BIOLOGICAL SAFETY and Bloodborne Pathogens Training

Presented by EH&S

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Definition/Purpose

† Information, Equipment, and Operating Procedures to reduce exposure to infectious material.

† In Addition

  † Contamination prevention
  † Regulatory compliance
  † Regulated Medical Waste (red bags)
  † OSHA (Bloodborne Pathogens Standard)
  † NIH “Guidelines”
  † Exposure Control Plan
  † Biosafety Manual
Biological Safety Levels (BSL)

- BSL 4  Front page news
- BSL 3  Serious disease, airborne transmission – (TB, SARS)
- BSL 2  Varying severity, direct contact - (Staph., Hep B)
- BSL 1  Not known to cause disease in healthy adults

- Also refers to appropriate set of safe practices

The appropriate biosafety level must also be determined for the use of microorganisms containing recombinant DNA. See http://www.ehs.columbia.edu/recombdna.html for information concerning safety and registration requirements for recombinant DNA use.
‘Biological Materials’

- Microorganisms
- Animal tissues/cells
- Human blood, body fluids, cell lines
- Recombinant DNA
Safety Practices-Types of Controls

- Substitution
- Engineering Controls
- Work Practices
- Personal Protective Equipment
Substitution

- ‘Safe Needles’ mandatory OSHA requirement to use safest ‘sharp’ possible, engineered-in safety features
- Replace glass with plastic transfer pipette instead of glass Pasteur
Substitution

Replace glass with plastic transfer pipette instead of glass Pasteur
NEVER break, bend, or recap needles.
Engineering Controls
Reduce Aerosols

Use BSC’s for aerosol-creating activities:

- Sonication
- Injections
- Vortexing
- Pipet Carefully

COVER WORK SURFACES (but not grills)
1. Turn off UV lamp, if one is there.
2. Turn on fluorescent work light, inspect air intake grilles and remove any obstructions
3. Adjust sash to proper height
4. Turn on blower, and allow 5 minutes to purge cabinet air
5. Wash hands and don appropriate PPE, including gloves and lab coat or gown
6. Before starting work, wipe interior surfaces with 70% isopropyl or ethyl alcohol and let cabinet run for 5 minutes; repeat at end of procedure.
7. Place a plastic-backed absorbent pad on work surface (do not block intake grilles)
8. Put all items to be used in the experiment in the BSC, taking care to segregate clean / dirty areas
9. Work from “clean” to “dirty” areas, working at least 6” from front intake grille
10. Minimize arm movement into and out of BSC to preserve air curtain
Avoid the use of flames in the cabinet.

- Air circulation in the cabinet runs counter to the upward air current produced by an open flame, resulting in turbulence that can decrease the cabinet’s effectiveness.
- The heat from a Bunsen burner will affect the integrity of the HEPA filters, which are critical to the cabinet’s proper functioning.
- Dipping cover slips, cell spreaders and other items for which contamination-free status is required, into alcohol followed by flaming with a Bunsen burner may cause a fire when ignited alcohol drips onto flammable material.

**INSTEAD of USING an OPEN FLAME**

- Use disposable cell spreaders, which may be collected and re-sterilized by autoclaving for subsequent use.
- Rather than dipping and flaming cover slips, autoclave batches in a glass petri dish. If kept covered in a biological safety cabinet, the slips will remain sterile.
INSTEAD of USING an OPEN FLAME (cont’d)

- Flaming pipets, bottle and tube necks and inoculating loops needlessly places an ignition source in the work area.
- The environment inside the cabinet is microbiologically sterile.
- Use wrapped sterile pipets to eliminates need to flame.
- Single use inoculating loops are available and like cell spreaders may be collected and re-sterilized by autoclaving for subsequent use.
- Opening and closing of tubes and bottles inside the cabinet eliminates the need for flaming the necks of these containers.
Bottom line: It is possible to work contamination-free without a heat source in a BSC.

If you must use a flame or heat, based on safety or contamination control issues either purchase a model that comes with a low-flame pilot light (left) or a micro-incinerator (right) that provides a heat source without an open flame.
WARNING

Cabinet not certified for infectious materials or contamination control

For additional information, call CU EH&S 305-6780
Cryovial safety

- Vials with a male stopper and “O” ring are less hazardous.
- Do not over tighten the “O” ring.
- Keep the vials in the vapor phase in the dewar.
- Loosen the vial cap immediately upon removal from liquid nitrogen. Place vial in secondary containment.
- Wear eye protection and cryoprotective gloves; do not remove until vials reach room temp.
Work Practices

Decontamination

- Beginning/End of Procedures
- After Spill
- Alcohol: limitations
- Bleach: fresh preparation
- Other (e.g. quaternary ammonium): follow instructions
- Match the decontaminant with the bioagent
SPILL RESPONSE

- Alert people in immediate area
- Put on protective equipment and 2X gloves
- Cover with paper towels/other absorbent
- Carefully pour an appropriate decontaminant around edges of spill
- Allow 10-20 minutes contact time
- Use forceps or other mechanical means to place towels in red bag  DO NOT TOUCH DIRECTLY
- Re-clean area, if necessary
Occupational Exposure Response

- Wash area w/soap and water, 10 minutes.
- Rinse eyes/mucous membranes.
- CUMC: Obtain medical attention:
  - Student Health Services, Workforce Health and Safety
  - After hours, NYP ER
- Morningside: Obtain medical attention:
  - Student Health Services, John Jay, 4-2284
  - After hours, St. Luke’s Roosevelt ER
- Complete Accident Report Form
- Notify EH&S 854-8749 or 305-6780.
Occupational Exposure

- Baseline HIV, HBV, HCV
- Anti-retroviral treatment may be indicated
- HIV Testing: 6 & 12 weeks, 6 & 12 months.
- HBV Vaccination/IG may be indicated
- Anti-HCV titer; assess liver enzymes

- Recognize constitutional symptoms
  - San Francisco VA lab meningitis fatality (*Neisseria meningitidis*)
Biological Safety Program

Regulatory Items

Bloodborne Pathogens

- Provide of Hepatitis B vaccine-(free for employees)
- Medical Surveillance/Accident Response & Testing
- Annual Training
- ‘Universal Precautions’ for the laboratory
- INCLUDES ALL HUMAN CELL LINES
Occupational Exposure

Accident Reporting

- Required for compensation benefits.
- Complete ASAP after emergency medical issues addressed.
- Valuable investigative tool.

http://www.hr.columbia.edu/hr/forms/workers-comp/pdf-ver.pdf
Bloodborne Pathogens (HBV)

- Jaundice, abdominal pain, fatigue, anorexia, vomiting OR cold/flu-like OR may be unapparent
- Percutaneous (sharps), mucus membrane, non-intact skin exposure
- Community risk factors: illicit drugs, needle sharing, mother>>baby
- Chronic infection develops ~5% of the time, increasing risk for cirrhosis, liver cancer; can transmit to others, even w/o symptoms
- Environmental persistence on surfaces
Bloodborne Pathogens
HBV Vaccination

- Free to all employees with occupational exposure
- Declination form
- 3 IM injections over 6 months; check titer 1-2 months later
- 90-95% of recipients develop titer
- Few side effects; yeast allergies to be considered
Bloodborne Pathogens (HCV)

- Symptoms and transmission similar to HBV
- Much higher prevalence (3.2 million), 15,000 deaths in 2007
- Untreated, chronic infection ~85% of the time with the same risks as HBV
- SOC (ribavirin + IFN) ~50% cure
- #1 reason for transplants
- No vaccine
Routes of Exposure

- Injection
- Ingestion
- Ocular via Splash
- Dermal
- Inhalation
Biological Safety Program

Routes of Exposure for Blood/Body Fluid

- **Percutaneous**
  - Hollow-bore needles
  - Scalpel
  - Suture needle
  - Broken vial
  - 10,378 (82%)
- **Non-intact skin**
  - 352 (3%)
- **Bite**
  - 131 (1%)
- **Mucous membrane**
  - 1817 (14%)
  - Splashes to eyes, nose or mouth

**CDC estimates that approximately 1,000 percutaneous injuries a day are sustained by healthcare personnel in US hospitals.**
Bloodborne Pathogens
Transmission Probabilities

Stick contaminated from blood + for:

- HBV: Up to 30% if unimmunized
- HCV: 2%
- HIV: 0.3%, 0.1% splash
- Dependent on viral titer and nature of injury
Regulated Medical Waste Disposal (Morningside)

- Picked up Tuesdays and Fridays; replaced with equivalent number of clean empties. Put out waste “morning of”, not the night before.
- Sharps containers for ALL pipets and tips; don’t overfill
- Red bags: items that will not puncture bag
Regulated Medical Waste
(Medical Center)

- Reusable Sharps containers: needles, syringes, blades, ALL pipets & tips. Don’t overfill
- Red bags: items that will not rip bag (gloves, plastic tubes & flasks)
Regulated Medical Waste Disposal (Medical Center)
What Is Not Regulated Medical Waste

- For non-hazardous containers refer to campus bottle disposal guide. MS – yellow bins. CUMC - dispose as ‘regular’ trash using cardboard or other sturdy container for glass.
Post hazard signs on rooms and equipment used for infectious materials:

- Tissue culture rooms
- Biosafety cabinets
- Incubators
- Freezers
Choose the Correct Glove

Laboratory chemicals may compromise protection

- Wash hands upon removal
- Change ASAP if contaminated
- Vinyl, nitrile to avoid latex sensitization

Remove when leaving lab
Recombinant DNA registration

- Not “Guidelines”, but actual requirements. Failure to adhere to the NIH Guidelines can result in suspension or termination of NIH funding to the Institution.
- Replication deficient ≠ exempt.
- The NIH Guidelines provide assistance in selecting the appropriate biological safety level for microorganisms contain rDNA.
- PI must designate the proposed biosafety level in rDNA application.
- Conduct all activities, including spill and incident response in accordance with the specifics of the particular biosafety level.
Recombinant DNA registration

- All rDNA work must be described in submittal to CU’s Institutional Biosafety Committee (IBC).
- RASCAL>>Hazardous Materials>>Appendix A
- IACUC related: attach to proposal.
- In vitro: select appropriate box on form.
- Human Gene Transfer: contact EH&S.
- See: [http://www.ehs.columbia.edu/recombdna.html](http://www.ehs.columbia.edu/recombdna.html)
- Replication deficient ≠ exempt.
- Report to IBC immediately:
  - any significant problems pertaining to the operation and implementation of containment practices and procedures
  - violations of the NIH Guidelines
  - any significant research-related accidents
Regulatory Items

Shipping Biological Materials

- National (DOT) and International (IATA) Regulations
- Regulated Items:
  - High and low risk pathogens
  - Genetically modified microorganisms (GMO’s)
  - Clinical material
  - Dry Ice
- Training on RASCAL
- Between campuses-not regulated as long as transported by foot, but use triple performance packaging

http://ehs.columbia.edu/ShippingBiological.html
Bioterrorism-Select Agents

- Administered by CDC or USDA
- ~25 Pathogens, 12 toxins
- Registration, security clearance, training, emergency drills
- Quantity limit for toxins

1,000 mg: T-2, diacetoxyxscirpenol
100 mg: abrin, conotoxin, ricin, saxitoxin, shiga-like ribosome inactivating proteins, tetrodotoxin, C. pefringens epsilon toxin, shigatoxin
5 mg: Staphylococcal enterotoxins
0.5 mg: botulinum neurotoxins
QUESTIONS ??