## **University of Pavia**

**Biological Agents** Safety in the Laboratory

## **Environmental and Safety Division**

### **GENERAL PRECAUTIONS**

#### For the handling of infected or potentially infected specimens/biological material

- Treat all biological specimens as potentially dangerous and apply the universal precautions.
- Keep the laboratory clean and orderly and do not introduce foreign objects or substances in the workplace. When the work is finished, decontaminate the countertops with a well-known effective disinfectant; it is advisable to periodically rotate the disinfectants (table 2).
- Do not smoke in the laboratory or conserve and consume food and beverages; do not bring objects to your mouth or put on makeup and contact lenses.
- Respect the basic rules of hygiene such as washing your hands after each procedure, always at the end of your work, and before leaving the laboratory. If your gloves tear you must **immediately** wash your hands.
- Display the "biological risk" sign on the doors of laboratories where groups 2, 3 and 4 biological agents are used. Put the same warnings on thermostats, refrigerators, equipment, waste containers, etc., which are used for infected or partially infected biological material.
- It is forbidden to use mouth-drawn pipettes; use mechanical systems (automatic pipettors).
- Always wear shirts and gloves, and when there is a risk of splashing or aerosol wear masks and protective glasses. Take off your protective clothing when leaving the laboratory. While wearing gloves do not touch common objects such as telephones, PCs, photocopiers, etc.
- Handle potentially infected material so as to minimize the formation of aerosol. Exercise caution when opening vials of freeze-dried or frozen material.
- Reduce the use of needles and sharp objects, and in all cases avoid replacing the cap on needles after use. Dispose of needles in puncture-proof containers near the work area.
- Use appropriate biological safety cabinets for handling biological agents, and always during procedures involving agents from groups 3 and 4.
- Conserve biological specimens in sealed tin containers; traces of the contents must not remain on the outside after hermetically sealing the containers; clearly indicate the contents with appropriate labels.
- In the event of spills involving potentially infected biological material, cover the surface with a cloth or absorbent paper soaked in disinfectant and wait for it to act; then wipe the surface taking appropriate precautions and reapply the disinfectant. Always notify the laboratory supervisor of the accident.
- Disinfect laboratory equipment before any maintenance or reparation procedures.
- Properly disinfect and eliminate solid and liquid infected waste so that there is no risk for the lab personnel. No waste is to be disposed of in normal waste bins, unless it is ascertained that these are non-infectious and chemically and environmentally safe.
- Always keep the containers of specimens to be analyzed separate from accompanying documents (requests for

type of exam, lists, etc.).

• Always consider the collateral risks from the presence of chemical substances, compressed gases, eqiupment, UV, radioactive elements, etc.

## ... when taking blood samples

- Blood samples should be taken by qualified personnel; personnel in training must be under the constant supervision of qualified personnel.
- Use throw-away nonallergic latex gloves (or similar gloves), and dispose of these immediately after taking the blood sample.
- Use vacuum-sealed devices that do not use needles to take blood samples; lay the butterfly pouch flat to avoid its rolling up after use.
- Do not handle the syringe needle after use or replace the cap; dispose of it in the container for needles and sharp objects.
- Transport blood and biological samples in properly labelled airtight containers specially designed to avoid spillage and leaks.

### ... use of laboratory animals

- Laboratory animals, even if not treated with or inoculated against biological agents, can be asymptomatic carriers of transmittable diseases (zoonosis). Use the following precautions:
- Get animals from bonified suppliers or breeders who can provide proper health certificates.
- Allow only trained personnel to look after, handle and undertake surgical techniques on the animals.
- Understanding animal behaviour and recognizing signs of aggression, stress and disease is important.
- Handle the animals in a safe but decisive manner based on the species, using appropriate techniques.
- Use constrictive devices (collars, forceps, muzzles) and individual protective devices (gloves, glasses, mitts, boots).
- Avoid raising dust when cleaning the cages and emptying the litter boxes.

# CLEANING, DISINFECTING AND STERILIZING

# **Disinfection:**

drastic reduction of micro-organisms from the workplace, objects, surfaces, and parts of the body.

# Sterilization:

total destruction, using chemical or physical agents, of all micro-organisms (pathogenic and non-pathogenic), including the most resistent bacterical spores found on inanimate objects.

Always verify that materials have been sterilized by using chemical or biological sterilization indicators.

# Always follow the manufacturer's instructions and consult the safety cards of the utilized products.

COMPOUND	USES AND WARNINGS
Ethyl alcohol, ethanol 70%	Integral skin antisepsis; keep on washed hands for at least 2 minutes; do not use on wounds. Causes skin dryness and irritation. When used with other active substances (chlorohexidine, iodine and derivates, quaternary ammonium salts) its effectiveness is increased. Volatile and inflammable compound.

### Table 1 – Disinfectants for the skin

Chloro derivates: Electrolytic chloro oxidant 5%	Wide range of uses; antisepsis for superficial wounds and burns. Inactive in the presence of organic substances. Concentrated solutions can be irritating and toxic.
Chloroexidine 4%	Can be used with ethyl alcohol 70% and cetrimide. Antisepsis for wounds, burns, antiseptic hand wash. Avoid contact with eyes and ears. No undesirable effects with normal concentrations.
Iodine and iodophors	Iodine and iodophor based solutions are potentially irritating to the skin. In small concentrations in water solutions they are recommended for small wounds, and in a detergent base as an antiseptic hand wash.

## Table 2 – Disinfectants for equpments, objects and surfaces

COMPOSITION	USES AND WARNINGS
Glutaraldehyde 2% active	High-level disinfectant activity. It is used cold as a sterilizing chemical agent. Active in the presence of organic substances, it does not corrode metals. Recommended for plastic, rubber, laboratory material, lenses, and optic fibers. Do not use on surfaces and workbenches. Toxic product, avoid exposing skin and mucous membrane to vapors and solutions. Wear gloves and protective glasses.
Phenolic derivatives	Disinfectant for floors, surfaces, furniture, objects. Stable in the presence of organic material. They are absorbed by plastic, rubber and silicone and difficult to subsequently remove. Irritating to skin and mucous membrance; use gloves and protective glasses.
Chloro derivatives: chloramine T, sodium hypochlorite (bleach)	High-level disinfectant for objects, contaminated surfaces, blood stains or organic material; in such cases concentrations of 5,000-10,000 ppm are recommended. They damage metals. Do not use with acids. Toxic products: use gloves and protective glasses.

### **BIOLOGICAL SAFETY CABINETS (CBS)**

#### **Correct use**

- Assure they are suitable to the specimens being treated and to the operation to be carried out, and that they are perfectly functioning.
- Turn on the laminar flow and leave it operating at least 15' before starting a procedure.
- Eliminate all but the necessary material from the workbench so as not to reduce the flow of air under the workbench.
- Plan your work so as to avoid having to place other material under the hood after starting a procedure; avoid sudden forearm movements inside the hood; do not use Bunsen burners. These can all cause alterations to the laminar flow.
- Carry out all operations in the middle or toward the back of the work surface.
- All potentially infected or contaminated material must be removed from the hood and placed in airtight containers that are cleaned on the outside.
- Leave the hood running for 15' at the end of the procedure.
- Clean and disinfect the hood after every procedure, removing if necessary the dimpled filter panel.

CLASS	% recirculating air	MAIN FEATURES	USES	PROTECTION operator environment sample	
Ι	=	Containment from external air inlet through front aperture. HEPA filter for air outlet.	low risk; groups 1-2 microorganisms	good excel poor	
II A	70	Vertical laminar flow in work area. Frontal air inlet aperture.	medium risk;		
II B 1	30	HEPA filters for air inlet and air	groups 2-3 microorganisms	good excellent excellent	
II B 2	0	outlet. Based on type of biological sample, in the presence of mutagenic, carcinogenic or			
III Glove box	=	Sealing gas, work in negative pressure, access allowed by gloves. HEPA filter in entrance, double HEPA filters out put	High risk Group 4 microorganisms	excellent excellent good	

# DEFINITIONS

**Biological agent:** any micro-organism, including genetically modified ones, cellular culture and human endoparassite apt to cause infections, allergies or intossication; therefore, bacteria, funghi, parassites and viruses.

**Microorganism:** any microbiological entity, cellular or otherwise, capable of reproducing itself or transferring genetic material.

Cellular culture: the result of *in-vitreo* growth of cells derived from multicellular organisms.

**Genetically modified microorganism (GMO):** a microorganism whose genetic material has been altered in such a way that it does not occur in nature through cross-fertilization and/or natural recombination.

**Containment level / biosafety:** the set of structural features, equipment and procedures that allow biological agents to be handled in conditions of safety for personnel and the work environment.

Risk group	Features	Containment level	Examples
1	agent that rarely causes diseases in humans	good microbiological laboratory procedures	
2	agent that can cause diseases in humans and represents a risk for workers; highly unlikely that it spreads to others; therapies and preventive treatment available	second	H. Pylori, Cytomegalovirus, Herpes simplex virus
3	agent that can cause serious diseases in humans and represents a serious risk for workers; can spread to others; therapies and preventive treatments available	third	BSE, Salmonella typhi, Hepatitis B virus, AIDS virus, M. Tuberculosis
4	agent that can cause serious diseases in humans and represents a serious risk for workers; high risk of contamination for others; effective therapies and preventive treatments not available	fourth	Ebola virus, Crimean- Congo hemorrhaging fever virus

#### Confined use of genetically modified microorganisms (GMO)

Risk class	
1	Confined use with no or negligible risk; that is, operations for which level 1 containment is sufficient to protect human health and the environment.
2	Confined use with low risk; that is, operations where level 2 containment is sufficient to protect human health and the environment.
3	Confined use with moderate risk; that is, operations were level 3 containment is sufficient to protect human health and the environment.
4	Confined use with high risk; that is, operations where level 4 containment is sufficient to protect human health and the environment.

# **CLEANING OF HANDS**

### Hands must be washed:

- in the event of accidental contact with biological liquids or materials;
- after work gloves have been removed;
- before bringing anything to your mouth, touching personal objects, and putting on makeup or contact lenses;
- after using the toilet;
- upon completion of work;
- when moving from one patient to another

#### **Proper washing procedure:**

- remove any bracelets, rings, watches, etc.;
- carefully lather your hands (fingers, palm, dorsum, wrist, nails) for at least 30 seconds;
- thoroughly rinse with running water;
- dry with throw-away towels;
- close taps using throw-away towels if there are no pedal, crank or photocell devices for operating the taps

#### Do not use bars of soap

Do not use paper towel dispensers or any comunal apparatus Do not wear rings, bracelets or watches when working

#### To prevent problems with irritation, dryness and chapped hands:

- Use appropriate amounts of soap and antiseptics
- Carefully dry your hands thoroughly
- Alternate the available antiseptics
- At the end of the work day use protective creams (emollients).

#### **USEFUL REFERENCES**

Local emergency number	tel. 113
Health emergency number	tel. 118
Fire brigade	tel. 115
Poison center	
Fondazione S. Maugeri, PV	tel. 0382 24444

In the event of accidents/injuries involving the risk of personal contamination, always immediately notify the laboratory head to initiate the proper corrective measures.

#### http://www.unipv.it/safety

### **INDIVIDUAL PROTECTIVE DEVICES (IPD)**

Any equipment to be kept and worn by laboratory personnel for protection against risks from work activities that threaten their safety or health at work, as well as any accessory or complementary article intended for that purpose. These devices are to be prescribed only when it is not possible to undertake other risk prevention measures (reduction of risk from the source, substitution of dangerous agents with less dangerous ones, limited use of such agents), use collective protective measures or methods or procedures to reorganize laboratory activities. The worker must correctly use these devices and take care not to modify them in any way, as well as notify the proper personnel in the event of defects or specific problems.

Minimum equipment to be worn in the laboratory (to be used according to specific needs):

- 1. long-sleeved shirts, preferably with clinging cuffs;
- 2. eyeglasses: spectacles with side shields for protection against chemicals, UV rays and laser rays;
- 3. visor or face shield to protect against splashes and aerosol; Biologico-opuscoloFINAL versione inglese.doc 27/09/2013

- 4. protective goggles: for dust, active carbons, facial filtrants;
- 5. gloves: throw-away and made from analergic material appropriate for the substances being handled.

# SAFETY SIGNS

# Safety signs have specific colors and symbols

Danger	Do not do	Fire Extinguisher
Warning	Prohibited	
Obligatory		
Prescription	Safety and First-Aid Device	S

Biological risk sign