Laboratory Safety
Safe Handling of Biological Hazards
Department of Environmental Health and Safety

Bob Holthausen
Biosafety Officer
631-632-9672
robert.holthausen@stonybrook.edu

Responsibility for Safety?
What is a Biohazard?

Infectious agents or hazardous biological materials that present a risk or potential risk to the health of humans, animals or the environment.

Biohazardous materials include:
- organisms and viruses infectious to humans, animals or plants (e.g. parasites, viruses, bacteria, fungi, prions, rickettsia)
- cultured human and animal cells
- certain types of recombinant DNA
- biologically active agents that may cause disease in other living organisms or cause significant impact to the environment or community. (i.e. toxins, allergens, venoms)

Development of Biosafety Practices

- **1941 - Meyer and Eddie**
  - 74 lab associated brucellosis infections in U.S.
- **1949, 1951, 1965, 1976 - Sulkin and Pike**
  Surveys for lab-associated infections:
  - Fewer than 20% associated with known accidents
  - Exposure to infectious aerosols plausible (but unconfirmed) for >80% of reported cases
  - Most commonly reported:
    - Hepatitis
    - Brucellosis
    - Tuberculosis
    - Tularemia
    - Typhoid
    - Venezuelan Equine Encephalitis
**Biosafety in Microbiological and Biomedical Laboratories – CDC/NIH**

- **Biosafety**: The application of combinations of laboratory practice and procedure, laboratory facilities, and safety equipment when working with potentially infectious microorganisms.

- Designed to protect human health and prevent release of pathogens into the environment.


**Biosafety Levels – CDC/NIH**

- Four levels of controls appropriate for research with infectious agents with different levels of risk.

- Ranges from minimal containment (BSL 1) to maximum containment when working with agents with high risk of life threatening disease (BSL 4).
Agent Risk Groups

- **Risk Group 1** – agents are not associated with disease in healthy adult humans. *Example: E. coli K-12 strain*

- **Risk Group 2** – agents are associated with human disease which is rarely serious and for which preventive or therapeutic interventions are *often* available. *Example: Hepatitis B virus*

Agent Risk Groups

- **Risk Group 3** – agents are associated with serious or lethal human disease for which preventive or therapeutic interventions *may be* available. *Example: Mycobacterium tuberculosis*

- **Risk Group 4** – agents are likely to cause serious or lethal human disease for which preventive or therapeutic interventions are *not usually* available. *Example: Ebola virus*
Risk Assessment

- Same agent can have different containment levels at different stages of protocol:
  - Procedures that produce aerosols have higher risk
  - Procedures using needles/other sharps have higher risk
  - Purified cultures/cell concentrates may have higher risk
  - Handling blood, serum or tissue samples may have a lower or higher risk
  - Larger volumes (10 L) have higher risk

Know Your Agents

- For each agent that you use or have a potential for exposure, know:
  - Pathogenicity
  - Host range
  - Infectious dose
  - Mode(s) of transmission
  - Effective disinfectant / physical inactivation
  - Recommended containment (BSL)
  - PPE
Where to find information

- Health Canada MSDS for Infectious Agents
- ABSA Risk Group Listing
  [http://absa.org/resriskgroup.html](http://absa.org/resriskgroup.html)
- CDC
  [http://www.cdc.gov](http://www.cdc.gov)
- ATCC Web Site
  [http://www.atcc.org/Home.cfm](http://www.atcc.org/Home.cfm)
Biosafety Level 1

- BSL 1 – represents a basic level of containment that relies on standard microbiological practices with no special primary or secondary barriers recommended, other than a sink for hand washing.
  - Examples: *Bacillus subtilis*, *Naegleria gruberi*, *E. coli*, Infectious canine hepatitis virus
Standard Microbiological Practices

- **Limiting Access** – Access to the lab is limited or restricted when work with cultures and specimens are in progress.
- **Hand washing** – Persons wash their hands after they handle viable materials, after removing gloves, and before leaving the laboratory.
- **No Eating, Drinking or Smoking** – as well as handling contact lenses, applying cosmetics, food storage. Those wearing contact lenses should wear goggles or a face shield.
- **No Mouth Pipetting** – mechanical pipetting devices are used for all pipet work.
- **Minimization of Aerosols & Splashing** – all procedures are carried out carefully to minimize the creation of aerosols or splashes.

Standard Microbiological Practices (Continued)

- **Decontamination of Work Surfaces** – all work surfaces are decontaminated at least once a day and after any spill of viable material.
- **Sharps** – policies for the safe handling of sharps are instituted.
- **Wastes** – all cultures, stocks, and other regulated wastes are decontaminated before disposal by an approved decontamination method such as autoclaving.
- **Biohazard Sign** – is posted whenever infectious agents are present.
BSL 1 – Safety Equipment

- **Lab coats** — recommended to prevent contamination or soiling of street clothes.
- **Gloves** — are worn if the skin on the hands is broken or if a rash is present.
- **Protective Eyewear** — is worn when conducting procedures in which splashes of microorganisms or other hazardous materials can take place.

BSL 1 Lab Facilities

- **Door to control access.**
- **Sink for hand washing.**
- **Lab design for easy cleaning. (No rugs.)**
- **Bench tops are impervious to water, resist heat and chemicals.**
- **When opening windows are present, they are fitted with fly screens.**
Biosafety Level 2

- BSL 2 is suitable for work involving agents of moderate potential hazard to personnel and the environment. It differs from BSL1 in that:
  - Lab personnel have specific training in handling pathogenic agents.
  - Access to the laboratory is limited when work is being conducted.
  - Extreme precautions are taken with contaminated sharp items.
  - Procedures in which infectious aerosols may be created are conducted in a biological safety cabinet or other physical containment.

Generation of Aerosols in BSL 2 work

- Procedures that may generate aerosols include:
  - Pipetting
  - Centrifugation
  - Tissue culture
  - Sonication
  - Blending of tissues
  - Inoculation of animals intranasally
Biosafety Level 2

- All BSL1 practices plus:
  - Restricted access when work is in progress. At risk persons not allowed in lab.
  - Policies and procedures on communication of hazards and specific entry requirements (e.g. immunizations)
  - Posted Biohazard sign with PI, agents, and contacts.
  - Immunizations are provided as appropriate. (e.g. TB)
  - Baseline serum sampling, when appropriate.
  - Biosafety procedures are included in SOPs.

Biosafety Level 2

- All BSL1 practices plus: (Continued)
  - Training is provided on hazards, precautions, and exposure evaluation procedures. Annual updates.
  - Increased sharp awareness and precautions. Only used when there is no alternative. Plastics used in place of glass whenever possible.
  - Needles, sharps, broken glass are decontaminated before disposal.
  - Cultures, tissues, & OPIM in sealed containers to prevent leakage when transported.
  - Decontamination of equipment and surfaces with *effective disinfectant*. 
Biosafety Level 2

- All BSL1 practices plus: (Continued)
  - Decon all equipment prior to repair or maintenance.
  - Proper packaging & training for transport of all infectious materials per government regulations.
  - Spills and accidents that result in exposures are reported. Medical evaluation, surveillance, and treatment are provided. Written records maintained.
  - No animals not involved in the work allowed in the lab.

BSL 2 Safety Equipment

- All BSL1 equipment plus:
  - BSC, preferably Class II, or other physical containment when:
    - Creation of infectious aerosols or splashes.
    - Opening pressurized containers with pathogens.
    - Harvesting infected tissues or embryonate eggs.
    - High concentrations or large volumes of infectious agents.
  - Face protection when handled outside BSC.
  - Lab coats, gowns, or smocks are used. Removed before leaving the lab. (Never taken home for laundering.)
  - Gloves worn when agent contact is possible.
BSL 2 Lab Facilities

- All BSL1 facilities plus:
  - Lockable doors to restrict agent access.
  - Labs located away from public areas.
  - BSCs installed away from doors, windows, heavy traffic areas.
  - Eyewash station readily available.
  - Adequate illumination / reduced glare.
  - Negative pressure is recommended.

OSHA Occupational Exposure to Bloodborne Diseases Standard

- All work involving the use of human blood, body fluids, tissue, cell lines, etc. is carried out using BSL 2 practices and procedures, because it is unknown if these materials contain bloodborne diseases such as HIV, HBV, HCV.
- Based on UNIVERSAL PRECAUTIONS!
- Bloodborne Pathogen Training Required!!!
Biosafety Level 3

- BSL 3 – is suitable for work involving indigenous or exotic agents which may cause serious or potentially lethal disease as a result of exposure by inhalation. It differs from BSL 2 in that:
  - All work takes place in a BSC or other physical containment.
  - The lab has special engineering and design features.
  - SOPs for all work which include special biosafety procedures to provide suitable containment for the hazards presented by the agents in use.

Biosafety Level 4

- BSL 4 – required for work with dangerous and exotic agents that pose a high risk of aerosol-transmitted laboratory infections and life-threatening disease.
- Not allowed at SBU.
- For details see BMBL.
Recombinant DNA Work

- All rDNA work at Stony Brook University **MUST** be reviewed by the Institutional Biosafety Committee (IBC) regardless of funding.
- Non-exempt work under NIH Guidelines must be approved by the IBC.
- IBC will set containment requirements.
- Exemption is based on IBC review!

Lab Registration Program

- Labs are required to register with EH&S by Nov. 30, 2008 or before initiating operations (new labs).
- Registration via web based portal.
- Provide lab contacts, chemical, biological, radiological, laser hazards and info on wastes generated.
- Information at > [http://www.stonybrook.edu/ehs/](http://www.stonybrook.edu/ehs/)
For all labs above
BSL1: Signs are required with necessary precautions / required PPE, emergency contact information, and nature of the hazard.

Color Code
BSL1 BSL2 BSL3 BSL4
Green Blue Red Magenta

Laminar Flow Cabinet (clean bench)

- For product protection only.
- This is not a Biosafety Cabinet – it does NOT protect the worker!
- Air flows from back of cabinet, across work surface, and onto user.
Biological Safety Cabinets

A. Class I
- Inward airflow protects worker
- Exhaust to outside (w/wo HEPA filter)

B. Class II
- Worker, product, environmental protection
- "Sterile" work area
- Use for work with aerosol-transmissible microorganisms and for tissue culture/virology

C. Class III
- Totally enclosed, ventilated, air-tight (glove box)
- Suitable for work with BSL3/4 agents

Biosafety Cabinet Use

- Ensure UV lights are off before starting work.
- Run BSC blowers for >5 min before work.
- Place only required materials in the BSC.
- Minimize in and out operations.
- Don't block front grill with arms, papers, materials.
- Work at least 4 inches inside the grill, work as far back as comfortable.
- Use horizontal waste trays for pipets, etc. to minimize in and out operations.
Biosafety Cabinet Use

- Remove potentially contaminated materials only after disinfection.
- Reduce splatter and aerosol generation.
- Use touch plate microburners. No open flames in BSC.
- Include overflow collection with disinfectants and HEPA filters in vacuum lines.
- Use plastic backed absorbent towels on work surface to handle spills. (Don’t block grill!)
- Close drain valves to contain spills. Pour in disinfectant if spills occur. Wait – drain.
- Disinfect BSC with 70% ethanol or bleach when work shift is completed.

Biosafety Cabinets

- BSC Certification
  - BSL 1 – recommended annually.
  - BSL 2 & 3 – Required annually.
  - And after moving of the BSC.
- More info on BSCs see “Primary Containment for Biohazards: Selection, Installation, and Use of Biological Safety Cabinets”
  - Available on CDC Website
Personal Protective Equipment

- **Examples of PPE:**
  - Safety Glasses
  - Lab Coats
  - Gloves
  - Chemical Splash Goggles
  - Face Shields
  - Sleeve Covers
  - Aprons
  - Respirators

Masks vs. Respirators

Masks are designed to protect others from contamination produced by the wearer.

Respirators are designed to protect the wearer from contaminants in the air.
Waste Disposal

- Do not put chemicals / agents down sink drains without EH&S approval.
- Separate different wastes, don’t mix unless you are sure that it is appropriate. (RMW vs. trash)
- Waste generators need hazardous waste training. (Available online at EH&S Website)
- NO Chemicals, RMW or sharps in trash!
- Autoclave or disinfect waste for safety or convenience.
- Autoclaved waste must go into RMW.
- When in doubt ASK!

Prevent Sharps Injuries

- Always put sharps in a sharps container
- Never recap needles by hand
  - Always use mechanical devices to recap
Keys to Autoclave Safety

- Ensure that autoclaving is appropriate for materials
- Don’t use an autoclave if you suspect there is a problem.
- Wear appropriate PPE – Eye and face protection, Lab coat, heat resistant gloves.

Emergency Procedures – Biohazard Exposure

- Sharps injuries – first aid:
  - Promote bleeding if present.
  - Wash area thoroughly with warm soapy water.
- Splash to face, eyes, mouth – first aid:
  - Rinse thoroughly with water.
- Report exposures to your supervisor / PI immediately.
- Seek medical attention based on nature of the agent.
Emergency Procedures – Biohazard Spills (BSL 1 & 2 Labs)

1. Alert people in immediate area of spill.
2. Put on protective clothing/PPE: closed lab coat, disposable gloves (double glove), disposable shoe covers, safety goggles, face shield, or respirator, as necessary.
3. Cover spill with paper towels or other absorbent materials.
4. Carefully pour a freshly prepared 10% dilution of bleach (or other appropriate disinfectant for the agent) around the edges of the spill and then into the center of the spill. Avoid splashing or creating aerosols when pouring the disinfectant.
5. Allow 30 minutes for the disinfectant to inactivate the material.
6. Use paper towels to wipe up the spill, working from the edges into the center.
7. Clean spill area with fresh towels soaked in disinfectant.
8. Place towels and disposable protective clothing in a plastic bag (red) and dispose of as regulated medical waste.

➢ Contact EH&S (Call 911) – if you do not have the proper materials or need assistance.

BSL 3 labs respond as per their site specific Biosafety Manual.

Emergency Procedures – Chemical Spills / First Aid

➢ Know where the nearest emergency shower and eyewash are located.
   • Call for help or assist the victim.
   • Splash to eyes – immediately flush with lots of water for at least 15 minutes.
   • Splash to body – remove contaminated clothing and flush with water for at least 15 minutes.
   • Seek medical help and report the incident to your supervisor.
Most important …. When in doubt…
ASK SOMEONE!