Roosevelt Biosafety Training

Created 10/2015

Objectives

* Identify risks and hazards in biological laboratories
* Understand biosafety levels for laboratories and the proper procedures for working in them
* Learn ways to reduce risks and hazards by understanding the use of aseptic technique and proper use of biological safety cabinets
* Learn proper procedures for cleaning biohazardous spills
* Learn proper disposal procedures for biohazardous waste in biological laboratories

Identifying Risks

* Risk assessment:
  * What are the biological and physical hazards of the organism/agent?
  * What procedures may spread the organism/agent?
  * What is the best method for inactivating and containing the organism/agent?
  * What is the pathogenicity of the organism/agent?
  * What are the potential deficiencies in practices of lab workers?

Identifying Risks

* Risk assessment:
  * Identify agent hazards
    * CDC and WHO have guidelines
    * Biosafety level
  * Identify laboratory procedure hazards
    * Aerosols
    * Volume and concentration
    * Use of sharps or animals
    * Complexity of experiment
  * Determine appropriate biosafety level and additional precautions
  * Evaluate staff and equipment for safe practices
  * Review risk assessment with biosafety professionals

Identifying Risks

* Risk Groups
  * Organisms are categorized by:
    * Potential effect of the agent on a healthy human adult*
    * Ease and route of transmission
    * Infective dose
    * Stability in environment
  * Knowing the risk group of the organism can determine the procedures necessary for reducing risk
  * May differ between strains of the same organism, depending on pathogenicity of the different strains
  * NIH and WHO definitions differ slightly
  * Do not equal biosafety levels (though they are correlated)

Risk Groups

* Risk Group 1
  * Agents not associated with disease in humans or animals
* Risk Group 2
  * Agents associated with human or animal disease, but are unlikely to cause serious hazard to lab workers
  * If exposure happens, preventive or therapeutic interventions are usually available
* Risk Group 3
  * Agents associated with serious or lethal human or animal disease
  * Treatments may exist, and the agent isn't easily spread
  * Use is discouraged at Roosevelt University
* Risk Group 4
  * Agents associated with serious or lethal human or animal disease that can be easily passed between individuals
  * Treatments are not usually available
  * Use is prohibited at Roosevelt University

Identifying Risks

* Routes of laboratory transmission
  * Inoculation from contaminated sharps
  * Spills and splashes onto skin and mucous membranes
  * Ingestion (mouth pipetting)
  * Animal bites and scratches
  * Inhalation of infectious aerosols
    * Considered to be a serious hazard and likely culprit of most laboratory exposures

Reducing Risks: Aerosols

* Procedures that produce aerosols:
  * Pipetting
  * Blending
  * Centrifugation
  * Sonicators and vortex mixers
* Vigilant workers can reduce the amount of aerosols by being cautious with above procedures
* Use biological safety cabinet when these procedures must be performed

Reducing Risks: PPE

* Minimum Personal Protective Equipment:
  * Safety glasses
  * Gloves
  * Lab coat
* Other equipment that may be necessary based on risk assessment:
  * Mask or face shield
  * Respirator
  * Gown

Reducing Risk: Equipment

* Biological Safety Cabinets (BSC)
  * Produce a sterile field to protect both the worker and the culture
* Centrifuge safety cups
* Sealed rotors
* Maintenance of equipment is essential

Reducing Risk: Facility

* Engineering controls to prevent release of hazards
  * Directional airflow in the lab
  * Substitution of lower risk agent for higher risk agent if possible
* Limited access to building or labs
* Training

Biosafety Level 1

* Well characterized strains of agents not known to cause disease in healthy adults
* Basic containment with sink for hand washing
* Laboratory doors kept closed during experiments
* Decontaminate work surfaces daily and after spills
* All waste decontaminated before disposal
* Mouth pipetting prohibited
* Eating, drinking, smoking, and applying cosmetics are not permitted in lab
* Wash hands after handling materials and when leaving the lab
* PPE use recommended (required at Roosevelt)
* Spills reported to lab manager to ensure proper documentation and clean-up

Biosafety Level 2

* All as in BSL-1
* Moderate-risk agents
* Can be used on the open bench if risk of aerosols and splashes is low, higher risk organisms must be manipulated in BSC
* PPE required, cannot be worn in non-laboratory areas
* Access to sink for hand washing and decontamination
* Access to laboratory is limited or restricted
  * Persons with increased risk of acquiring infection or for whom infection is unusually hazardous should not be allowed into labs
  * Only individuals that meet entry requirements (e.g. immunizations) may enter
* Workers advised of potential hazards and given proper training in handling agents
* Biohazard signs clearly posted on doors and equipment
* Sharps use minimized

Biosafety Level 3

* All as in BSL-1 and -2
* Agents with a potential for respiratory transmission with serious or lethal infections
* Work is discouraged at Roosevelt
* Lab personnel must have specific training in handling agents and are supervised while conducting experiments
* All work done in BSC or other enclosed equipment
* Controlled access to lab and special ventilation to prevent accidental release
* Lab must have specific design and containment equipment
  * Air lock, shower, or changing room required between unrestricted areas and lab
  * Surfaces of walls, floors, and ceilings must be water resistant
  * Windows are closed and sealed
  * Exhaust system provided to prevent release of agents
* PPE required and may include respirator, double gloves, gowns, etc. Lab coats are not suitable
* Workers must comply with entry and exit procedures
* Vacuum lines protected with HEPA filters and liquid traps

Biosafety Levels 4

* All as in BSL-1, -2, and -3
* Work is *prohibited* at Roosevelt
* Agents with high risk of lethal disease, easily transmitted (aerosol), with little or no treatment options
* Class III BSC or full-body, air-supplied positive pressure suit required for working
* Controlled access to labs, specialized ventilation, and waste management systems

Biosafety Cabinets

* Use when procedures are likely to produce aerosols or when high concentrations or large volumes of agent are being used
* Different models provide protection in different ways; must be chosen according to needs of the lab and agents used
* Lab workers must be trained in proper use of BSC

Biosafety Cabinets

* For proper use of BSC, watch following video:

  http://www.youtube.com/watch?v=ZnUW1N-JJz8

* Lab workers may require in-person training depending on procedures, experience, and agents being used
* Instructors MUST train their students (students cannot train other students)
* In-person training will be provided by instructor or laboratory manager

Aseptic Technique

* Method of laboratory work that prevents contamination by (unwanted) microorganisms
* Provides barrier between sterile cell cultures and microorganisms in the environment
* Varies depending on whether working on the bench or in a BSC

Aseptic Technique

* For proper bench-top aseptic technique, watch the following video:

  http://www.youtube.com/watch?v=bRadiLXkqoU

* Lab workers may require in-person training depending on procedures, experience, and agents being used
* Instructors MUST train their students (students cannot train other students)
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Biohazardous Spills

* Basic biological spill kit should contain:
  * Disinfectant (e.g. bleach 1:10 dilution, diluted quaternary solution, or other suitable disinfectant)
  * Absorbent material (paper towels, spill pillows, etc.)
  * Waste container (biohazard bags and sharps containers)
  * PPE
  * Mechanical tools (forceps, dustpan and broom)
* Procedure can depend on agent spilled and where

Biohazardous Spills

* BSL-1 spills:
  * Notify others
  * Wear PPE
  * Surround spill with disinfectant
  * Clean up with paper towels (if large, use spill pillows)
  * Re-apply disinfectant to the surface and let sit for 10 minutes. Clean again.
  * Put contaminated waste in biohazard bags for autoclaving
  * Wash hands
  * Notify lab manager to assure proper cleanup

Biohazardous Spills

* BSL-2 spills:
  * Evacuate the room and close doors; notify lab manager
  * Remove any contaminated clothing and decontaminate body surfaces
  * Allow at least 30 minutes for potential aerosols to be reduced before re-entering
  * Don protective clothing and respiratory protective equipment
  * Decontaminate spill with appropriate disinfectant and allow 10 minutes of contact time
  * Clean spill with paper towels or spill pillows and dispose in biohazard bag
    * Pick up sharps with forceps or tweezers, never with hands, and dispose of in autoclavable sharps container
  * Reapply disinfectant and clean after 10 minutes.
  * Wash hands and/or shower after cleaning spill

Biohazardous Spills

* Biosafety Cabinet Spill
  * Keep cabinet running during the cleanup
  * Remove any contaminated PPE and replace with clean
  * Apply appropriate disinfectant to the spill (bleach can be used but should be used with caution; it will corrode the stainless steel)
  * Wipe up spill and dispose of paper towels in biohazard bag
  * Reapply disinfectant and clean again
  * If bleach is used, clean the surface of the cabinet with water to remove traces of bleach
  * Items that must be removed should also be decontaminated before unloading from cabinet
  * Run UV/germicidal lamp for at least 15 minutes for final decontamination (formaldehyde gas can also be used)

Waste Disposal

* Sharps
  * Items capable of puncturing, cutting, or abrading the skin (e.g. broken glass or plastic ware, scalpels, razor blades, needles, etc.)
  * Never place sharps in regular trash
  * Dispose of in puncture proof containers
  * Clean broken glass can go into broken glass containers
  * Any sharp contaminated with blood or other biohazard must be decontaminated (autoclave or bleach) and disposed of in an appropriate container
    * Leak proof, rigid, puncture-resistant
    * Tightly sealed
    * Labeled with biohazard symbol

Waste Disposal

* Biohazardous waste
  * Waste containing infectious or potentially infectious substances (e.g. blood, bacterial cultures, liquid waste from cell culture, etc.)
  * All waste must be disposed of in bags marked with biohazard symbol; bags can go into labeled, leak-proof containers to await autoclaving
  * Autoclaved before disposal in regular trash
  * Autoclave should be checked regularly for proper functioning (reaches temperature and pressure, etc.)

Obtaining Biohazardous Materials

* Lab Manager approval required for new organisms
* Check risk group and recommended biosafety level
* Determine if necessary or if a lower-risk organism can be used instead
* Submit for lab manager approval
  * Will check requirements to determine if Roosevelt has appropriate facilities
  * Currently no facilities for BSL-3
  * Fill out required paperwork in order to obtain organism
* Training with new organism must be conducted by lab manager or instructor

Resources and Sources

* Center for Disease Control's Biosafety site - www.cdc.gov/biosafety
* NIH Office of Science Policy for Biosafety - http://osp.od.nih.gov/office-biotechnology-activities/biosafety/nih-guidelines
* Roosevelt's CHP - https://www.roosevelt.edu/~/media/Files/pdfs/Policies/Safety/ChemicalHygienePlan.ashx