an overcoat, an airway protection device, goggles, and a second pair of gloves with sleeve covers if required.

At the conclusion of experimental procedures and after collecting materials to be autoclaved, the outermost pair of gloves and any sleeve covers can be discarded within the laboratory. The gown cover should be removed and disposed of, along with shoe covers and any remaining disposable materials, at the exit immediately after the yellow door within the filter area. Operators are responsible for autoclaving the used gowns, foot covers, etc.

1C. Microbiological standard operating procedures

1. It is strictly forbidden to eat, drink, smoke, handle contact lenses, and apply cosmetics in the laboratory.
2. Do not touch your face while handling biological materials.
3. Pipetting by mouth is prohibited; only use automatic pipetting machines.
4. Labels must not be moistened by licking them, and no objects must be brought to the mouth.
5. Take measures to minimize the generation of aerosols during operations.
6. Work surfaces must be decontaminated at the beginning and end of work using 70% ethanol. In the case of material spills, immediately decontaminate with 5% sodium hypochlorite (commercial bleach).
7. Door handles should be decontaminated at least once a day using disposable paper wipes soaked in 70% ethanol.
8. All contaminated materials must be placed in closed containers to prevent leaks and autoclaved before disposal.
9. All experimental operations must be conducted within a biosafety hood or other appropriate physical containment structure. Do not operations with infectious materials on open benches.
10. Only use pumps that are equipped with suitable filters and have an intermediate trap.
11. The use of glass pipettes, glassware, and instruments with sharp points (such as syringes, scissors, scalpels, Pasteur pipettes, etc.) is prohibited, except in cases where they cannot be replaced by alternative tools (e.g., tissue preparation). These items must be disposed of in appropriate sharps containers and autoclaved.
12. Use leak-proof containers for transporting biohazardous materials.
13. Virus-producing cells, virus-containing body fluids, and culture fluids must be inactivated with 5% sodium hypochlorite before being transported from the laboratory.
14. Once staff members leave the laboratory after removing their gloves, they must wash their hands. Exiting the laboratory while wearing gloves is not permitted. Any material transported out of the BL3 must be appropriately decontaminated with ethanol and/or hypochlorite and placed in a clean container, which does not require the use of gloves, in the passage before entering the BL3.

15. Materials, instruments, reagents, and samples used in the laboratory can only be removed after decontamination, as discussed with responsible personnel.
16. The laboratory must be kept clean, organized, and free from any objects that are not relevant to the work being conducted.
17. Before maintenance or repairs, all equipment must be decontaminated.

1D. Pipetting techniques

1. Pipetting by mouth is strictly prohibited. Automatic pipetting devices must be used instead.
2. Avoid forcefully expelling liquid from the pipette and prevent spilling the last drop.
3. When discarding liquid from the pipette, keep the tip as close as possible to the discard point or allow the liquid to flow along the container walls. This helps prevent the generation of aerosols caused by liquid droplets.
4. Avoid mixing liquids through alternating movements of suction and ejection. Instead, mix or agitate them in a closed container using a vortex or other appropriate method. The use of parafilm alone is not sufficient to close a container.
5. Do not aerate cultures by generating air bubbles through the culture medium.

1E. Internal rules to be scrupulously followed

1. Each group within the BL3 laboratory is responsible for procuring consumables such as plastics, media, Ficoll, FCS, antibiotics, etc.
2. At the end of their work, each operator must ensure that work surfaces are clean, hoods are as free as possible from materials and reagents, and bags to be autoclaved are properly sealed. They should also organize and restore finished materials.
3. Do not touch telephones and door handles while wearing work gloves.
4. Before leaving the laboratory, the last operators must check that all instruments and lights are turned off and ensure that incubators and refrigerators/freezers are fully closed and functioning properly.

2. Laboratory entry and exit procedures

2A. Entry procedures

1. Verify the pressure gauge to ensure that negative pressure is maintained in the laboratory.
2. Minimize the amount of time the first door remains open.
3. After dressing according to point 1B (personal protection procedures), access the laboratory. Note that the doors in the filter area cannot be opened simultaneously. Therefore, check that the previous door is closed before opening the next one.
4. Keep the laboratory doors closed during work.

2B. At the beginning and at the end of the experimental procedures

1. Check if the hood is working properly.
2. The hood should be turned on half an hour before work starts in the morning and turned off half an hour after work ends in the evening.
3. Clean the hood with a disinfectant (such as ethanol) before and after each use. Use hypochlorite for decontamination.
4. Ensure that the suction grille is not blocked by any material.
5. Avoid storing excessive materials under the hood.
6. Collect the material to be autoclaved in the designated waste container.
7. Properly store the materials that need to be kept.
8. Dispose of the used outer pair of gloves in the designated waste container.

2C. Exit procedure

1. the reinforced overcoat and all other PPE must be disposed of in the appropriate containers of solid waste with infectious risk in the BL3;
2. enter the first filter room (with the autoclave);
3. discard the shoe covers and the second pair of gloves in the trash bins supplied with an autoclave bag;
4. disinfect your hands before leaving the filter area.
5. In the outer filter room hang the gown on the clothes rack or in the locker

3. Procedure for decontamination and elimination of infected material

Authorized operators are responsible for decontaminating all material to be discarded. Strictly observe the following procedures:

3A. Solid waste must be inactivated before leaving the BL3, specifically:

1. Flasks, plates, and test tubes must be emptied and sealed, and then placed in an autoclave bag inside another autoclave bag set up in large trash container next to the hood.
2. Serological pipettes must be treated with hypochlorite overnight and collected in special long Aliboxes.
3. Pipet tips are collected in small Aliboxes. Any materials that present even a minimal risk of cutting are collected directly in Aliboxes.
4. All solid material used during experimental procedures must be autoclaved before being removed from the BL3 and disposed of.
    - The Aliboxes must be placed in autoclave bags that are then sealed with autoclave tape (not too tight).
    - The autoclave bags in the trash containers next to the hoods must be filled only halfway (in the presence of a large quantity of plastics) or at most two-thirds of the volume. The inner bag is sealed with autoclave tape (not too tight).
    - Items to be autoclaved are placed on the metal cart positioned in front of the autoclave door passing between the BL3 and the kitchen-washing glassware room.
5. The task of placing solid waste in the autoclave is assigned to an operator on a weekly basis. The operator must strictly follow the instructions for opening, loading, and closing the autoclave, as indicated on the autoclave itself on the BL3 side.
6. The pass-through autoclave is operated by personnel external to the BL3. At the end of the sterilization cycle, the autoclaved bags are placed in yellow bags contained in cartonplast boxes, which are labeled with the date and lab information and left in the corridor for disposal by the housekeeping personnel.

3B. All liquid waste generated in the BL3, residual blood samples in vacutainers and other tubes containing liquids from biological samples, is considered to be infectious, and is disposed of as follows.

1. must never be decanted.

2. They must be tightly closed and placed in a rigid black bin with a pressure lid designed for this type of material. The bin should be marked with the label "Special medical waste at infectious risk (C.E.R. 18.01.03*)" and the white label should specify that it is liquid waste by writing "04 liquid".
    - The weight of the waste should not exceed 7 Kg.
    - The external surface of the container must be cleaned with 70% ethanol and labeled with the 'closed' date and lab information.
    - Finally, the container should be positioned outside the BL3 laboratory to be handled by the specialized company.
3. Tissue culture medium is collected in a plastic container containing at least 1/5 volume sodium hypochlorite solution under the hood. After at least 24 hours, the container can be removed from the hood and placed in the rigid black bin with a pressure lid marked with the label "Special medical waste at infectious risk (C.E.R. 18.01.03*).
4. The solution containing hypochlorite used to inactivate the serological pipettes is also collected in a plastic tank as described above. The filled tank is closed tightly and placed inside an autoclave bags within a rigid black bin with a pressure lid, marked with the label "Special medical waste at infectious risk (C.E.R. 18.01.03*)". The white label on the bin should indicate that it is liquid waste by writing "04 liquid".

3C. Decontamination procedures

The decontamination procedures vary based on the pathogens involved and the material to be decontaminated.

1. To decontaminate work surfaces in the case of small accidental spills and splashes of liquids containing retroviruses, sodium hypochlorite followed by 70% alcohol should be used.
2. The most effective and straightforward method to inactivate microorganisms on instruments that are declared autoclavable is sterilization using an autoclave.
3. For instruments that may be damaged by heat, specific procedures should be considered that involve hydrogen peroxide sanitization as well.

3D. Storage of vials containing infectious material

Vials of infectious material should be labeled and sealed before being stored in liquid nitrogen.

Personnel should wear eye and hand protection when retrieving vials from storage and refrigeration.

The external surfaces of the vials stored in this manner should be disinfected when removing them from storage.

3E. Transport of samples and cultures

1. Samples to be transported outside the BL3 must be placed in closed, decontaminated containers and then placed in another clean container that can be handled without gloves.
2. Viable cell cultures in flasks or plates should be transported in waterproof, unbreakable, and sealed transport boxes.
3. All samples, viral stocks, etc., must be transported in sealed tubes.

4. Procedures for the correct use of instruments and equipment

4A. Biosafety hoods

All experimental procedures involving infectious agents must be conducted within biosafety hoods. Certain procedures, such as pipetting, transferring, mixing, centrifugation, sonication, and stirring, can generate significant aerosols. It is important to exercise caution during these operations and maintain sufficient distance between the sample and the open part of the hood facing the operator. Remember that a biosafety hood provides effective containment only when correct microbiological techniques are applied. The following tips are helpful for containing microorganisms:

1. Keep the front grille of the hood clear. Blocking the airflow through the grille compromises the maximum protection provided by the hood.
2. Do not clutter the hood with unnecessary objects.
3. When handling infectious agents, use two pairs of gloves. Remove the outer gloves once manipulations within the hood are complete.
4. Items that are reused without autoclaving, such as containers, test tubes, and small tools, should be cleaned with alcohol or hypochlorite disinfectants before being removed from the hood.
5. Bunsen burners are not permitted inside the hood.
6. Thoroughly clean the hood before and after use. It is also advisable to leave the hood free of materials to facilitate the next operator’s work.

7. Regular maintenance of the hood is necessary, including filter replacement and functional record-keeping.
8. It is mandatory to keep a container with bleach for liquid waste and a separate container for used tips, which should be disposed of in small plastic bags before sterilization in an autoclave.
9. Biosafety hood filters should be replaced as per the manufacturer's instructions, while ventilation system filters should be replaced annually after sanitizing the facility.

4B. Centrifugation procedures

During loading and unloading operations of the centrifuges, always wear gloves. All low-speed centrifugations must be performed in closed tubes with caps, not with "parafilm," placed in the appropriate centrifugation adapters equipped with covers or in rotors equipped with gaskets. Such containers should be used because, in case a tube breaks, the infectious material will be contained in the bucket, which can later be opened and decontaminated under the hood, thus avoiding the contamination of the entire centrifuge.

Follow these criteria for centrifugation:

1. Before centrifuging, inspect the tubes for any breaks or cracks.
2. Ensure that the correct type of tube adapter for the tubes being used is inserted into the bucket.
3. Fill and decant all tubes and bottles under the biosafety hood. Clean the outside of the tubes with disinfectant before placing them in the rotor.
4. Do not fill the test tubes to the brim as liquid may leak through the cap. Close the caps of the tubes securely before centrifugation. Make sure to properly close the lid of the rotor.
5. To decontaminate a rotor or bucket, soak it in 70% ethanol or 5% sodium hypochlorite for 20 minutes, provided the solution is non-corrosive to the material. Then, immerse the rotor in a mild detergent and rinse it thoroughly.

<u>When using microcentrifuges</u>:

Microcentrifuges with enclosed, double-lidded features are appropriate for use in the BL3. Ensure that the gaskets on the lids are intact before use.

<u>Regarding the use of ultracentrifuges:</u>

Operators planning to use the ultracentrifuge must carefully review all operating instructions and procedures beforehand. It is crucial to balance tubes precisely before placing them in the rotor to ensure proper functioning of the ultracentrifuge. Insertion and removal of open tubes to and from buckets/rotors must be carried out under the biosafety hood. After use, buckets should be inspected for the presence of any residual liquid. The buckets and lids are rinsed with 70% ethanol and exposed to the UV lights under the hood.

4C. Centrifuge decontamination procedures

Adapters, buckets and rotors that come into contact with infectious material are to be decontaminated as follows:

To decontaminate adapters and rotors, fill them with 70% ethanol and let them sit for 20 minutes. Afterward, clean the outside by spraying with 70% ethanol and wiping with paper towels. In the event of test tube breakage or leakage of biological liquids inside the centrifuge:

1. Disinfect the affected area using appropriate disinfectant solutions.
2. After disinfection, clean the area while wearing thick protective gloves or, alternatively, double gloves, along with an overcoat and an anti-splash visor.
3. Remove any jagged pieces very carefully using pliers, and dispose of the fragments in an Alibox.

4D. Guidelines for using freezers at -20°C and -80°C:

Store tubes, vials, and test tubes in clearly labeled freezer boxes or test tube holders. Discard any unlabeled material after sterilization.

1. Ensure that the freezer doors are tightly closed. Promptly report any malfunctioning of the freezers to the relevant personnel.
2. It is advisable to periodically clean and defrost the freezers, checking the condition of the contents and removing any broken containers.
3. During the cleaning process, wear heavy rubber gloves, an overcoat, and an anti-splash visor. Use tongs to remove any sharp-edged material and place it in an Alibox. After cleaning, proceed with disinfecting the surfaces of the freezer.

4E. Magnetic, rotating, vibrating stirrers (vortex)

Before using an apparatus to stir/shake a sample, please follow these guidelines:

Ensure that the rotation speed is appropriate to avoid splashes or container breakage.

- Check that the sample container is intact and tightly sealed, not relying solely on parafilm.
- If it is necessary to hold the container or lid with your hands, ensure you have a secure grip.
- Open containers under the hood, allowing a few minutes for aerosols to settle before lifting the lid.

4F. Thermostatic baths:

- Install the thermostatic bath away from any live electrical sources such as sockets or appliance cables.
- Fill the thermostatic bath with distilled water, preferably adding an anti-mold or antimicrobial agent.
- Replace the water at least once a week and treat it as infectious waste when it appears dirty or contaminated. Periodically clean and disinfect the bath thoroughly while wearing gloves.

4F. Homogenizers and sonicators:

1. Use equipment specifically designed for laboratory use within a biological safety hood.
2. Fill and open the container under the hood, allowing approximately 10 minutes for aerosols to settle before opening the container.
3. Always check the condition of containers and closures before use, ensuring they are free of cracks. Do not use glass containers in the BL3.
4. Avoid overfilling containers.
5. Always wear gloves, face protection, and disposable gowns.
6. For sonicators, the user must also wear an individual hearing protection device, such as earplugs or headphones.

4H. Use of the autoclave:

The pass-through autoclave located in the BL3 laboratory is loaded with contaminated materials through the door inside the laboratory. Autoclaved materials for disposal are removed by opening the autoclave from the adjacent room (sterilization area). Please refer to the operating instructions for proper use. Select a cycle that ensures complete inactivation of the pathogenic material. After the cycle, remember to turn off the main switch.

5. Accidents

When it comes to accidents in the BL3 laboratory, as well as in all other labs, it is essential to have a spill cleaning kit available. The kit is located in the corridor next to the -80°C ULT freezer. The kit contains the following items:

- Absorbent cloths (at least 7)
- Sharps containers (1)
- 5% sodium hypochlorite (commercial bleach)
- Gloves (vinyl/latex)
- Waterproofed overcoat
- Safety goggles
- Headphones
- High-efficiency filtering facepiece (FFP3)
- Cleaning instructions
- Sign prohibiting entry for decontamination

5A. Accidental spillage of infected material

In the event of an accidental spillage of infected material, it is important to follow established procedures to minimize the risk of exposure:

For small quantities of spilled material (<10 ml):

1. Cover the spill with absorbent paper soaked in disinfectant.
2. Pour additional disinfectant around the spill area, ensuring that the spilled material remains in contact with the disinfectant for at least 20 minutes.

3. All materials used to clean up the spill must be autoclaved. The only exception is waste containing phenol/Trizol, which must be collected and sealed in a resistant bag and removed from the BL3 without autoclaving.

For spills involving large quantities of material (>10 ml):

1. To avoid inhaling aerosols, leave the room. Notify the other individuals present and the laboratory manager about the accident, and close the door.
2. Rescue exposed personnel: Remove contaminated clothing and dispose of it. Wash any exposed skin with soap and an antiseptic solution. In case of splashes in the eyes, rinse the eyes with the appropriate eye wash station for 15 minutes. A complete shower should also be available.
3. The potentially contaminated individual should activate the procedures specified for occupational accidents by contacting the laboratory supervisor or the responsible person.

In the event of accidental exposure to infected material, the worker should go to the nearest Emergency Department and follow the procedures outlined in the document "Management of biological risk injuries" I_SPP_P02, available on the IRCCS IOV Intranet site, following all the instructions provided. The operator must immediately notify the Unit Director and/or the responsible person, as well as the IOV Preventive Medicine (medicina.preventiva@iov.veneto.it, Tel. 049-8215965) and the IOV Prevention and Protection Service (spp@iov.veneto.it, Tel. 0423-421362).

Cleaning procedures

To ensure proper handling and decontamination of a spill of infected material, please follow the corrected instructions:

1. Immediately affix a sign warning others not to enter the area and allow any aerosols to settle for at least 30 minutes.
2. Always wear appropriate protective clothing, including reinforced gowns, gloves, overshoes, headphones, and a filtering facepiece (FFP3). Collect the spilled material for decontamination using thick gloves, disinfectant, absorbent cloths, biohazard bags, and pliers.

3. Pour sodium hypochlorite onto the absorbent material and any areas that may have been splashed with contaminated material. Ensure that all spilled material remains in contact with the disinfectant for at least 20 minutes.
4. Remove the absorbent material and clean the affected area three times using cloths soaked in 4% sodium hypochlorite. Start cleaning from the outer edges towards the point of contamination. In the case of vertical surfaces, clean from top to bottom. Finish with a dry cloth. Use pliers to carefully remove any fragments, which should be placed in sharps containers.
5. Autoclave all materials worn or used during the cleaning process to ensure proper decontamination.

5B. Cleaning spills inside the biosafety hood

1. Keep the hood running during the cleaning process.
2. Use paper soaked in disinfectant to spray or clean the walls, surfaces, and tools within the hood.
3. Cover the work surface with disinfectant and let it sit for at least 20 minutes. Remove the disinfectant using a sponge or absorbent paper. Clean the grids by using paper soaked in disinfectant, then proceed to clean the bottom of the hood.
4. Autoclave all materials used for cleaning to ensure proper decontamination (see exception above for phenol/Trizol).

6. Cleaning and sanitizing the laboratory environment:

1. Floor cleaning and sanitization activities should be conducted after laboratory personnel have eliminated any specific risks present.
2. Access the protected area only while wearing personal protective equipment (PPE) such as an overcoat on top of the uniform with trousers, gloves, overshoes, and a cap.
3. Follow the designated path through the filter area.
4. Clean the floors daily using a wet method. At least three times a week, use a cloth soaked in a 1% chlorine solution (commercial hypochlorite diluted 1:5) for the final step of cleaning.
5. Do not manipulate any equipment without proper authorization or supervision from the operators.
6. In the event of an accident, immediately notify the laboratory manager and your supervisor.
7. Do not attempt to independently resolve any accidents that may occur during service. Follow the instructions provided by the laboratory personnel.

8. Leave the laboratory only through the designated routes and dispose of the PPE in the appropriate containers before exiting the protected area.
9. The sanitizing tools used within the protected area should not be taken outside, and the damp cloth used for cleaning must be autoclaved and disposed of after each use.

6. Emergency protocols

1. in the event of accidental spills of small or large quantities of infected material, follow the procedures indicated in the paragraphs above.
2. In case of accidental exposure to the HIV virus or a Genetically Modified Microorganism (MOGM), take the following steps:
   - Immediately wash the affected part with water if it is a mucous membrane or with 70% ethanol if it is skin.
   - Go to the nearest Emergency Department and follow the procedure outlined in the document "Management of biological risk injuries" I_SPP_P02, available on the IRCCS IOV Intranet site. Follow all the instructions provided in the document.
   - Immediately notify the Unit Director and/or the responsible person, as well as the IOV Preventive Medicine (medicina.preventiva@iov.veneto.it, Tel. 049-8215965) and the IOV Prevention and Protection Service (spp@iov.veneto.it, Tel. 0423-421362).
3. In the event of an electrical blackout that compromises the maintenance of negative pressure in the laboratory, take the following step: leave the laboratory immediately using the keys located near the interlocking doors.
4. In case of a fire, follow the instructions below:
   - Communicate the alarm to the laboratory supervisor, who must immediately call the operations room (2525) and the Fire Brigade. Provide precise information about the location of the fire (department, floor, building), as well as the name, surname, and telephone number of the caller.
   - Communicate to department employees to make themselves available for emergency management and follow their instructions.
   - Safely evacuate personnel not involved in the emergency (students, trainees, the public) through emergency exits and direct them to the designated external safe collection area.
   - Wait for the arrival of the technical operators from the emergency response team. Direct them to the fire site, inform them of the procedures to access the BL3 laboratory, and assist them in donning the appropriate personal protective equipment (PPE) as per the access procedure shared with 2525 and the training performed in March 2023.

- Remove any injured person(s) from the laboratory and provide assistance as directed by the emergency response team.
- Wait for the arrival of the medical operators from the emergency evacuation team to rescue the injured person(s).
- Cooperate with the Fire Brigade, Emergency Coordinator, and Laboratory Coordinator.

The Emergency and Evacuation Management Plan of the IDMO Building, drafted by the Prevention and Protection Service of IOV, by the IOV Fire Safety Technical Manager, and by the Qualified Expert of IOV, is available on the company intranet (Azienda Informa - Prevention and Protection Service).

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