

# MOUNT SINAI SCHOOL OF MEDICINE

## **BIOSAFETY PRINCIPLES--SYLLABUS**

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## INTRODUCTION

You are reading this material because you are taking the training course in Biological Safety, or Biosafety, as it is commonly abbreviated. This course will serve to acquaint you with some of the principle concepts and terminology found in Biosafety, and present the basic practices to use to protect yourself from the hazards of handling biological agents.

It is by no means comprehensive in scope or content. Hopefully, your awareness will be heightened, and you will want to go to the resources given at the end of this syllabus, or to the MSSM Institutional Biosafety Program Website- <http://www.mssm.edu/biosafety/>, for more information.

Ultimately, you as a researcher have to decide that you want to work as safely as possible while performing your research--no safety program, training, presentations, or lectures can succeed otherwise, no matter how well planned and delivered. Literally, **your** safety rests ultimately in **your** hands.

### 1. WHAT IS BIOSAFETY?

#### a. History

Biosafety has an old and a new beginning. The “old” beginning was initiated with the first studies of microorganisms, disease and pathology. Aseptic technique, disinfectants and culturing methods and rudimentary safety devices were developed during the late 1800’s-early 1900’s.

The “new” beginning came in the 1970’s at the time when researchers developed the abilities to “cut-and -splice” DNA, and transfect microorganisms with these novel recombinants, requiring the development of a new biosafety on the framework of the old principles.

#### b. Principles

If one looks at the principle guidance documents for Biosafety in existence today, the key principles practiced today are the same as those of the “old” period of biosafety, specifically **Good Microbiological Technique**. There is no substitute, and all of the different Biosafety Levels used by the NIH and the CDC are based on these specific practices:

- Possess *beforehand* the knowledge, skill and ability to work with specific organisms safely and in a manner that does not present risk to self and others
- Handwashing after handling cultures or infectious materials
- Good “Housekeeping”- keeping work surfaces and equipment clean and disinfected, with proper storage of infectious materials
- Decontamination of stocks, cultures and wastes by autoclave
- Controlling aerosol-generating practices and splash hazards
- Needles, syringes, glass-items and other sharps are handled with caution and disposed of accordingly

By consistently using these basic practices, you are creating a relatively safe work place to work with your biological agents.

#### c. Microorganisms

Microorganisms encountered in research can run the spectrum of the different kingdoms, phyla and orders that organisms occur in, and include those that defy classification such as prions, *Actinomyces* etc.

By and large, most of the organisms found in research today are of the following:

- Bacteria
- Chlamydiae
- Rickettsiae
- Fungi
- Viruses
- Prions
- Protozoans (Parasites)

We could even throw a few more in, but these are sufficient for our current purposes.

#### d. Risks

With each project there are *risks* associated with a given *hazard* that a microorganism possesses. To clarify further, a **HAZARD** is a characteristic that is inherent within a particular microbe. The microbe may produce a toxin, or is extremely invasive due to receptors on its surface-this is the hazard.

The chance that an exposure or circumstance that will bring this microbe and its hazard into contact with you as the recipient, is the **RISK**. The balancing of **Hazard** and **Risk** is the main activity of Biosafety. The need to perform research with a hazardous microbe in order to gain knowledge is leveraged against the risks to personal health and safety of the researcher.

Biosafety attempts to clarify the hazards and the risks associated with microorganisms, and seeks to provide an operating framework in which research can proceed safely. A framework is used instead of rigid rules, because flexibility is key in working with different groups, and sometimes, within individual genera of microorganisms.

To provide some examples of hazards, blood-borne pathogens are spread through direct contact with human blood, and are always assumed to be present in every human blood and body fluid specimen. Airborne pathogens do not need direct contact *per se*, but if inhaled can set-up an infection in the recipient. Food-borne pathogens require ingestion of contaminated food and have different hazards and risks associated with these exposures.

Bioaerosols, or aerosols of infectious agents pose the greatest hazard of exposure to a pathogenic agent in the laboratory. Of special concern are droplets, or particulates of 1-5 microns in diameter. These objects on inspiration,

can penetrate deep into the alveolar region of the lungs, giving ample opportunity for an infectious agent to start growing and cause disease. Simple lab techniques such as mechanical mixing, using a blender, using a sonicator, or centrifuging can release aerosols within the specified diameter-range. Fluid-borne agents, when aerosolized can behave in a similar manner to airborne-agents if the right pathogenic mechanisms exist for setting up infection by that route of exposure.

Next to inspiring aerosols, needle sticks, direct contact with infectious material, injuries with broken glass or other sharps, aspiration through a pipette (by mouth), and bites/scratches from animals are all causes of lab-acquired illness in descending order of incidence. Prudent practices in the laboratory seek to avoid or eliminate outright these sources of exposure.

#### e. Regulatory Agencies

In the United States alone, the following agencies have jurisdiction over the acquiring, handling, storing and disposal of microbiological agents:

- CDC\*
- NIH\*
- USDA
- USPHS
- USDOT
- US Postal Service
- USEPA
- OSHA
- FEMA

<http://www.mssm.edu/biosafety/> has links to some of these agencies

The two asterisked Agencies, the National Institutes of Health and the Centers for Disease Control and Prevention publish specific guidance documents for Biosafety and Recombinant DNA research. Researchers should become familiar with these two agencies and their requirements since they impact on research activities and Grant funding.

## 2. PRIMARY CONTAINMENT

Primary containment is the set of practices that **you** perform in the laboratory to contain and isolate the hazards associated with a given organism in order to reduce the risk of personal occupational exposure to that organism. It is the **primary** or first-line of defense.

Examples of primary containment are:

- Using self-sealing closures, screw-cap closures on tubes, flasks etc
- Using Biological Safety Cabinets (Tissue-Culture “Hoods”)
- Using Glove boxes for high-hazard operations
- Using lab coats, respirators, gloves, gowns, suits and other forms of Personal Protective Equipment (PPE)

-Using special safety caps on centrifuge buckets and rotors

All of these are active/interactive practices you utilize in the lab

### 3. SECONDARY CONTAINMENT

Secondary containment utilizes a more passive, less interactive group of practices and equipment to create or enhance the safety of the work area. The equipment is usually facilities-associated, and usually extends from the laboratory to adjacent areas and even external to the whole facility.

Examples of secondary containment are:

- self-closing, sealed doors
- double-door access anti-rooms under negative pressure
- sealed walls and floors, with no open penetrations to the outside
- directional flow ventilation systems
- differential pressures between low and high-hazard areas
- contained plumbing systems with HEPA-filtered vents

All of these are examples of equipment / facilities that are designed / engineered in such a manner as to prevent the release of pathogens to surrounding areas within and outside of the research building.

### 4. BIOLOGICAL CONTAINMENT

Whereas some of the previous containment practices and equipment have been around from the earliest days of microbiology / infectious disease research, this type of containment was developed by the NIH in response to the emerging science of biotechnology and Recombinant DNA Research of the 1970's. The basic underlying concept is to use bacteria, plasmids, fungi and viruses that were restricted in replication and / or propagation, that in the event of accidental release to the external environment, these organisms would be incapable of survival outside of the laboratory, and would not imperil the community at large.

*Appendix I* ([http://www4.od.nih.gov/oba/RAC/guidelines/appendix\\_i.htm](http://www4.od.nih.gov/oba/RAC/guidelines/appendix_i.htm)) of the *NIH Guidelines* covers this concept in some detail and gives examples of specific Host-Vector systems that have been certified since the earliest days of R-DNA research.

### 5. HEALTH AND SAFETY PRACTICES

For many of the microbes that have been studied or used in the past, the CDC and the NIH have together and separately developed health and safety practices to protect individuals working with these agents. In *"Biosafety in Microbiological and Biomedical Laboratories"* and referred to by biosafety practitioners as *"BMBL"*, the NIH and CDC present the specific BioSafety Levels (BSLs) and Agent Summary Statements for many biological agents.

(See: <http://www.cdc.gov/od/ohs/biosfty/bmbl4/bmbl4toc.htm> ).

The NIH in its “*Recombinant DNA Guidelines*” has Appendices which cover Risk Groups for the microorganisms, Physical Containment and Biological Containment to be used with the Risk Groups, and Large Scale Containment Practices.

(See: <http://www4.od.nih.gov/oba/rac/guidelines/guidelines.html> ).

Together, both references constitute the bulk of the health and safety practices required to work safely. Much has been learned about which classes of microbes present which hazards, the associated risks, and the required levels of protection based on recommendations from the Sulkin and Pike studies given in the “*BMBL*”.

## 6. APPLYING BIOSAFETY PRINCIPLES

When you are designing your projects, take a close look at the biological agents that you will be using and how you will be using them. The first step is to identify what diseases the microbe is associated with. As stated above, there is already a large base of information on many agents. This will aid you in determining what safety procedures and practices to use in your project.

If you have the fortune of working with an agent that has not been characterized well, look at what diseases have been associated with the agent. *Serratia marcescens* was used as a surrogate in studying the movement of biological aerosols, and was considered a “safe” bacteria, until it was associated with human meningitis cases.

Use Risk Groups to determine what a potential exposure to a given bacteria, virus, etc could do to you if it came out of containment. Then use the BioSafety Level practices and procedures recommended in either the “*NIH Guidelines*” or “*BMBL*” that give you the greatest protection from your microbe of choice. Remember that these levels are to be considered as **minimal practice levels**- they do not include all practices possible, and do not exclude other methods of arriving at the same level of protection. Congratulations, you have just performed a Job-Hazard Analysis with appropriate safeguard recommendations.

For most practical lab work, the use of a Biological Safety Cabinet (BSC) eliminates most potential exposures in a similar way that a chemical fume hood protects researchers from the hazards of chemical exposure. Using the BSC, and screw-cap containers, sealed centrifuge buckets in combination provides a higher level of protection together.

In the paradigm being used, it is necessary to consider **the Source, the Pathway** and **the Receiver** in designing a safety procedure. Any interdiction we make at these points to interrupt the exposure will give us the result we seek.

**The Source:** by containing the microbe in sealed containers, centrifuging or vortexing a suspension will not result in aerosol release.

**The Pathway:** by using a Biological Safety Cabinet, any aerosols generated will be contained within the cabinet and directed away from the Receiver.

**The Receiver:** if none of the above options are available to you, then we have to start hanging protective equipment on you--i.e., gloves, gowns, respirators, bonnets, shoe-booties etc. to provide equivalent protection.

Any one of these examples can be used, or all of them, in combination to provide a safe level of protection. It is more preferable to use passive systems such as the special containers or the BSC, as opposed to using interactive systems which rely on your ability to put on, use, and discard when contaminated, any or all of the items in the system.

A good BioSafety Level (BSL-) to use for most microbiological, recombinant-DNA, tissue culture, or blood-work is BioSafety Level 2 (BSL-2). This set of practices and procedures should be considered the **minimum operating procedures** for laboratories working with these materials.

(See :<http://www.cdc.gov/od/ohs/biosfty/bmbl4/bmbl4s3.htm> ).

## 7. EMERGENCY PROCEDURES

Every laboratory working with a pathogen, irrespective of its biosafety level or risk group designation should prepare an emergency procedure to follow in the event of a spill or exposure. The MSSM Institutional Biosafety Program web-site has a Spill Guide that can be used for developing your procedures. Even if the procedure is no more than holding your breath and exiting the area, everyone should know this and be trained in this response while working in that lab. Phone numbers for the MSSM safety officers, the Emergency Department, Paramedics should be handy by the phone. For BSL-3 or Risk Group 3 Agents, a Standard Operating Procedure manual must be prepared and everyone trained in the practices and procedures contained in that plan. All procedures have to be included, from when the agent or specimen arrives, through how it is manipulated, incubated, cultured, stored and finally destroyed and disposed of.

If you have had a spill or exposure, wash the site of the exposure with soap and water for fifteen minutes. Be sure to remove any contaminated articles of clothing. Notify the Biosafety Officer at once at 241-1451. Go to the Employee Health Service, or the Emergency Department after-hours and weekends.

If you are stuck by a needle, or cut with contaminated glass, follow the needlestick procedure and call the Page Nurse at 4118, and inform the nurse that you have had a needlestick injury. Notify the Biosafety Officer after being treated. Use the form on the IBP website to report your injury in compliance with OSHA regulations.

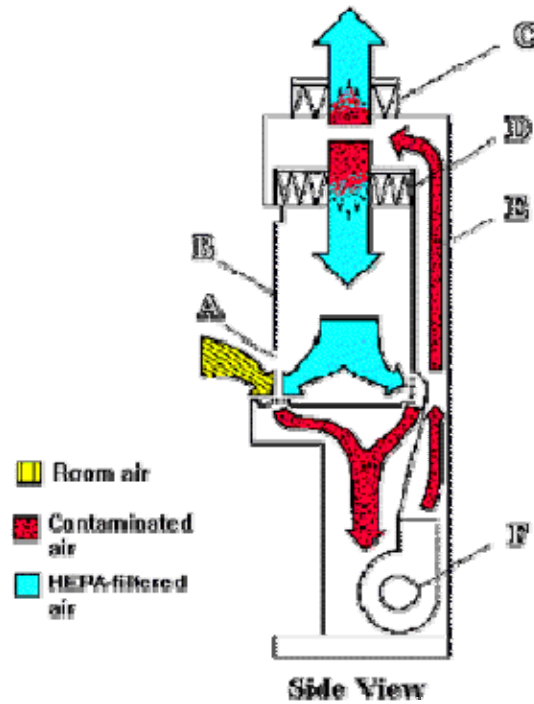
GoTo: <http://www.mssm.edu/biosafety/forms/BSO-1.doc>

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Figure 3. The Class II, Type A BSC.



- A. front opening
- B. sash
- C. exhaust HEPA filter
- D. rear plenum
- E. supply HEPA filter
- F. blower

Source: CDC / *Primary Containment for Biohazards: Selection, Installation and Use of Biological Safety Cabinets; 2<sup>nd</sup> Edition*