Laboratory safety S1

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BIOSAFETY

There are four biosafety levels (BSLs) for working with live organisms; each BSL consists of combinations of laboratory practices and techniques, safety equipment and laboratory facilities. Each combination is specifically appropriate for the operations performed, the suspected routes of transmission of the organisms and the laboratory function or activity.

Biosafety Level 1 represents a basic level of containment. It is suitable for work involving well-characterized agents not known to cause disease in healthy adult humans. The potential hazard to laboratory personnel and the environment is minimal.

Biosafety Level 2 is suitable for work involving agents that can cause human disease and have a moderate potential hazard to personnel and the environment. Precautions must be taken for handling and disposing of contaminated material, especially needles and sharp instruments. The laboratory must have limited access.

Biosafety Level 3 is used in laboratories where work is done with pathogens, indigenous or exotic agents which may cause serious or potentially lethal disease as a result of exposure by the inhalation route. Such microorganism can present a serious hazard to workers and a risk of spreading to the community, but there is usually effective prophylaxis or treatment available. BSL 3 requires special facilities with self-closing double doors and sealed windows, decontamination of clothing before laundering and controlled access.

Biosafety level 4 is required for work with pathogens which pose a high individual risk of aerosol-transmitted laboratory infections and life-threatening disease, for which there is no effective prophylaxis or treatment available. Such organisms present a serious hazard to workers and may present a high risk of spreading to the community. The BSL 4 facility is generally a separate building with specialized ventilation and waste management systems to prevent release of live pathogens to the environment.

GENERAL SAFETY RULES FOR WORKING IN THE LABORATORY

The following basic safety rules should be observed at all times in the laboratory:

1. Wash your hands with liquid soap and dry them with paper towels upon entering and prior to leaving the laboratory.
2. Wear laboratory coat and gloves. Tie back loose hair.
3. Do not carry your personal belongings in the laboratory; place them in specified locations – never on bench tops.
4. Do not smoke, eat, drink, apply cosmetics or insert contact lenses in a laboratory.
5. Keep doors and windows closed during the laboratory session to prevent contamination from air currents.
6. Contaminated spots on clothes or body can be sprayed with disinfectant and washed with water. Contaminated material should be put into special containers.
7. If you had any contact with hazardous chemicals while wearing your gloves, change the gloves before you touch other laboratory equipment, do not touch your face or your clothes with contaminated gloves.

8. Do not allow water or any water-based solution to come into contact with electrical cords or conductors. Make sure your hands are dry when you handle electrical equipment.
9. Report all accidents immediately to the instructor.

RULES FOR HANDLING CHEMICALS

Almost all chemicals can be harmful in some way and prolonged exposure may cause long-term effects as yet unknown. Preparation of hazardous chemicals must be conducted under the fume hood.

When handling chemicals the following rules must be strictly met:

1. Always read labels before handling any chemical. Learn hazard warning symbols which are displayed on the labels.
2. Take care to avoid spillage - if this occurs, neutralize any hazard and clean up immediately, including the outside of the container.
3. Some chemicals have a delayed or cumulative effect. Inform the instructor if any feeling of being unwell occurs when using chemicals.
4. Chemicals must not be disposed of by indiscriminate washing down the sink. Carefully read the appropriate material safety data sheet and follow the instructions.

CHEMICAL HAZARD SYMBOLS

Explosive

Mutagenic, carcinogenic, causing respiratory sensitization

Oxidizing

Corrosive

Extremely/Highly flammable

Dangerous for the environment

(Very) Toxic

Pressurized gases

Harmful

PIPETTING TECHNIQUE

Pipetting is one of the most frequent tasks in the laboratory and it directly affects the success and repeatability of the experiments. It is critical to follow good pipetting practice techniques.

ASEPTIC TECHNIQUE

Aseptic technique is a combination of procedures designed to reduce the probability of infection. In spite of the introduction of antibiotics, contamination with microorganisms remains a problem in tissue culture. Bacteria, mycoplasma, yeasts and fungal spores may be introduced by operator, atmosphere, work surfaces, solutions and many other sources. In order to avoid contamination aseptic technique should be used while handling cell cultures.

Correct aseptic technique provides a barrier between microorganisms in the environment and the culture within its flask or dish. Hence, all materials that will come into direct contact with the culture must be sterile and manipulations designed in such manner that exclude direct link between the culture and its nonsterile surroundings.

The elements of aseptic technique are a sterile work area, good personal hygiene, sterile reagents and media, and sterile handling.

Rules for sterile work:

1. Start with completely clear work area and wipe the surface with 70% alcohol and a sterile gauze.
2. Spray and wipe your hands with 70% ethanol.
3. Clean the outside of the containers and other objects with 70% ethanol before placing them in the microbiological safety cabinet.
4. The work surface should be uncluttered and contain only items required for a particular procedure; it should not be used as a storage area.
5. Remove everything that is no longer required and clean with 70% alcohol before the next procedure.
6. Arrange items to have easy access to all of them without having to reach over one item to get to another.
7. Work within your range of vision, e.g., insert a pipette in the pipetting device with the tip of the pipette in your line of sight continuously and not hidden by your arm.
8. Clean up any spillage immediately with absorbent tissues and wipe with 70% alcohol.
9. Remove everything when you finish and wipe the work surface with 70% ethanol.
10. Use ultraviolet light to sterilize the air and exposed work surfaces in the microbiological safety cabinet between uses.

GMO

GMO is an abbreviation for genetically modified organism. GMO is an organism that is created when a recipient (host) organism, with the help of a vector, successfully incorporates the insert in its genetic material and can transfer it to its descendants.
Closed system is a laboratory or some other closed room for GMO work.

Recipient (host) organism = cell/organism which accepts genetic material from the original organism or the environment, replicates and expresses it and can transfer it to its descendants.
Parent organism = recipient organism before the genetic change
Original organism = organism from which the genetic material for transfer in the host is acquired
Vector = DNA tool used in genetic engineering to harbour genes of interest and transfer them to the host
Insert = genetic material that is integrated into a vector

Example: In cell and molecular biology, the GFP **(**green fluorescent protein) gene **[insert]** is frequently used as a reporter of expression. GFP is a protein that exhibits bright green fluorescence when exposed to light in the blue to ultraviolet range. It was first isolated from the jellyfish *Aequorea victoria* **[original organism]**, although many marine organisms have similar green fluorescent proteins. It is carried on plasmids **[vector]** to the target cells **[parent organism].** The cells that manage to express the protein are called **host organisms** (GMO).

When working with GMO, traceability is essential. For that it is necessary to keep a good operating and autoclave log book. Operating log is used for writing down essential GMO information, work procedure, solid and liquid waste management and potential work related accidents. Autoclave log is a record of all waste that has been autoclaved.

GMO waste can be deactivated in two different ways – thermic or chemical treatment. Deactivation prevents the GMO`s to migrate out of the closed system. Sterilized liquids can be washed down the sink, dry sterilized solid waste can be thrown in municipal waste.

In case of a GMO accident the biosafety commissioner needs to be informed and his/her directions should be followed. If a spillage occurs there has to be enough absorbent material to absorb all the liquid. Work surfaces should be decontaminated with a disinfectant.

FURTHER READING:

Freshney R. I. Culture of animal cells: a manual of basic technique.3rd ed. Wiley-Liss, Inc. New York, 1994.

http://www.biotechnology-gmo.gov.si/eng/genetically_modified_organisms/index.html