GUIDELINES FOR BIOHAZARD LABORATORIES

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1. INTRODUCTION: BIOHAZARD

Biohazard refers to the probability that an individual comes into contact with potentially contaminated biological material. Actually, this risk is always present in all living and working environments, but here we are concerned with the deliberate use of the same when biological agents

are intentionally introduced into the work cycle to be treated, handled or transformed, or to exploit their biological properties in any capacity.

Legislative Decree No. 81 of 2008 , in Art. 267, sets the following definitions:

- Biological agent: any microorganism, including genetically modified ones, cell culture and human endoparasite which could cause infections, allergies or intoxications;;
- Microorganism: any microbiological entity, cellular or otherwise, capable of reproducing or transferring genetic material;
- Cell culture: the result of the in vitro growth of cells derived from multicellular organisms.

The subjects exposed to biohazard may contract an infectious disease, that is, a health disorder caused by a biological agent capable of penetrating, multiplying and producing harmful effects in a living organism. The same biological agent may then be able to transmit itself to other organisms. The most frequent methods of contamination in the laboratory are represented by the following:

- exposure to infected material through skin and mucous membranes;
- ingestion of infected material through hand contamination;
- formation of aerosols following the opening of containers, test tubes and capsules or the use of agitators, centrifuges, etc.

The measures to be adopted in order to work in safety must be aimed at protecting the human health, safeguarding and protecting the environment, and no task may be considered so important that it be done neglecting the necessary safety measures.

2. RULES OF CONDUCT IN THE USE OF BIOLOGICAL AGENTS

The biohazard is a professional risk related to the possibility of contracting diseases from bacteria, fungi, viruses, and parasites present in the material to be analysed, which arises whenever there is contact between the aetiological agent of the disease and the host.

The common ways of penetration of the biological agent, and some measures to be taken in order to minimise the risks are listed below:

<u>Mouth (ingestion)</u>:

• It is forbidden to do mouth pipetting;

• It is forbidden to eat or smoke in the laboratory;

• Avoid putting the pens used in the laboratory in your mouth.

<u>Nostrils (inhalation)</u>:

• Avoid monitoring critical operations, such as sowing and opening of plates and tubes, centrifugation, and homogenisation, without wearing PPE (personal protective equipment).

Skin (injection):

• Handle needles, Pasteur pipettes, broken glassware, etc., very carefully;

• Carefully protect cuts, wounds or abrasions on the skin.

Eyes (splashes):

• Always protect your eyes.

Therefore, it is necessary to treat all biological samples as potentially dangerous and to apply the following appropriate precautions:

1. Wear PPE (gloves, helmets, spectacles, goggles, masks, lab coats, shoe covers, waterproof aprons, etc.), check them, clean them, and disinfect them at the end of each working cycle. Protective clothing must be disinfected before washing.
2. Wash your hands after each procedure with disinfectant soap, always at the end of the work, and before leaving the laboratory. In addition, hands must be washed immediately if gloves are torn.
3. Keep your hands away from your face, especially nose, mouth, eyes, and wash them often. Therefore, it is implicitly forbidden to smoke, store or consume food or beverages inside the laboratory. Do not apply cosmetics or contact lenses.
4. It is forbidden to perform mouth pipetting, and to use mechanical systems, such as automated pipettes, etc.
5. Reduce the use of needles and sharp objects, and absolutely avoid putting the needle caps back on the needles after use; dispose of them in special containers placed in the laboratories.
6. Keep the laboratory clean and tidy and do not use foreign substances and objects in your work activities. At the end of the work, decontaminate the work surfaces with an approved chemical disinfectant (see below); it is advisable to periodically rotate the disinfectants. It is advisable to choose equipment that may be easily decontaminated.

3. CLASSIFICATION OF BIOLOGICAL AGENTS

The hazard level of biological agents is established on the basis of the following:

- Infectivity: understood as the ability of a microorganism to penetrate and multiply in the host;

- Pathogenicity: understood as the ability to produce disease following an infection;
- Transmissibility: understood as the ability of a microorganism to be transmitted from an infected subject to a susceptible subject;
- Neutralisability: understood as the availability of effective prophylactic measures to prevent a disease, or therapeutic measures for the treatment of the same.

Depending on their hazard level, biological agents are divided into the four Risk Groups, defined in the following description, pursuant to Art. 268 of Legislative Decree No. 81 of 2008, and Annex XLVI of Legislative Decree No. 81 of 2008, from which the following examples are taken:

1. A GROUP 1 biological agent (none or low individual and collective risk) - an agent that represents a low risk of causing a disease in humans.
2. A GROUP 2 biological agent (moderate individual risk, limited collective risk) - an agent that may cause diseases in humans, and that represents a risk for employees; it is unlikely to spread in the community; effective prophylactic or therapeutic measures are normally available. Examples: *Clostridium tetani, Klebsiella pneumoniae, Hepatitis A Virus, Vibrio cholerae, Streptobacillus moniliformis* (Rat bite fever), etc.
3. A GROUP 3 biological agent (high individual risk, low collective risk) - an agent that may cause serious diseases in humans and that represents a serious risk for employees; this biological agent may spread in the community, but effective prophylactic or therapeutic measures are normally available. Examples: *Mycobacterium tuberculosis*, HIV Virus**, Hepatitis B virus**, Hepatitis C virus**, Yellow Fever Virus, etc.
4. A GROUP 4 biological agent (high individual and collective risk) - a biological agent that may cause serious diseases in humans, and that represents a serious risk for employees, and may present a high risk of propagation in the community; as a rule, effective prophylactic or therapeutic measures are not available. Examples: Ebola Virus, etc.).

Some agents classified in Group 3 and indicated with a double asterisk (**) in Attachment XLVI of Legislative Decree No. 81/08 may involve a limited risk of infection, because they are normally not carried by the air.

In the event that the biological agent subject to classification may not be unequivocally attributed to one of the above groups, it must be classified in the higher risk group between the two possibilities.

4. GENETICALLY MODIFIED MICROORGANISMS

The contained use of genetically modified microorganisms is regulated by Legislative Decree No. 206 of 2001, to which reference is made for specific obligations. Below are the definitions set in the aforementioned decree, for the purpose of recognising the activities that fall within such scope of application:

- Microorganism: any cellular or non-cellular microbiological entity (including viruses, viroids and cultured animal or plant cells), capable of replicating or transferring genetic material;
- Genetically modified microorganism (GMMO): a microorganism whose genetic material has been altered in a way that does not occur naturally by mating or natural recombination;
- Contained use: any activity in which microorganisms are genetically modified, or in which such GMMOs are cultured, conserved, used, transported, destroyed, disposed of or otherwise used, and for which specific containment measures are employed, in order to limit their contact with the population or with the environment.

5. BIOHAZARD ACTIVITIES

Title X of Legislative Decree No. 81 of 2008 and subsequent amendments, identifies the prevention and protection measures that must be adopted for the risk of exposure to biological agents, in the case of deliberate use or potential exposure.

A deliberate use of biological agents takes place when the latter are intentionally introduced into a working cycle in order to exploit their biological properties in any capacity (raw material, substrate, catalyst, reagent, or a product in a working process, even if partial).

At Unicam, the main working activities involving deliberate use of biological agents are the following:

- research and testing of new materials and processes using biological agents (including biotechnological processes);
- research and testing of new diagnostic methods;
- research and testing of new drugs with anti-microbial activity;
- use and testing of drugs containing biological agents;
- general and clinical microbiology laboratories;
- biological testing on animals or cells.

Conversely, potential exposure to biological agents takes place when the activity does not involve direct manipulation of microorganisms, but includes coming into contact with them indirectly, by

means of biological materials, infected people or animals. In these cases, the presence of biological agents represents an unwanted, but inevitable epiphenomenon, rather than being the intended and specific object of the work.

The main work activities involving potential exposure risk are the following:

- chemical-clinical diagnostic laboratory activities (excluding microbiology laboratories);
- veterinary activities;
- zootechnical and agricultural activities.

6. OBLIGATIONS OF THE EMPLOYER

The preliminary obligation of the employer is the assessment of the biohazard level, which must comply with the requirements of Art. 271 of Legislative Decree No. 81 of 2008 and subsequent amendments, starting from the description of the work cycle in order to identify the phases and areas of work at biological risk, the identification of the hazardous biological agents present or presumably present, and their classification within the 4 Risk Groups.

The presence of activities with exposure to biological agents must be reported to the Occupational Physician, who will collaborate in the risk assessment, also for the purposes of planning, where necessary, health surveillance and vaccination programs.

Below are the main requirements set out in Title X of Legislative Decree No. 81 of 2008 concerning the employer.

First of all, it is necessary to carry out the risk assessment, taking into account both the characteristics of the biological agent and the working methods, with particular reference to the classification of the agents, to the information on the diseases that may be contracted, and to the potential allergic and toxic effects.

The name and address of the company (Unicam), the owner (the Rector, as the Employer), and the biohazard assessment document for Risk Groups 2,3 and 4 must be communicated to the local healthcare authority [*Italian acronym:* ASL], at least 30 days before the start of the activities. the name and address of the company (Unicam), the owner (the Rector as employer) and the biohazard evaluation document for Risk Groups 2,3 and 4. Only for the deliberate use of Group 4 biological agents should a specific authorisation be requested from the Ministry of Labour and Social Policies. This request must be accompanied by all the required information, including the agents you intend to use.

On the basis of the risk assessment, the employer must take technical, organisational, procedural and hygienic measures to avoid any exposure of employees to biological agents.

When assessing the risks, the employer in healthcare and veterinary facilities shall pay particular attention to the possible presence of biological agents inside the animal organism, and in the pertaining samples and residues, and to the risk that such presence entails in relation to the type of activity carried out, adopting specific measures for healthcare and veterinary facilities.

The employer must adopt specific measures for laboratories and animal housings, and emergency measures in case of emergency.

He/she shall prepare and carry out health surveillance, as established in the healthcare protocol drawn up by the Occupational Physician.

7. SPECIFIC MEASURES FOR LABORATORIES AND ANIMAL HOUSINGS

Article 275 of Legislative Decree No. 81 of 2008 sets specific measures for laboratories and animal housings.

In laboratories involving the use of biological agents of Groups 2, 3 and 4 for research, educational or diagnostic purposes, and in premises intended for laboratory animals deliberately contaminated with such agents, the employer must adopt appropriate containment measures, in accordance with ANNEX XLVII of the above mentioned decree, as indicated in the following paragraph.

The employer must ensure that the use of biological agents is carried out under the following conditions:

a) in work areas corresponding to, at least, Containment Level 2, if the agent belongs to Group 2;

b) in work areas corresponding to, at least, Containment Level 3, if the agent belongs to Group 3;

c) in work areas corresponding to, at least, Containment Level 4, if the agent belongs to Group 4;

In laboratories involving the use of materials with possible contamination by biological agents that are pathogenic for humans, and in premises intended for experimental animals, which are possible carriers of these agents, the employer must adopt the measures corresponding at least to those of the Containment Level 2.

In premises where biological agents that are not yet classified are used, but the use of which might cause a serious risk to employees' health, the employer, duly informed by the Head of the Laboratory, must adopt measures corresponding at least to those of the Containment Level 3.

8. BIOSAFETY LEVELS

Biological laboratories are divided into basic laboratories (Biosafety Level 1 and 2), containment laboratories (Biosafety Level 3) and maximum containment laboratories (Biosafety Level 4). When assigning a Biosafety Level, the following is taken into account: structural characteristics of the laboratory, the containment capacity, the available equipment, the activities carried out, and the operating procedures necessary to work with agents belonging to the various Risk Groups. Therefore, the assignment of a given level of Biosafety for laboratory activities with a specific microorganism must derive from a careful risk assessment, rather than being carried out automatically based only on the Risk Group to which the pathogen belongs, (ISPESL [Italian National Institute for Prevention and Safety at Work], 2005).

In any case, the provisions of the previous paragraph must be complied with.

The ""Biohazard" sign must be displayed on the entrance door to the premises where activities involving Group 2 (or higher) biological agents, or activities for which the Risk Assessment has reported a potential risk of exposure to these biological agents.

The construction characteristics of the laboratories corresponding to the different containment levels are set in Annex XLVII of Legislative Decree No. 81/08 and are as follows.

CONTAINMENT MEASURES	CONTAINMENT LEVELS		
	2	3	4
The work area must be separated from any other activity in the same building	No	Recommended	Yes
The air introduced into the work area and the extracted air must be filtered through a HEPA ultra-filter	No	Yes, on the extracted air	Yes, on the air introduced and the air extracted
Access must be limited to authorised persons only	Recommended	Yes	Yes, through a clearing house
The work area must have the possibility to be sealed tight, so as to allow disinfection	No	Recommended	Yes
Specific disinfection procedures	Yes	Yes	Yes
The work area must be kept at a negative pressure compared to the atmospheric one	No	Recommended	Yes

Effective control of vectors, e.g. rodent and insects	Recommended	Yes	Yes
Hydrophobic and easy-to-clean surfaces	Yes, for the workbench	Yes, for the workbench and the floor	Yes, for the workbench, the furniture, the walls, the floor and the ceiling
Surfaces resistant to acids, alkalis, solvents and disinfectants	Recommended	Yes	Yes
Safe deposit for biological agents	Yes	Yes	Si, safe deposit
Inspection window or other device that allows you to see its occupants	Recommended	Recommended	Yes
The laboratories must contain all the necessary equipment	No	Recommended	Yes
Infected materials, including animals, must be handled in safety cabinets, insulators, or other suitable containers	Where appropriate	Yes, when the infection is carried by the air	Yes
Incinerators for the elimination of animal carcasses	Recommended	Yes (available)	Yes, on the spot
Means and procedures for the treatment of animals	Yes	Yes	Yes, with sterilisation
Wastewater treatment	No	Optional	Optional

The premises with intended use for “Laboratories”, located inside the University facilities, must be considered, from a structural point of view, as Containment Level 1 or 2.

9. ANIMAL HOUSINGS

Experimental activities, i.e. the use of animals for experimental or educational purposes, can and must only take place in the authorised structures of the University:

- University animal housing, located at the School of Pharmaceutical and Health Products Sciences, Experimental Medicine, via Madonna delle Carceri 9, Camerino (rats, mice, guinea pigs and rabbits);
- University animal housing, located at the School of Biosciences and Veterinary Medicine – University premises in Matelica, University Teaching Veterinary Hospital, coded as Unit A - small animals (porcine and ovine animals), located in Via Circonvallazione 93/95, and

Unit B - large animals (porcine animals, ovine animals, and equids), located in Casette San Domenico snc;

- ➢ University insectarium at the Department of Biosciences, Via Gentile III da Varano No. 5.

In an animal housing or an insectarium, the main risk factors are represented by the following:

- animals (allergies, transmission of zoonotic diseases, aggression and traumatic events, such as bites and scratches, presence of potentially infected biological material);
- environmental conditions;
- type of procedures (e.g. experiments with the inoculation, in animals, of pathogens transmissible to humans);
- physiological conditions of the operators.

The most frequent damages for operators are the following:

- allergies caused by allergens of animal origin;
- infectious processes caused by animal pathogens (zoonoses);
- bites, scratches and other accidents caused by animals;
- damage caused by improper use of the material and equipment.

9.1 Specific rules for animal housings and insectariums

The risk of contracting zoonotic diseases depends both on the health status of the animals and that of the employees, and on the prevention measures adopted in animal housing environments aimed at minimising the sources of contagion and/or infection.

Basic preventive measures include the following:

- the purchase of healthy animals with health certificates;
- the purchase of uncontaminated feed and bedding;
- resorting to quarantine, and clinical and laboratory exams on animals;
- regular disinfection and decontamination of the environment;
- the timely recognition of a disease or malaise of the animals;
- risk assessment for the work environment;
- health surveillance of operators.

The main rules of conduct to be followed by personnel assigned to work at animal housings and insectariums are indicated below:

- access to the premises of the animal housing in Camerino is controlled and registered by means of a biometric system;
- access to the premises of the animal housing in Matelica it is reserved exclusively for personnel authorised by a ministerial project;
- access to the premises is allowed only to personnel officially included in research projects authorised by the Ministry of Health, and to the technical staff of the animal housing. Once authorised, the staff will have free access only to the premises in which the animals are housed, subject to adequate clothing and observance of the agreed rules of conduct. Those who access an animal housing or an insectarium must not have had contact with animals housed in other establishments or kept as pets, in the days prior to the access, as indicated by the Animal Welfare Specialist and the Appointed Veterinarian;
- access authorisation for undergraduate students, PhD students, interns, etc., is released, upon request, by the Head of the animal housing/insectarium. It should be noted that limited access is intended to ensure a good level of hygiene and cleanliness of the premises, and a reduction of all risks deriving from operations carried out by the not properly trained personnel. The prior assessment of their own health conditions (allergies, etc.) is the responsibility of the authorised personnel;
- access to premises is prohibited to pregnant women;
- it is mandatory to wear the footwear or clogs for the exclusive use in the animal housing; alternatively, disposable shoe-covers can be used, put on at the entrance to the establishment, and disposed of at the exit;
- it is mandatory to use disposable scrubs or lab coats other than those worn in other environments; the protective clothing must be changed whenever it is necessary to keep it clean, and every three days as a minimum;
- wash your hands both when entering and leaving the animal housing;;
- use disposable gloves every time you come into contact with animals to perform dressings, take samples, administer drugs, or change the bedding; gloves must be changed every time they break, and when visiting animals housed in different rooms or stalls;
- do not eat, drink or smoke on the premises of the animal housing;
- if an animal present in a user or breeding establishment shows clinical symptoms referable to a disease, infectious or not, it must be immediately separated from the others, even if subjected to a research protocol. After a timely visit by a Veterinarian, if it proves necessary

and following the latter's advice, the animal must be treated or killed, if it presents a health risk, or if it is in conditions of significant malaise, pain or suffering. The cage must be carefully cleaned and disinfected in the shortest possible time; if it is a case of an infectious disease, the measures indicated by the Veterinarian must be adopted;

- the same measures must be taken by the internal or external visiting staff members, who, even if occasionally, use animal housings and insectariums.

10. GENERAL RULES FOR BASIC LABORATORIES (CONTAINMENT LEVEL 1 and 2)

Most contaminations with infectious agents that occur in the laboratories are the consequence of a human error. To eliminate or limit the risk of contamination, it is possible to adopt a series of hygiene and operational standards ("Good Laboratory Practices"), which take into consideration every aspect of the work, from the organisation of the laboratory to the conditions under which the work is programmed, and the rules of conduct that each operator must comply with during the activities.

On the basis of the best practice procedures and the most up-to-date scientific knowledge available, the Employer, in collaboration with the Head of the Laboratory, must do the following:

- provide containment measures for each working phase, by adopting optimised safety procedures proportionate to the potential exposure relating to the type of biological material present;
- adopt, as much as possible, standardised practices and procedures;
- ensure that the equipment meets its purpose, is fully functional, and subject to regular maintenance;
- provide all visitors with specific information and training in relation to the risks associated with their work activities, and in particular to the following:

- actual or potential health risks for each individual work phase;
- rules of conduct and precautions to be observed in order to avoid exposure;
- procedures for safe handling and treatment of hazardous biological agents or samples of potentially hazardous biological material;
- hygiene requirements;
- measures to be taken in the event of accidents and injuries, and how to prevent them;
- correct use and maintenance of Personal and Collective Protection Equipment.

- provide all visitors with means, aids, and materials for the implementation of the protection rules;
- before starting the activities and whenever significant changes occur, identify all those exposed to risk and provide the Occupational Physician with all the information, so that he/ she may carry out, in the context of the implementation of the health surveillance program, immune prophylaxis interventions against infectious diseases for which vaccines are available;
- supervise the correct application of prevention and protection measures by all laboratory visitors, with particular attention to students.

The main rules of conduct that each operator must follow in order to eliminate or limit the risks present in the work environment and to guarantee the quality of his/her work, are shown below and concern the access to the laboratory, protection measures, procedures. and work areas.

1. Access to the laboratory

Access to the laboratory must be allowed only to personnel authorised by the Head of Teaching Activities and Laboratory Research (*Italian abbreviation:* RADRL).

Women of childbearing potential must be informed of the risks to the foetus resulting from exposure to microbial agents. Any pregnancy should be notified immediately to the Employer and to the Head of the Laboratory. In any case, during pregnancy and up to the 7th month of age of the child, it is forbidden for mothers to work in the laboratory.

Only persons who have been provided with specific information / training regarding the potential risks associated with the work activities, may access the work areas of the laboratory.

Never work alone in the laboratory, but avoid crowding in the laboratory as much as possible.

The laboratory doors must be kept closed during work activities.

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10.2 Protection measures

The following protection measures must be taken:

- At all times while working in the laboratory, it is obligatory to wear lab coats, scrubs or suitable laboratory clothing with long sleeves, trousers and preferably closed shoes;
- Work clothes should not be worn in areas other than laboratories, such as offices, study rooms, reading rooms, bars, etc.

- Long hair must be tied behind the head. The use of contact lenses is not recommended and, if that is the case, it is advisable to wear protective spectacles.
- Do not keep scissors, steel spatulas, glass tubes or sharp material in the lab coat pockets.
- For all procedures involving direct or accidental contact with blood, biological liquids, animals or other potentially infected materials, suitable PPE (gloves, glasses, etc.) must be worn. In the event of visible contamination, the equipment must be removed and replaced.
- While wearing gloves, do not touch the objects that are not part of the procedure you are carrying out (computers, telephones, switches, etc.).
- After use, gloves and other equipment must be removed carefully so as not to contaminate the skin.
- Staff members must wash their hands before and after contact with biological, animal, or infected / potentially infected material, and before leaving the laboratory work areas.
- safety spectacles, face shields, and other protective equipment must be worn when it is necessary to protect the eyes and face from splashes, collisions and sources of artificial ultraviolet radiation.
- It is forbidden to eat, drink, smoke, apply make-up, and handle contact lenses in the laboratory work areas. It is also forbidden to store food or drinks in any area of the laboratory.
- The material used in the laboratory (including pens, pencils, scissors, etc.) must not be taken elsewhere..
- Protective clothing that has been used in the laboratory should not be stored in the same lockers or cabinets used for personal clothing.

10.3 Procedures

The following procedures must be implemented:

- All procedures must be carried out so as to minimize the formation of aerosols or di aerosol or droplets (closed containers must be used in centrifugation, homogenization, and sonication procedures).
- The procedures that have a high probability of creating aerosols (mixing, shaking, pipetting, etc.) must be carried out inside a biological safety cabinet.
- Do not perform mouth pipetting, only use mechanical systems.

- The micro-pipettes must always be kept in a vertical position and never placed on the workbench; at the end of each work activity, each micropipette must be properly disinfected.
- Do not recap the needles, and do not walk or move with uncapped needles in your hand. The use of hypodermic needles and syringes to withdraw the contents from diaphragm bottles should be limited to a minimum. Cannulas should be used instead of needles whenever possible.
- All sharp objects must be stored, awaiting disposal, in special needle boxes; they must be placed in a suitable position (close and comfortable) with respect to the various workstations.
- Do not leave unidentifiable material in the work areas.
- Do not place containers, bottles or appliances near the edge of the workbench.
- Do not leave ongoing reactions and working devices unattended.
- Correctly label and affix the date on all containers so that their contents can be recognized at any time.
- Specific internal procedures must be prepared for the management of any accidental spillage of liquids contaminated with pathogenic material, or accidental exposure to infected material.
- Handling of the samples inside the laboratory, with the aim of subjecting them to the various process stages, must take place in secondary containers (boxes, trays, etc.) that ensure the vertical position of the samples. The secondary containers must be made of autoclavable material, resistant to chemical disinfectants, and must be regularly decontaminated.
- The equipment must be decontaminated before any maintenance or repair work.
- The instruction manuals for the equipment used must be kept in a place known to all users, so that they can be easily consulted in case of need.
- All episodes involving contamination with potentially infectious biological materials (needlestick injuries, cuts, getting stained with biological liquids, splashes, etc.) must be notified to the Head of the Laboratory and to the Logistics, Heritage and Prevention Office [*Italian acronym:* ULOPP] and, in any case of injury, it is necessary to comply with the provisions of Chapter 13.

10.4 Work areas

The laboratory must be kept tidy, clean and free of materials that are not strictly necessary for the work activities.

Work surfaces must be decontaminated with a suitable germicide at the end of each work activity, and always after spilling potentially dangerous liquids. For correct decontamination, it is necessary to know the sensitivity to germicides of the biological agents that are being used (for example, alcohol is ineffective for some bacteria).

Containers suitable for collecting infected or potentially infected special waste should be placed near each workstation. See, on this subject, the Operational Guidelines for the management of special hazardous waste.

The Head of the Laboratory and the Administrative executive in charge are required to supervise the correct application of prevention and protection measures by all laboratory visitors, with particular attention to students, research fellows, interns, postgraduate students, etc.

10.5 PPE and biohazard protective clothing

In terms of biohazard protection, a fundamental role is played by the means intended for the direct protection of the operators against specific accident / disease risks. This category includes the means defined in the legislation as Personal Protective Equipment (PPE).

PPE refers to any equipment intended to be worn and held by the worker in order to protect him/her against one or more risks likely to threaten their safety or health during work, as well as any supplement or accessory intended for this purpose.

Biohazard PPE and protective clothing are, in general, disposable. They must be changed frequently and generally after manoeuvres and operations at risk. Non-disposable PPE must be washed, disinfected and possibly replaced (e.g. in case of damage or wear).

During the undressing operations, particular attention must be paid not to touch parts of the body, surfaces or uncontaminated equipment.

Biohazard PPE must have CE marking as Category III personal protection equipment, according to Directive 89/686/EEC, and must be accompanied by information leaflets on their use and maintenance.

Below is a list of PPE:

1) Disposable hand protection equipment, such as latex or nitrile gloves, and non-disposable equipment, such as work gloves made of rubber or more resistant cut-resistant materials (e.g. to prevent injuries, capable of transmitting infectious agents).

2) Gloves for protection against microorganisms must comply with the technical standard EN 374:2003.
3) Respiratory protective equipment, in particular filtering facepieces or disposable respiratory masks, equipped with FFP2 filters for the protection from fine particles (dust, microorganisms etc.). In case of the presence of particularly dangerous agents transmissible by air, FFP3 masks are recommended.
4) Respiratory protection equipment, compliant with the technical standard EN 149:2001, and CE certified, pursuant to Directive 89/686/EEC, is considered suitable for protection against airborne biological agents.
5) Face and eyes protection equipment against splashes of biological liquids, such as protective spectacles, goggles, and face shields, must comply with the technical standard EN 166:2001.
6) Body protection equipment, such as lab coats, overalls, fluid-resistant scrubs, etc. Commonly used cotton lab coats are not, in general, PPE but protective clothing, as they do not protect against specific risks. They should have a snap closure, sleeves with elastic cuffs, and they should be knee-long. Disposable PPE comprises, e.g., certain fluid-resistant lab coats and scrubs, and nonwoven fabric overalls offering full body protection. Specific clothing for protection against infectious agents must comply with the technical standard EN 14126:2003.

It is mandatory to use closed shoes in the laboratory, it is absolutely forbidden to use open footwear, e.g. sandals.

10.6 Biosafety cabinets and usage rules

Biosafety or microbiological safety cabinets, often called Biohazard hoods or cabinets, represent primary collective protection equipment, present in every biological laboratory, as they serve to protect the operator and the work environment from the risk of exposure to aerosols of pathogens. Some types are also used to guarantee operator safety and sterility to the product handled, as in the case of cell cultures..

Biosafety cabinets must comply with UNI EN 12469:2001 and, like all instruments, must be correctly installed, taking into account any interference with other equipment in the laboratory. In addition, they must be used and subjected to regular maintenance according to the instructions given in the manual.

Biosafety cabinets are classified, according to the EN 12469:2001 standard, into three Classes: I, II, III, which guarantee different levels of safety.

Cabinets of all three classes are equipped with a HEPA (High Efficiency Particulate Air) filter for the exhaust air, while the Class II and III cabinets are also equipped with a HEPA filtering system for the incoming air directed to the work surface.

HEPA filters (class H14 or higher, in accordance with the technical standard EN1822-1:2009) are able to guarantee a 99.97% filtering of particles with a diameter equal to or greater than 0.3 microns. These filters are ineffective against gases or vapours.

Some cabinets have UV lamps inside, which perform germicidal action.

Class I cabinets guarantee operator protection through a flow of air drawn in from a front opening without a pre-filter. Once the air has passed through the work surface, it is not re-circulated, but exhausted outside, after passing through HEPA filters.

Class The cabinets are able to protect the operator from contamination (biological agents with low risk of infection), but they do not protect the samples from possible external contamination. Class II cabinets have a front opening through which an air flow is introduced, which is sucked under the work surface, filtered with a HEPA filter, circulated from top to bottom (vertical laminar flow of sterile air, a "barrier" between the inside of the cabinet and the operator), then exhausted outside, after being filtrated. Based on the percentage of recirculated air, the cabinets are divided into: Sub-class IIA (70% recirculated air, 30% exhausted air), and Sub-class IIB (30% recirculated air, 70% exhausted air), or 100% exhausted air. The IIB cabinets are connected to ducts for the exhaust of air outside the building.

Class II cabinets provide a good sample-operator-environment protection compromise.

Class III cabinets are hermetically sealed "glove boxes". The incoming air is introduced through a HEPA filter on the work surface, then exhausted through a double HEPA filter system, ensuring negative pressure to the internal environment.

They are equipped with sleeve gloves, incorporated in the front structure of the cabinet, which ensure a total barrier between the operator and the work surface.

It is essential, for the protection of the operator's health, and the protection of samples from contamination, to know the operating principle of the cabinet in use, and the good practice techniques that must be adopted for the use of the same. Before use, it is essential to read the manual supplied with the equipment, and to define the operating protocols for each single work phase.

Below are some indications on the correct use of biosafety cabinets:

- First of all, wear appropriate PPE;
- The cabinet must be suitable for the sample to be treated and to the operations that must be carried out, and must function correctly;
- All biosafety cabinets must be periodically checked to verify the efficiency of the HEPA filters, and appropriate certification must be issued;
- Before starting to work, check that the UV lamps are off;
- Turn on the suction engine at least 10 minutes before the start of activities in order to stabilise the laminar flow (this delay will also allow the chamber to be purged of the dust collected inside it during the rest period);
- In order to guarantee the correct speed of the air flow, in particular for the Class II cabinets, make sure that the intake grids are not blocked by materials, equipment, or devices;
- Make sure that the front glass shield (if sliding) is at the correct height (20-30 cm);
- Reduce the presence of objects, containers and equipment inside the cabinet; Inside Class II and Class III cabinets, the use of Bunsen burners or other types of burners is prohibited in order to avoid deviation of the internal air flow and possible damage to the HEPA filters;
- Work, as much as possible, in the central area of the cabinet;
- Keep activity in the room to a minimum. The continuous opening of doors and windows, and the passage of people, can cause turbulences that allow microorganisms to cross the air barrier. For the same reason, avoid abrupt movements of the arms;
- Waste from the processing must be placed in suitable containers for biological waste, positioned inside the cabinet. The containers may be transferred outside the laboratory after checking the sealing of the cap, the existence of the label with the biohazard signal and the absence of residues on the external surface;
- At the end of the activities, decontaminate the work surface with the cabinet turned on, with a suitable disinfectant;
- At the end of the operations, leave the turned on for about 10 minutes.

In case of spillage of biological material inside the cabinet, perform the following procedure:

- Do not turn off the cabinet;
- Wear protective gloves;
- Immediately remove spills from the work surface with a cloth-paper soaked in disinfectant;

- decontaminate walls, surfaces and tools; if the work surface is a continuous surface, apply the disinfectant and leave it to act for a few minutes, otherwise (for example, in perforated surfaces), remove the components and clean them thoroughly with disinfectant.
- Periodically, sanitise the entire cabinet.

11. SPECIFIC RULES FOR WASTEWATER TREATMENT

Wastewater carries various microorganisms (viruses, bacteria, fungi), protozoa, helminths, etc. which, due to the formation of aerosols during the various stages of wastewater treatment, may be dispersed in the surrounding environment.

Besides microorganisms that are generally harmless to humans, pathogenic microorganisms, such as *Salmonella spp., Vibrio spp., Escherichia coli, Leptospira interrogans*, and enteric viruses (enterovirus, rotavirus, hepatitis A virus, etc.), as well as intestinal parasite eggs, may be present and survive in urban waste water. The microorganisms commonly detected in the wastewater treatment plants fall into Groups 1 and 2, as indicated in Legislative Decree No. 81/08 (Annex XLVI).

Thus, employees in wastewater treatment plants may be exposed to aerosols containing a high concentration of potentially dangerous biological agents.

Contamination of employees may occur by inhalation of contaminated water droplets, particles, or dust, dispersed during the processes; or by contact with skin and mucous membranes (splashes, direct contact with damaged skin, eye contact); or via the faecal-oral route (accidental contagion due to bad personal hygiene).

The biohazard assessment for employees who deal with wastewater must be carried out in collaboration with the Occupational Physician, who must evaluate if there is the need for any health and vaccination protocols.

12. PROCEDURES IN THE EVENT OF AN INJURY OR ACCIDENT

In each laboratory where pathogenic biological agents, or potentially infected biological materials, are used, specific procedures must be prepared to be followed in the event of an accident or injury.

The rules of conduct to be followed in the laboratory in order to protect the operators' health in the event of accidents involving contamination with biological material, such as accidental punctures with needles, cuts with scalpels or other cutting tools, bite injuries, contacts between organic materials and mucous membranes (e.g. oral cavity, conjunctival mucosa), are described below:

- In case of punctures or cuts with the tools being used, favour the bleeding from the wound, and clean it with running water and neutral soap, then proceed with the thorough disinfection of the wound with electrolytic chlorine oxidant, sodium hypochlorite or with a povidone iodine-based product;
- In case of contact with biological liquids, but which do not involve wounds, clean the skin or the mucosa with running water and neutral soap, and proceed with further rinsing with electrolytic chloride oxidant, or with 10 vol hydrogen peroxide;
- In case of contamination of the conjunctival mucosa with biological liquids, proceed with abundant rinsing of the mucous membranes with running water or with physiological solution;
- In all cases of injuries due to accidental contact (punctures, cuts, contact through the skin and / or mucous membrane) with biological material of unknown or certain origin, the operator must notify the Head of the Laboratory and the Administrative executive in charge, and go immediately to the Emergency Room, where the appropriate prophylaxis treatments will be administered, and the accident report will be filed;
- The Emergency Room certificate / medical certificate must be immediately sent to the Employer for the communication / report of the accident, also for the purposes of insurance coverage;
- Within 48 hours from the receipt of the ER certificate / medical certificate, the Employer shall report the work-related injury, as required by law, and at the same time shall transmit a copy of the documentation on the work-related injury to the Occupational Physician for the follow-up.

In case of accidents that may cause a biological agent belonging to Groups 2, 3 or 4 to be released into the environment, employees must immediately leave the affected area, which may only be accessed by those involved in the necessary interventions, with the obligation to use suitable means of protection.

13. BIOLOGICAL WASTE DISPOSAL

In biohazard laboratories, particular attention must be paid to waste management. Biological waste is the waste the origin and/or composition of which justify the existence of the risk of the transmission of diseases and infections. Therefore, the attribution of the hazard code HP9 should not be done as a precaution, but based on a careful assessment of the actual risk that the

management of that particular waste entails. Hazardous medical waste produced by the University, posing an infectious risk, is mainly composed of the following:

- disposable laboratory material (e.g. gloves, syringes, pipettes, test tubes, plates);
- sharp and pungent materials (e.g. needles, scalpels);
- materials contaminated with recognisable traces of blood, faeces, urine and other biological liquids (e.g. gauze, cotton wool).

In particular, as such materials represent a source of potential exposure to biological agents, all the necessary precautions in their handling must be taken, and, before delivering them to the company appointed for the disposal of the same, they must be autoclaved or covered with disinfectant powder for infectious waste (chlorine, sodium hypochlorite, ortho-phenyl phenol, etc.), or r disinfected using another procedure, specifically identified in agreement with the competent offices of the University.

This procedure, in addition to ensuring the decontamination of the waste and therefore the safety of waste management operations, prevents the spread of bad smells generated by contaminated materials in the work environment.

Sanitary waste posing an infectious risk must be disposed of by delivering it, within five working days, to a waste transportation and disposal company appointed by the University.

For the correct management of special waste produced in the laboratory, please refer to the specific Guidelines for waste management.