S.I.S.S.A. I.S.A.S.

Health and Safety Services

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SISSA – ISAS

Trieste

Laboratory Safety

1 Introduction

The research laboratory has traditionally been an environment where the last thing to cross your mind is the thought you are working. Whoever works there – in this case, the researcher – does so of their own free will, and the commitment, hard work, schedule and even danger, seem like a small price to pay in reaching the ultimate goal: research results.

In the past, the unconscious tendency to neglect or at times underestimate the danger, along with limited *information* on its real significance, created consequences for the researchers - serious ones for their health in numerous cases. Many of these consequences became apparent only as time passed.

Research laboratories are very much work environments where various dangerous agents and work equipment may be present. These agents and equipment may put the *safety* and *health* of the individual working with them in danger, depending on the risk level associated with their use.

This does not necessarily mean lab employees will suffer injury - whether serious or otherwise - as a consequence of their activities. The likelihood of this happening depends on how they organize their work, and on the precautionary safety measures they adopt while carrying it out. These measures are obviously set out in relation to the nature of the danger, and after an acute assessment of the entire operation has been made.

Legislative decree no. 626/1994 requires just this: safety must play an integral part of any work activity and, as in the case of the latter, must be completely organized and planned. All employees, or more generally, all parties in any way involved in the operation, need to be informed about these measures.

Written on the basis of risk assessment findings, this brief manual is an information guide to lab risks. Most of all it is a handbook explaining precautionary safety measures and operating procedures that should be adopted in order to eliminate risks, or reduce them to their lowest possible levels.

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2 Risks and Safety Measures in the Research Lab

In any kind of research lab, even in ISAS's molecular neurobiology and biology labs, risks arise due to the presence of a series of dangerous factors or agents, material or otherwise:

- Danger of materials used: Dangerous chemical, physical, or biological agents;
- Danger of equipment; High voltage equipment, high-velocity centrifuges, pressure systems, high and low temperatures, etc.;
- Overcrowding, lack of space;
- Sometimes inefficient training of personnel, particularly non-permanent staff, students, interns, doctoral students, fellows, and guests.

Risk levels may vary, requiring different safety standards. In any situation, whatever the risk level, you are expected to possess an understanding of everything having to do with the work, so you can act with responsibility and prudence to protect yourself and others.

In addition to possibly suffering or provoking injury, by not following safety norms you subject yourself to full penalties under the law, as well as possibly losing insurance coverage in the case of accidents.

The danger and ensuing risk connected to the use of the ***agents*** listed above can be two-fold:

Health Risk: linked to long-time exposure to these agents (onset of various ailments including occupational disease)

Risk of Injury: the likelihood that an accident ends in injury, as in the case of poisoning, acute intoxication (toxicants), asphyxiation (inert gases), fire or an explosion (flammable solvents), serious and unserious burns (corrosives), eye damage (UV and laser), etc.

The risk and danger connected to the use of ***equipment*** can also be two-fold:

- ***Risk of Injury*** this is the most common risk, and is the likelihood that the equipment leads to an accident and injury (abrasions, wounds, bruises, crushing, grinding, electrocution, etc.).
- ***Health risk:*** normally linked to malfunctioning equipment increasing the likelihood of exposure to agents with the effects listed above.

The main goal of these safety measures is:

to avoid or reduce to a minimum contact between lab workers and the dangerous agents and their sources

This automatically reduces or eliminates the related risk.

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2.1 General Safety Measures

Employee protection is ensured by applying specific provisions under the law, and by following technical standards and common sense. To achieve a safe environment, respect the priorities of the law's protective measures (art. 3, D.Lgs. no. 626/1994), give preference to collective priorities over the individual's, and enforce workplace protection, equipment safety, individual employee rights, conditions of use, safety signs, and personal protection equipment.

The measures cover the following:

1. Hazardous work - or work dangerous to your health - must be carried out, whenever possible, in ***separate areas*** so as to avoid needlessly exposing employees working on other projects to the same materials:
   - → Work presenting risk of explosion, fire, poisonous and toxic gases, or harmful radiation, must be undertaken in isolated areas adequately protected against the spreading of the harmful substance;
   - → The floor and walls of this area must be in condition to allow for easy and complete removal of the hazardous materials that may accumulate;
   - → The work area must be kept clean;
2. There must be ***limited spreading*** of the hazardous agent in the work environment:
   - → The employer must take steps to prevent or reduce toxic, flammable, or unbreathable gases in activities where they develop, and wherever fumes of any kind are created.
   - → Ventilate gas, vapour, odours, and fumes near where they are produced;
   - → Any dangerous gas-emitting equipment, or tools emitting vapour, liquid, and dust must be fitted with the appropriate vacuum devices near the emission source.
   - → ***The chemical or biological hood is the most common protective device used in labs to limit the spread of hazardous agents.***
3. Install automatic **alarms and indicators** to warn you when dangerous concentrations or conditions are reached in the work area. This area can be the site of hazardous accumulations of gases, vapours, explosive powder, flammables, asphyxiates, and toxicants. Whenever possible, carry out frequent controls and measurements.
4. If the danger cannot be eliminated by following the aforementioned measures, put suitable ***safety signs*** in place to signal the presence of persistent risks for the employees;
5. Operators must use suitable ***personal protection equipment (PPE)*** to further reduce these risks;
6. Establish thorough ***operating procedures***, including behavioural standards, which the employees must apply and respect.
7. Provide ***information and training*** to the employees about the risks they are exposed to, the safety and control measures in place, and the additional ways to reduce them.

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2.2 Personal Protection Equipment

Personal Protection Equipment (PPE) stands for any piece of equipment (and any accessories) that lab workers wear to protect themselves against risks that might threaten their safety and health in the workplace.

Use PPE when risks cannot be avoided or sufficiently reduced through technical safety measures, collective safety, or work reorganization methods and procedures.

Employees are required to correctly use and care for PPE. You cannot modify PPE in any way, and must make known any defects or inconveniences. If the PPE is a possible contamination source, use it exclusively for handling and never let it come in contact with clean areas.

PPE must have the EC conformity marking and a pamphlet containing its technical data, including information on its care and use.

It is worthwhile to remember to place appropriate signs in the near vicinity when using PPE for work requiring these precautions.

You must normally use PPE during the following activities in ISAS labs:

- *Use of dangerous chemical agents;* lab coats (always), gloves (always), masks (when there is powder, vapour, fumes, clouds), goggles (if the risk of splashing or projection exists)
- *Use of hazardous biological agents;* lab coats (always), gloves (always), masks (when aerosols are present), goggles (if the risk of splashing or projection exists)
- *Use of radioisotopes;* lab coats (always), gloves (always), masks (when there is powder, vapour, fumes, clouds), goggles (if the risk of splashing or projection exists)
- *Use of hazardous non-ionizing radiation:* goggles (always), gloves (if hands are involved)
- *Use of cryogenic liquids;* in the case of spills: lab coats, aprons, gloves, face screens.

The following PPE is normally available and in use:

Body protection

- Lab coats (always)
- Aprons (use when there is risk of spray, e.g. liquid nitrogen);

Eye Protection

- Generic goggles (protection against solid particles or non-aggressive liquids)
- Goggles guarding against aggressive liquid spurts (e.g. sodium hydroxide; the guard is a small mask for the face)
- Goggles protecting against damaging radiation (laser and UV)

Inhalation Safety

- Surgical masks for thick dust
- Surgical masks for thin dust
- Surgical masks with filters for hazardous fine dust, vapour, fumes and fog due to dangerous liquid.

Hand Safety

- Disposable latex gloves to protect against agents that are not particularly aggressive
- Gloves for protection from aggressive solvents and chemicals
- Gloves to protect against burns (liquid nitrogen)

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2.3 Safety Signs

The goal of safety signs is to make lab workers and anybody else who might be in the work area aware of the risks that cannot be eliminated by simply adopting technical or preventive measures.

Each sign is made up of the combination of the following symbols:

- A geometric form (circular, triangular, square, rectangular)
- A colour (red, blue, green, yellow or yellow-orange)
- A symbol or pictogram (an image depicting a situation or prescribing specific action).

You can also use light or sound warnings, which usually signal an immediate or imminent danger.

The signs are divided into several groups depending on the type of warning: forbidden, danger warning, instructions or requirements, rescue and assistance, information, and extra. ISAS's signs are shown here as an example. They conform to the indications and requirements as laid out by safety regulations.

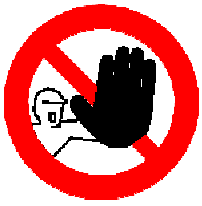
Pohibitory Signs

They have a circular form; the safety sign is characterised by a red edging (covering at least 35% of the surface) on a white background. The symbol and the wording – if any - appear on the white background in black. These signs signal that some activity or action is prohibited because it may cause danger for lab workers.

NO smoking or use of free flame

Do NOT put out with water

Prohibited access to moving trolleys



Access Prohibited to unauthorized persons

NO Smoking

Do NOT Touch

Mandatory or Warning Signs

They have a circular form where the safety warning is blue and covers the entire surface. The symbol and the wording – if any - appear on the background in white. These signs give instructions on how to safely carry out work.


Compulsory eye protection


Compulsory inhalation protection


Compulsory use of Safety Gloves


General Requirement (with possible additional sign)


Compulsory ear protection


Compulsory body protection

Danger Signs

They have a circular form; the safety warning is yellow or yellow-orange (covering at least 50% of the surface). The symbol, wording, and edges are all in black. These signs warn you about the dangers in the work area. Yellow and black signs warn of continuous danger areas (collision, obstacle, etc.).


Attention


High Voltage Danger


Low temperatures


Careful: Obstacle


Risk of falling: Gradient


Hazmat


Corrosive


Flammable


Combustible


Explosive


Toxic Substance


Ionizing Radiation


Biohazard Agents

Laser

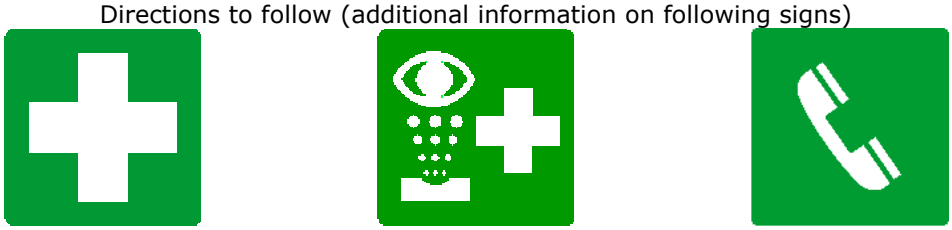

Fixed Obstacles

Rescue and Emergency Signs

These signs are square or rectangular in shape; their background is green, with symbols and wording in white. They point out the nearest emergency exit and how to reach it, the first-aid kit, infirmary, telephone, etc.

Emergency Exit/Route

Directions to follow (additional information on following signs)

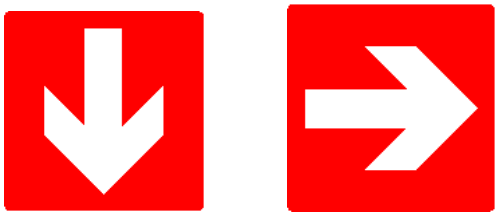


First Aid | Eye Wash | Rescue and First Aid Phone

Fire emergency signs

These signs are square in shape; their background is red, with symbols and wording in white. They give information on fire protection

Fire Extinguisher | Hose | Emergency Telephone



Directions to safety

Additional Information Signs

These signs are square or rectangular in shape; their background is blue, with symbols and wording in white. Place them with the previously mentioned signs to give additional information.

* * *

2.4 General Operating Rules

Apply the following general operating rules in any kind of lab:

- Any research activity is subject to laws and regulations, which the researcher must follow right from the initial planning phase – in collaboration with the health and safety officer - HSO. The latter must carry out risk assessments according to the laws in force;
- All the technicians and researchers must be informed and updated as to the risks present in lab activity;
- Only authorized personnel can access the lab;
- Avoid overcrowding; when inevitable, organize the placement of the lab workers and their activities accordingly;
- You are forbidden to work by yourself, especially during off-hours. You are absolutely prohibited to work by yourself in refrigeration or radioactive rooms, or when engaging in complex and dangerous operations;
- Smoking is strictly forbidden in the lab;
- No eating or drinking is allowed in the lab;
- You are forbidden from keeping food and drink in the lab, particularly in refrigerators where lab substances are stored;
- Avoid wearing open shoes, sandals, or high-heel shoes; long hair should be gathered up and held back; dangling jewellery (earrings, bracelets, etc.) may present risks;
- Contact lenses are discouraged since they may be subject to an accumulation in harmful substances; when an accident occurs, they may make conditions worse or undermine first aid application.
- Do not keep scissors, steel knives, glass test-tubes, blunt material or instruments in your pockets;
- Before using any kind of agent, you should read all the information about its characteristics and dangers in the accompanying documentation;
- All receptacles must be adequately labelled so that you can correctly identify their contents; *never use the contents of a container not bearing a label, and never leave unidentifiable material in the lab or inside the hood;*
- *Regularly use personal protection equipment (PPE)* in the lab. PPE is available for every risk level (lab coat, disposable gloves, goggles, surgical masks, protective masks, footwear, etc.); use PPE correctly, and keep it in good condition;
- Any type of manipulation of a hazardous, chemical or biological agent must be undertaken inside a hood;
- Advise the other persons present (colleagues, students, visitors) whenever an experiment involves the use of hazardous agents;
- Always refer accidents or unsafe conditions to the supervisor quickly;
- Keep the lab in order and clean. Quickly remove glassware and equipment when it is no longer in use. Do not introduce foreign objects or substances into the lab activity;
- Do not leave chemical reaction experiments unattended;
- Do not touch door handles or other lab objects when you are wearing gloves you used to handle hazardous chemical and biological substances and agents, or radioactive isotopes. Use of the gloves outside the lab is strictly prohibited;
- Never block the emergency exits, circuit breakers, or first aid equipment;
- Notify the lab supervisor as soon as possible in case of pregnancy. Follow the procedures for protecting pregnant workers on the basis of the risk assessment. If necessary, require compulsory cessation of the lab activity that entails risks to pregnancy or nursing.

2.5 Staff Training

1. Working with the HSO, the lab supervisor has the task of adequately training the lab staff, including students, interns, fellows, guests and other non-permanent personnel. This staff must receive adequate information on the following:
   - → Risks pertaining to the workplace and duties;
   - → Possible damage due to the use of dangerous substances and equipment;
   - → Safety and control measures for each specific situation;
   - → Fire measures and fire escape plan
2. The supervisor makes sure he/she furnishes every necessary piece of information, in order to reach the established goals.
3. *Whether permanent or not, all lab staff must:*
   - → Constantly report to the supervisor;
   - → Observe the safety operating rules in place and follow the provisions meant for collective and individual protection;
   - → Immediately refer any problems with the protection systems to the officer responsible;
   - → *Non-permanent lab staff* in particular must:
     - Actively work with the permanent staff to efficiently maintain the safety system in place;
     - Attend all the courses offered, including the ones covering radioprotection;
     - Read the rules in this manual when making a request to use the ISAS labs.

2.6 Responsibility for Third Parties

Legislative decree no. 626/1994 (art. 7) imposes safety measures for contracting firms' employees or self-employed workers, whereby every lab officer must put in place safety and control measures to avoid work related risks, including risks to third parties.

As far as the responsibility for the janitorial personnel is concerned, toxic substances, biological or radioactive material must not be in a position to create danger during cleaning hours.

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2.7 Rules for Students and Guests

In order to assure safe lab activities, the student or guest must (in the scope of their assignment):

- Access labs only with the express authorization of the supervisor. This holds especially true for labs where specific dangers are present;
- Follow the safety operating rules in place in each lab, and strictly keep to the instructions given by the supervisor and responsible personnel on group and individual safety;
- Follow the no smoking signs in the designated areas, in classrooms and research/teaching labs. Food and drink is prohibited in the labs;
- Abstain from handling anything that might compromise safety, anything you have not been authorized to handle or for which you have not been adequately trained by the Supervisor.
- Correctly and appropriately use the safety equipment, substances, chemical compounds and devices, carefully conserving them and making sure not to tamper or remove them;
- Take care of your own safety and health, as well as the safety and health of others in the workplace who you may affect with your actions or omissions;
- Work actively with the Supervisor and university service employees to efficiently maintain the safety system in place;
- Undergo individual health check-ups as indicated, taking care in particular to submit to a final medical check-up, whenever so requested;
- Let the Supervisor or employees in charge know immediately of any malfunction in the safety measures, or of any dangerous situations you may become aware of. In this last situation, act directly but within your own qualifications to eliminate or reduce these deficiencies or dangers.

In the event of an **alarm or emergency**, the student and/or guest must:

- Keep calm; panic usually only makes the situation worse;
- Keep to the instructions given by the staff in charge, or to the rules relating to the specific situation;
- If at all possible, help individuals in difficulty;
- Move in an orderly way to the outside of the building and to the nearest safe area. Follow the shortest route as indicated by the signs, and close the fire doors; when a fire is in progress never use the elevators;
- Before leaving the scene but as quickly as possible, put dangerous substances into a safe place to reduce the accident's danger. Do this only when the situation allows, and never carry out any operation for which you have not been trained.

2.8 First Aid Instructions

- Wash your hands well with soap and water, or with alcohol;
- Wash and clean the wound with water;
- Let a few drops of blood come out; then dry, disinfect and cover the wound;
- If there is a lot of bleeding, press or tie above and/or below the wound while waiting for medical attention;
- If the injury is to the eyes, wash with water only and cover the affected area;
- In case of puncture or bite wounds, squeeze the wound and apply ammonia to it;
- For burns, wash the affected area well with cold water, dry, and apply the medicine for burns;
- If the injury is serious, consult a medical doctor immediately.

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3 Chemical Agent Risk

Given the types of research carried out at the ISAS, a great number of chemical agents are used in its labs, many of which are classified as dangerous. Nevertheless, the quantities in use are almost always small, and the work to be done with them simple, inasmuch as they do not involve specific chemical reactions.

Whether the chemicals are dangerous or not, all the containers must be labelled as required so that their contents can be easily identified.

Safety records for the chemical agents must be available in the lab and its immediate surroundings.

Consult the safety record *before using* an unknown chemical agent, or whenever you are unsure: pay attention to R and S Notes.

3.1 Handling Dangerous Chemical Agents

- Always use the PPE that is appropriate for the type of handling, and for the chemical agent's characteristics.
- Do not pipette with your mouth;
- All the operations involving the handling of dangerous products (volatile, toxic-harmful, inflammable, corrosive, etc.) causing vapours, fumes, aerosol, chemical clouds, dusts, must be conducted under a chemical hood
- Never leave chemical reaction experiments unattended, or dangerous equipment on.
- Before starting the reaction, understand the characteristics and behaviour of all the substances involved;
- All weighing of hazardous material dust must be done under a vacuum hood, or in an area made for scales under calm air conditions. If possible, protect the work area with cardboard to collect any dust residue. In the case of toxic, carcinogens or mutagenic compounds, it is always advisable to weigh the substances once and adjust the volume of the solvent to get the desired concentration;
- All known or suspected toxic or environmentally hazardous chemical substances must be eliminated according to the procedures for hazardous material disposal;
- Never dispose of environmentally toxic-noxious chemicals into the sewer;
- Collect chemical compounds and solvents in appropriate containers, and dispose of them according to the established procedures;
- Transport chemical substances and hazardous materials in an adequate way; use trolleys with containment receptacles;
- Immediately contain and clean chemical spills with the procedures indicated on the safety records.

3.2 Protection from Carcinogens and Mutagenic Agents

Common *carcinogen risks* are:

- R45: MMMay provoke cancer
- R49: May provoke cancer through inhalation
- R40: Possibility of irreversible effects

Common risks for *mutagenic agents* are:

- R46: May provoke hereditary genetic alterations
- R40: Possibility of irreversible effects

This group may also include ***toxic agents harmful to the reproductive cycle*** both when it comes to *fertility* (risks R60 and R61) and *development* (risks R62 and R63).

- In areas where carcinogens or mutagenic agents are handled, it is ***prohibited*** to:
  - → Eat or drink;
  - → Smoke;
  - → Wear contact lenses;
  - → Wear make up or cosmetic products;
  - → Touch handles, doors, telephones, etc. while wearing gloves;
  - → Wear protective clothing and other PPE outside the workplace;
- In areas where carcinogens are handled, adopt the following ***precautions***:
  - → If possible, substitute the dangerous products with other non-hazardous materials. Adopt changes in the working process; for example, when dealing with a situation involving the production of dusts, use the substances in a humid area;
  - → Always handle the substances presenting risks R45, R49, R46, R40, R60, R61, R62 and R63 under a Class A chemical hood;
  - → Mandatory use of long-sleeve protective lab coats; if necessary, use goggles and a surgical mask with a FFP3S filter;
  - → Place protective clothing in a separate place;
  - → Limit the number of exposed lab workers to the minimum;
  - → Limit the quantity of the substance to the minimum necessary for the operation;
  - → Operators must keep their hands away from their face, particularly nose, mouth, and eyes. They should wash their hands frequently;
  - → Use storage and transport-resistant sealed containers, labelled according to the legislation in force;
  - → Control access to the area, and put up a list of lab workers authorized to enter the area;
  - → Anytime operators use a carcinogens or mutagenic substance, they must fill out the form supplied by the HSS;
  - → Choose equipment that is easy to decontaminate;
  - → Place a basin or absorbent cardboard on top of your work area;
  - → Operators must systematically clean the work area and equipment after using carcinogens; employ non-reusable instruments for cleaning;
  - → Waste must be stored in separate containers and well identified as explained on the safety card;
  - → In cases of unplanned exposure, leave the affected area immediately and inform your Supervisor;
  - → Store carcinogenic and mutagenic agents in locked cabinets, or in a separate area with access reserved to authorized personnel. The area should be well marked by warning signs.

3.3 Storage of Chemical Agents

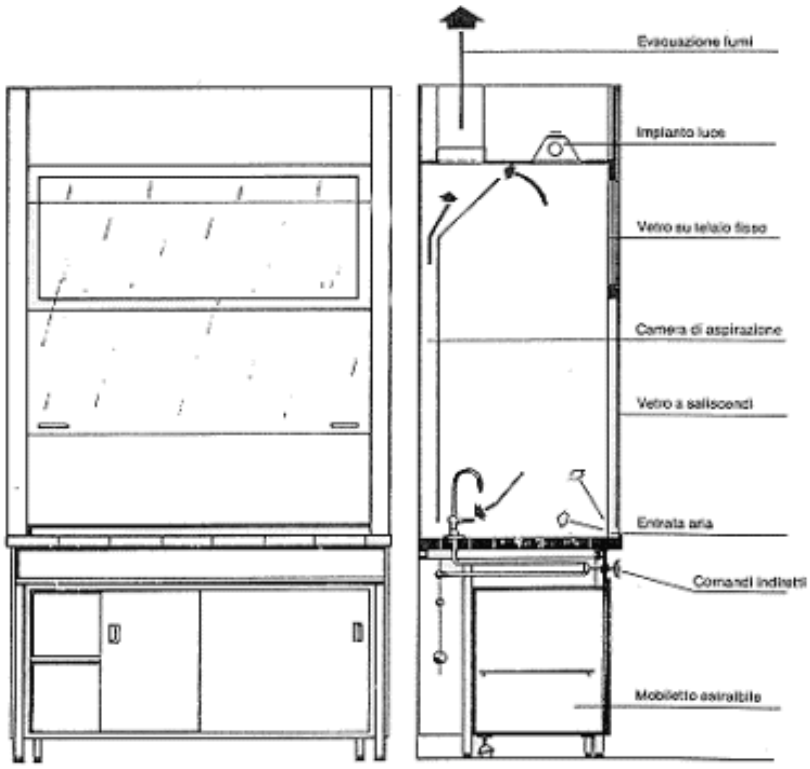
- **In the lab**, products should be kept in quantities strictly necessary for the experiments underway, and if possible, should be placed in locked cabinets;
- **Stockpiles** should be stored in separate places, preferably outside. They should be correctly divided, fixed with automatic fire alarms and sprinklers, and adequately ventilated (windows, forced ventilation systems) on the basis of their contents and quantity;
- The reagents cupboard must be a **cabinet with shelves**; it should be safe for particular products (acids, bases, inflammable and/or toxic), and must have lockable doors. It must also have:
  - → Shelves with a raised outer edge to keep containers from slipping and falling, and to keep spills contained;
  - → A basin at least at the base of the stack of shelves;
  - → Safety signs indicating the product dangers;
  - → Special fire resistant features, in case of fire resistant cabinet;
  - → The following **information** on every cabinet:
    - A list of the contents;
    - Instructions on where to find the safety records;
    - Lab supervisor's name and number;
- The products should be placed inside the reagents cupboard so that:
  - → Corrosives, caustics, and irritants are below eye-level;
  - → Larger containers and the most dangerous substances are on the lower shelves;
  - → Containers are not stored one on top of the other so the shelf is overloaded;
  - → Containers are labelled with at least the chemical name of the substance or compound, as well as the danger class and symbol;
  - → Special instructions found on the safety records are respected;
  - → Mutual incompatibility of chemicals is respected;
  - → Solids are separated from liquids;
  - → They are shielded from direct sunlight and other sources of heat;
  - → If shelves are used, they are adequately fixed down.
- Cabinets should be positioned away from corridors, work areas, lab or work area entrances, emergency exits, free-flame (Bunsen burners, stoves, etc.). They should not create an obstacle in reaching emergency equipment (fire extinguishers, first aid kit, eyewash fountains, etc.);
- Every reagent cupboard or storage area should have material for absorbing or neutralizing spills, as shown in the safety records;
- Label all the reagents with their exact *chemical name*, and the symbols corresponding to their toxicity and harmfulness; also label the *risks* and safety suggestions;
- Keep an inventory of all the chemical substances, especially for the carcinogens and mutagenic agents (R45, R49, and R40);
- Flammable substances should not be kept in home refrigerators, and in any other places where they can be subjected to sparks.

Several chemicals require special precautions:

Huge quantities of flammable liquids need to be placed by themselves in fire resistant cabinets. Store liquids requiring low temperatures in anti-deflagrating (AD) refrigerators, where both the inside and outside are protected. It is better if they are powered by separate circuit breakers;

Place **highly toxic agents (e.g. carcinogens)** separately in locked vacuum cabinets.

3.4 Correct Use of Chemical Hoods



- Before starting, make sure the hood is working;
- Avoid creating air drafts near the hood in use (opened doors or windows, people walking by frequently);
- Keep sources of chemical emissions at least 15-20 cm inside the hood;
- Do not put your head inside the chemical hood;
- Keep the front of the hood as low as possible during the experiment. The further the front is lowered, the less the hood is affected by drafts in the room;
- Keep the work space clean and orderly after each operation;
- Do not use the chemical hood as a storage space: Keep the materials necessary for the experiment under the chemical hood;
- Never obstruct the vacuum openings or the airway along the surface of the hood;
- Do not use the hood to dispose of reagents through forceful evaporation;
- Prepare an emergency plan of action for any malfunctions that might occur during an experiment, or for explosions or fires in the chemical hood;
- It is wise to clean the chemical hood after every use with special products depending on the substances handled. This is to avoid creating risks for the next chemical hood users;
- Close the front, and turn off the vacuum ventilator whenever the chemical hood is not in use.

3.5 Chemical Agent Accidents or Contamination

The amount of chemical agents in the ISAS labs is small, and their uses do not present any substantial risk of accident, particularly when it comes to environmental accidents. Just the same, when contamination occurs, you should:

- Apply first aid, if necessary;
- Substitute the protection equipment that has been contaminated;
- Decontaminate the exposed skin with running water, showers, eyewash fountains, antidotes, neutralizers, etc. depending on the substance;
- Do not spread the contaminants into the surrounding environment;
- Keep non-essential persons away;
- Remove the contaminants from the surface with absorbent materials, making sure to wear gloves that are compatible with the chemical substance in question;
- Whenever an irregularity occurs in the labs, inform the lab supervisors immediately. If they consider the situation as serious, they will inform Health and Safety Services.

3.6 Use of Cryogenic Liquids

The dangers involved in the use of cryogenic liquids (usually inert liquefied gases like nitrogen or argon) are connected with two of their important properties:

- They are extremely cold and any contact produces ***burns***; contact with the eyes can produce serious burns;
- Small quantities of liquid become large volumes of gas, and create the risk of ***under oxygenated conditions***.

Use of cryogenic liquids:

- Only use containers designed and certified for this specific use:
- When a "hot" container is filled, keep away from the evaporating liquid or discharge, and the gas that is developed;
- Use goggles with visor during the operations that may involve liquid spraying (spills etc.)
- Wear the appropriate gloves loosely, so you can easily remove them;
- Wear lab coats and long pants, or an apron to protect against splashes to the legs or other parts of the body. Do not wear open or porous shoes, or sandals;
- Do not touch the equipment's frozen parts with your bare hand;
- When vapour clouds form, the operator must move away (possible reduction in the air's oxygen concentration;
- Access to the areas where cryogenics are used must be limited to authorized personnel only.
- Ensure that the area has sufficient openings for the natural flow of air. As an alternative, install mechanical ventilation instruments in the floor or in the lowest areas, so as to eliminate any vapours that may form;
- When handling cryogenics in an inadequately ventilated environment, it is essential you use an analyzer (oximeter). It must have a light-sound alarm that goes off when the concentration of oxygen goes below 18%.
- Use goggles with visor during the operations that may involve liquid spraying (spills etc.)
- Wear the appropriate gloves loosely, so you can easily remove them;
- Wear lab coats and long pants, or an apron to protect against splashes to the legs or other parts of the body.

3.7 Using Compressed Gases

The containers for liquefied, dissolved, or compressed gases are single piece receptacles holding between 5 and 150 litres, and are referred to as cylinders.

General risks linked to cylinder use can be caused by:

- Cylinder instability
- Internal pressure
- Exposure to extreme temperatures (high or low).

Precautionary Measures

- Store cylinders in a safe place that is clearly labelled. It should be dry, cool, and well ventilated.
- Keep the empty cylinders separate from the full ones.
- Store the cylinders inside the lab so as to keep them from accidentally falling over.
- The laboratory staff in charge of controlling the use of compressed gases must understand the nature and properties of the gases contained in the cylinders.
- Personnel in charge of handling the cylinders must use protection equipment (appropriate gloves, steel-toe work shoes).
- Before taking a cylinder out of storage make sure of its contents by looking at its labels.
- Move the cylinders on special trolleys, even for a short distance.
- Never move a cylinder without a valve cap, and never remove valve caps that are fixed in place.
- Do not subject cylinders containing compressed, dissolved or liquefied gases to temperatures over 50°C.
- Do not subject cylinders to violent impacts.
- Do not use valve caps as casual receptacles.
- Never use cylinders whose review period has expired.
- Never use gases by simply opening the cylinder's valve. Use "pressure regulators" to manage the gas supply. These instruments must suit the gas's type and nature, and must fit the operating conditions.
- Before connecting the pressure regulator to the cylinder valves, make sure all the parts that need to be joined are free of contaminants (e.g. oil, grease, paint, dust, etc.), and that the threads and attachment stem's washer are in good shape. When dealing with a metal support, make sure the outlets you need to connect are in perfect condition.
- Inlets must be made out of material that is compatible with the gas in the cylinder.
- The regulator's connection to the cylinder must not require intermediary fittings.
- Open the cylinder valves slowly to avoid stress on the regulator (the latter should be closed).
- Do not lubricate the connections (valves, regulators, etc.) with oil, grease, or substances of any kind.
- When you do not need to supply gas, close the cylinder valve and loosen the regulator control screws.
- Put back the valve cap on the cylinder immediately after disconnecting it from its system.
- When handling flammable gases, keep fire extinguishers within reach. The extinguishers should be suitable for the particular type of gas.
- When dealing with toxic gases, keep personal safety equipment within reach (gas mask with filter, independent respirator, goggles, etc.) and use it if the lab work or experiment so requires.

4 Biological Agent Risk

The most important kinds of work where individuals run the risk of exposure to biological agents in university labs like ISAS's are the following:

- Work involving biotechnology
- Work involving gathering and disposal of potentially infected waste
- Work involving contact with animals and/or animal products

The main features to consider are:

- Virulence: the ability of the microorganism to penetrate and multiply in the host organism;
- Pathogenicity: the ability of the microorganism to produce a disease after infecting the organism, and its ability in determining the seriousness of the disease;
- Transmissibility: the transmission of a microorganism from an infected organism to a susceptible one;
- Neutralization: refers to the availability or lack thereof, of efficient preventive measures to avoid the disease, or of therapies to cure it.

Only microorganisms belonging to groups 1 and 2 are used in ISAS's labs.

Biological agents are used in labs where there is an adequate containment level protecting against the group the agents belong to.

4.1 General Safety Rules

- It is strictly forbidden to smoke, eat and drink, or keep food or tobacco in the areas where hazardous biological materials are handled and kept;
- Wear disposable latex or vinyl gloves every time you handle blood, biological fluids, or any other human or animal material. Wear protective clothing including long-sleeve lab coats, synthetic water-repellent overalls, goggles, and a visor;
- Take off the protective clothing and gloves when leaving the lab;
- Do not touch door handles and other lab objects with the gloves used in handling the potentially infected material;
- Follow the rules on hygiene by washing your hands frequently and every time you contaminate them, or immediately after removing the gloves;
- Do not pipette with your mouth. Only use mechanical pipettes.

- Take precautions to prevent injury due to cutting objects;
- Do not cover needles again - dispose of them directly in the special containers;
- Eliminate micropipette points in hard plastic containers;
- Use biological hoods that are suitable for the containment level and for activities provoking aerosols. The containment level depends on the microorganism's danger.
- Always use filter masks for biological materials when it is impossible to use a hood.
- Decontaminate your work surface and instruments every day, or after a spill. Use sodium hypo-chlorite solutions 5% common bleach concentrate), 70% ethanol or other disinfectants;
- If dealing with human material, you are recommended to get the hepatitis B vaccination.
- Biological risk warning signs must be put in place in areas where hazardous biological materials are used.

4.2 Safety Rules for Contact with Lab Animals

Risk of contact with lab animals affects the following personnel:

- Animal habitat workers: personnel in charge of animal operations;
- Lab technicians: persons in charge of handling animals during experimental studies, their suppression, or extraction of organic liquid and materials;
- Experimenters: the research project supervisor, degree-holding personnel participating in the research, fellows, etc.

Animals are handled using techniques appropriate to the species; they are handled with safety, and in a decisive manner. You are advised to use methods of constraint (collars, tongs, muzzles) and PPE (gloves, goggles, boots).

The risks can be classified as follows:

1. Risk of injury (caused by the animals)
2. Risks due to cleaning chemicals (ISAS hires an outside firm to clean the labs), or due to medical or toxic chemicals
3. Risks due to contact with animal saliva, urine, feces, serum, dandruff and fur
4. Biological risk due to animals carrying pathogenic agents that are transmittable to humans.

Observe the following rules to reduce risks:

- Use healthy animals: risks are lower if you use unconventional animals (SPF or GF); e.g. animals bred in a closed environment fully or in part deprived of microbes (normal practice at ISAS). Higher risks result from contact with conventional animals possessing natural bacterial flora. In their habitat animals can be host to saprophytic or potentially pathogenic bacteria through their skin, or mucous (mouth, nasal, rectal, urogenital); the latter may be transmittable to humans.
- Reduce environmental stress factors (overcrowding, adequate environmental criterion including ventilation, temperature, humidity);
- Gradually accustom the new animals to the presence of humans, and to their new environment (do not mix animal groups if at all possible);
- Use appropriate protection equipment when stabling and handling the animals (special suits, lab coats, gloves, surgical masks, goggles), and a hood to avoid dangerous aerosols;
- Correctly handle the animals to prevent transmission of diseases through bites and scratches: It is important to understand the animal's behaviour, and to recognize signs of aggressiveness;
- Properly handle animals of different species by using appropriate techniques; use a safe and decisive manner;

- Handle sick, exhausted, frightened animals with additional safety and care (e.g. animals just introduced into the habitat, exhausted by transport, and placed with a group of unknown animals causing aggressiveness);
- Take out of the habitat only the animals needed to carry out the experiment;
- Keep the animal in the lab under a hood, and carry out the entire experiment under a hood, if possible;
- You must use filtering masks, in addition to the required clothing in all cases where you do not handle the animal under a hood;
- Work in well-ventilated areas, under a hood whenever possible, and keep the work area clean;
- Correctly use sharp tools, including syringes, needles, surgical knives, and post-mortem instruments. Personnel must be trained in the correct use of these instruments. The instruments must be efficient and high quality, and should be cleaned and sterilized regularly (preferably single-use/disposable).

5 Physical Agent Risks

Physical agents commonly used in the ISAS labs are:

- Laser systems
- UV radiation
- Radioisotopes

When using these agents, refer to the general rules explained previously.

* * *

5.1 Rules for Laser System Use

RAGGI LASER

Radiation related eye hazards are divided into 5 levels: 1, 2, 3A, 3B, and 4. Each number corresponds to an increasing danger level: 1 is safe, 4 very dangerous. Special precautions must be taken for lasers included in classes 3A, 3B or 4, given the potential danger. Discourage use of powerful machines in cases where lower level systems would suffice.

Use of laser systems can also present additional risks, including electrical shock, chemical or non-ionized radiation. Electrical shock dangers are linked to high voltage power supply adapters used in many laser systems. The power supply for class 4 systems can cause electrocution during maintenance or calibration. Chemical risks are linked to gas or colour lasers, or to any vapours created by the laser-matter interaction in the target area. Non-ionized radiation side effects occur with almost all lasers. At times, X-rays may also be produced: special precautions must be taken in these cases.

Special provisions regulate the use of class 3A, 3B, and 4 lasers. Use of class 1 and 2 lasers, which are found in commercial products (e.g. laser printers, compact disk players, etc.), is not regulated. Operators must nonetheless follow the manufacturer's safety instructions, and cannot perform any maintenance repairs that involve access to the laser.

- Only personnel authorized by the supervisor may use lasers and gain access to controlled areas.
- The supervisor plans precautionary safety measures; in particular he/she should:
- Verify dangers in advance; reduce laser use and the number of exposed lab workers to the bare minimum required;
- Set out special procedures and personal protection equipment in advance;
- Display safety signs and see to other required indications;
- Cordon off and mark the controlled areas, including temporary ones; control access to the areas;
- Make sure the beam is not directed at the lab workers' eye level; see to the personal protection equipment (goggles, etc.);
- Avoid specular reflections; estimate and prevent accidental ones;

- Avoid freely scattering class 4 beams; avoid the interaction of these beams with flammables;
- Check for possible additional risks (gas under pressure, cryogenics, additional radiation, etc.); see to supplying adequate protection from these risks and avoid producing noxious gases, fumes, or particulates without suitable ventilation and vacuums.

Operators must follow the regulations and provisions as set out by the supervisor; in particular they should:

- Wear special goggles and masks for the laser in use, and save them with care;
- Not observe the laser through fibre optic or collection systems (telescope, microscope, etc.) without the expressed authorization of the supervisor; never directly look at the beam, even while wearing protective goggles;
- Take care to avoid accidental and uncontrolled reflections (do not wear watches, etc.);
- Check to make sure the planned safety conditions are all fully in place before shooting the beam into an area that is not under their direct control;
- Neither remove nor modify the protective and interlock equipment without the supervisor's authorization; not carry out manoeuvres for which they are not qualified, or which may compromise safety;
- Let the Supervisor or Director know about personal protection equipment that does not work or is damaged, in addition to any dangers observed; in the meantime, interrupt laser use and eliminate the immediate dangers; only the specifically authorized operators can carry out maintenance repairs, including adjustments to the beam made by opening the cover; students without a degree are prohibited from performing these operations;
- In case of an accident, contain the effects and immediately inform the Supervisor or Director. They will then inform the of the Health and Safety Division Supervisor and L.S.T. (Laser safety technician) and, where necessary, a medical doctor;
- Only sufficiently trained personnel may perform maintenance on fibre optic data transmission systems;
- Disable the laser when it is not in use to prevent unauthorized use. Lab cleaning can only take place when the lasers are off.

* * *

5.2 Using UV Rays

The most common sources of ultraviolet radiation in the lab are:

- Low mercury pressure germicide lamps (UVC): used to disinfect the air in adjoining areas, liquids, and in sterilizing materials.
- Low, medium, and high pressure fluorescent lamps: used in photochemical applications, in inducing reactions and creating damage to materials; also used in molecular polymerization, and in inducing fluorescence in materials.
- Transilluminators (312 nm): used in viewing molecular structures, DNA.

Protection against overexposure to electromagnetic UV rays can be achieved by introducing technical rules into the workplace, setting out recommendations on how to act, and by protecting the staff with the following:

- Limit access to areas with UV ray emitting equipment to assigned personnel.
- All the personnel using UV rays should be acquainted with the risks linked to their use. The work area must have signs relating to the correct methods of use and how to act;
- Indicate the presence of high intensity UV rays with bright signs installed in the entranceway. In all cases, arrange the signs so that they are visible and understandable to anyone.
- Operators should keep themselves as far away from the source as possible.
- The most useful safety measures are better introduced during the instrumental planning phase, and include light-tight containers, shutters, UV resistant glass, and plastic screens. If the UV source must be viewed, use closed housing and screening areas that are constructed so that all the openings for viewing are made out of UV resistant material (e.g. PVC, special glass and acrylic materials).

If all the technical measures have been correctly carried out, you do not have to use personal protection equipment. Nevertheless, the nature of the work results in inevitable exposure of the most at risk areas of the body: hands, arm, and eyes. In these cases, the protective measures require you to cover the exposed areas with the following instruments:

- **Face and eyes:** glasses, goggles, or plastic visor;
- **Hands and arms:** gloves and sleeve couplings; for long exposure, plastic is better than rubber; poplin and flannel are materials letting small amounts of UV pass through;
- **Head and neck:** cotton cap.

Although they let light through, all glass (except for special UV and quartz glass) and plastic substances like Perspex and polyvinyl acetate do not let short length UV rays pass through them. This guarantees complete protection.

UV rays with a wavelength less than 245 nm may provoke the formation of ozone due to the interaction between the rays and the oxygen in the atmosphere. Install a good ventilation system to keep this from occurring.

High-pressure lamps may explode if they suffer an impact. Pay particular attention when moving these UV sources.

* * *

5.3 Rules for Radioisotope use

Radioisotopes are used at the ISAS labs, and due to their characteristics are classified as unsealed radioactive sources.

Operators must:

- Read the safety and emergency rules;
- Keep the workspace clean and in order;
- Avoid working with unprotected cuts or bruises on the hands or forearm;
- Carry out a blank handling test before each new experiment;
- Never pipette by mouth any liquids of any kind or in any quantity;
- Use latex gloves or some similar product, masks, and goggles for handling unsealed radioisotopes;
- Never transfer contaminated materials outside the area at risk;
- Pay attention to the electrostatic build-up on the gloves while handling fine powders;
- When you have gloves on, never touch anything not having to do with the work;
- Handle all radioisotopes in the determined areas (under a hood), and follow all the precautions to avoid spreading the materials;
- Always keep all the solutions or radioactive compounds closed;
- Label all the containers with the relevant data, the dangers, and the date;
- When finished, put the radioactive compounds back in their shielded containers, or place them in storage;
- Always indicate the presence of radioactive material in a clear manner;
- Check for any surface and individual contamination while working. Always do so at the end of every experiment.
- Let the Supervisor know immediately about any contamination still present after decontamination;
- Collect radioactive waste in the special containers;
- After any kind of handling, wash your hands;
- Check for possible individual contamination before leaving the risk area;
- Do not move objects out of the risk areas before checking for possible contamination;
- Always fill out the inventory list accurately;
- Fill out the work registry: indicate the type of radionuclide used, as well as the time and type of experiment.

* * *

6 Lab Equipment Risks

Numerous different pieces of equipment are used in a research laboratory, both in the experimental and manipulation phase, as well as during the analysis and registration of results phase. Almost all this equipment is powered by electricity, which means the following precautions should normally be taken:

- Never use multiple converters to connect several instruments;
- Immediately let the supervisor know about any electrical equipment malfunctions or any damaged components or wires;
- In the case of faulty equipment, stop what you are doing, shut off the tool and ask a technician for help;
- All repairs to the electrical system and wiring, or to electric equipment must be carried out by specialized staff;
- Never hold flammable materials near equipment with electrical motors;
- Never unplug the equipment by pulling on the electrical cable;
- Always use certified components;
- Never make repairs on protective parts of electrical circuits.

6.1 General Safety Rules

Keep to the following precautions when using any type of equipment:

- When in doubt, consult the equipment's instruction manual. Instructions must accompany every piece of equipment, and must be available to all users;
- Never use equipment for purposes it was not designed for;
- Always follow the instruction manual;
- Never tamper with the equipment and their safety features;
- In the case of faulty equipment, ask a technician for help;
- Whenever a piece of equipment is left on outside the normal working hours of the lab, attach a card to it with information about its task, how to turn it off, and the name of the person to contact in case of emergency.

6.2 Centrifuges

These are among the most common pieces of equipment in ISAS's labs. A centrifuge may generate aerosols and material discharge during use. This means you need to:

- Check to make sure all the accessories (rotor, containers, test tubes) are intact and ready for use;
- Use centrifuges that can only be opened when the rotor is still;
- Use gloves while loading and unloading the centrifuge;
- Correctly balance the containers according to their weight with the test tubes in place;
- Avoid using saline solutions that corrode metal;
- Avoid overfilling test tubes, particularly when using fixed angle rotors with biological material that is infectious or potentially so;
- Always use shatterproof test tubes, supplied with an autolock cap (preferably a screw cap);
- Do not use plastic film to close test tubes
- Immediately clean liquid discharge and disinfect in cases of biological liquid;
- The accessories and the inside of the centrifuge should be regularly cleaned and disinfected. They should always be cleaned before making any repairs;

- If the centrifuge is placed under a hood, remember that the airflow originating in it generates turbulence, and that infected particles may fly out at speeds too high to be blocked by a Class I biological hood.

6.3 Autoclaves

Autoclaves work under pressure and exploit the sterilizing potential of superheated steam. They are used in sterilizing:

- Infected material that will be reused
- Material and liquid for sterile compounds
- Infected waste.

Normally avoid using the same autoclave to sterilize waste and to treat material for reuse. Whatever the case, do not load different materials together.

To correctly use the autoclave:

- Always check to make sure the materials you need to sterilize are compatible with the cycles and steps of the sterilization program (i.e. heat resistant or thermo stable);
- Set the heat resistance correctly;
- Use a setting with a cooling phase when sterilizing the hermetically sealed phials, so that you do not pull out the phials at temperatures greater than 70°C;
- Before beginning a sterilizing cycle check to make sure the manual release valve (if included) is well shut;
- Lab technicians in charge of the autoclave must wear clothing that will adequately protect them against potential risks arising from contact with the materials. They must wear gloves to protect against burns, as well as goggles.

6.4 Agitators

Before starting the cycle, make sure that:

- The rotating speed is such that it will not provoke spattering, or break the containers;
- The specimen container is intact and can be hermetically sealed;
- You can get a good grip on the container and cover if you need to hold it in place by hand;
- Open the containers under the hood after letting them sit for a few minutes until the aerosols settle;
- Use the appropriate PPE (lab coats, gloves, face protection).

6.5 Refrigerators and Freezers

- Install the appliances away from sources of heat, and removed from the wall;
- Do not open them frequently or uselessly;
- Use containers made for storage temperatures;
- Avoid overfilling the containers used for freezing;
- All the containers must be labelled;
- Never conserve normal flammable materials in refrigerators;
- Use gloves to extract containers at extremely low temperatures;
- Periodically clean the appliance, and disinfect the interior and exterior surfaces afterwards.

6.6 Automatic Analysis Tools

Automatic analysis tools present several different risk factors:

- Moving mechanical parts: the areas near the moving parts must be indicated and protected;
- Parts under strain: in this case protect with screens, which must not be moved;
- Fume and aerosol formation (chromatographic gases, flame analyzers, atomic absorption): install a local vacuum system;
- Non-ionizing radiations (laser, UV): block the light source so that the ray cannot be let out. Use screens that interrupt the emission of dangerous radiation when removed.

Decontaminate the appliances and their accessories before proceeding with repair and maintenance.

Collect liquid discharge in containers connected to the appliance, and get rid of it by following the procedures for waste collection.

6.7 Glassware

- Substitute glassware with disposable plastic materials whenever possible;
- Do not use broken or chipped glassware;
- Handle glassware with care, especially older materials;
- Do not use glassware of dubious cleanliness: In these cases, re-sterilize it:
- When using glassware with infected or potentially material, wash and sterilize it before use;
- If glassware breaks with dangerous agents inside, cover the area with paper towel or some other absorbent material. Pour disinfectant on the area when dealing with biological agents, and remove everything using lab tools (tongs, spade, brush). Do not use your hands even if you are wearing gloves. When dealing with chemical agents, follow the instructions on the safety records.
- Carry out disposal by following the appropriate procedures for the different types of waste.

6.8 Microscopes

When observing microorganisms, cell cultures, etc. you need to:

- Place and remove the slide with gloves;
- Clean and disinfect the contaminated parts of the microscope and the work area with an appropriate non-corrosive solution;
- Get rid of slides by following waste disposal procedures.

6.9 Bunsen Burners

- Use Bunsen burners equipped with a thermocouple. This feature shuts off the gas when there is no flame;
- Use UNI-CIG standard tubes, fixed down with straps;
- Never set a fire near flammable materials.

7 Precautions for safeguard maternity

As already mentioned in the general rules of conduct in paragraph 2.4, working mothers, specially during pregnancy, are exposed to particular risks and consequently receive a specific form of protection for their health and safety, which also takes into consideration that of the fetus and of the new born baby.

In line with specific laws regarding the protection of pregnant women, and with the results of the evaluation of the risks involved, women who work in SISSA's biochemical research laboratories are in general exposed at the least to the following ***risk factors that are not compatible with being pregnant***:

- dangerous chemical agents
- dangerous physical agents
- biological agents
- being in a standing position for more than half the working day

As a consequence, ***it is forbidden for them to work in the laboratories that contain these dangerous agents during pregnancy and up until seven months after the birth.***

The female worker who finds herself in these working conditions must:

☛ immediately inform the Director of her pregnancy.

She will be moved, if possible, to another job that is compatible with her condition, or her working environment will be changed in order to avoid risks to her health.

When this is not possible, the Director will suspend her from work resorting, if necessary, to the procedure for bringing forward her maternity leave through the Provincial Employment Office.

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