Biological Safety Training

October 1, 2014
I. What is a Biohazard
II. Who Should Receive Biosafety Training
III. History and Development of Biosafety Practices
IV. Institutional Biosafety Committee (IBC)
V. NIH Guidelines and rDNA
VI. Biological Safety Levels (BSLs)
VII. Risk Assessment (RA)
VIII. General Laboratory Biosafety
IX. Bloodborne Pathogens
X. Biosurety
I. What is a Biohazard
• An agent of biological origin that has the capacity to produce deleterious effects in humans, such as microorganisms, toxins and allergens derived from those microorganisms, and allergens and toxins derived from higher plants and animals.

• Examples
II. Who Should Receive Biosafety Training
General biosafety training is required for all personnel who work with potentially viable biological materials, including (but not limited to):

- Microbes
- Cells
- Tissue Cultures
- rDNA
- Animals
- Animal Tissue or Fluid
- Face-to-Face Contact
III. History and Development of Biosafety Practices
Prior to 1940s, no data was available on frequency or source of Laboratory Acquired Infections (LAIs)

From ‘49 to ‘51, Drs. Sulkin and Pike surveyed hundreds of hospitals, health clinics, medical schools and research facilities.

They found 4,079 LAIs, associated with 168 fatalities from:

- Brucellosis
- Q-Fever
- Hepatitis
- Typhoid Fever

- Tularemia
- Tuberculosis
- Dermatomycosis
- Venezuelan Equine Encephalitis
Follow-up retrospective studies conducted from ‘79 to ‘01 and from ‘98 to ‘02 reveal *common routes and mechanisms* of LAIs.

**Exposure route:**
- Percutaneous 135
- Aerosol 125
- Mucosal 6
- Cutaneous 5

**Mechanism of percutaneous exposures**
- Sharps edges 66
- Sharps 42
- Animal bites/scratches 22

*Biosafety* is the consistent application of safety measures to minimize or prevent exposure to the person handling the agent, the lab and building occupants, the community and the environment.
IV. Institutional Biosafety Committee (IBC)
The IBC works integrally with the Office of Research Services Grant and Contract Review section, the Institutional Review Board (IRB), the Institutional Animal Care and Use Committee (IACUC) and EH&S.

Protocol approval by the IACUC and the IRB are contingent upon IBC approval.

The LSUHSC-NO IBC, in coordination with Environmental Health & Safety (EH&S) and the Biological Safety Officer, (BSO) is tasked with oversight and review of all research conducted at LSUHSC-NO for biological safety issues.
Prior to Initiation of Research

- Principal Investigators (PIs) must submit all protocols to the Institutional Biosafety Committee

- Fill out an **IBC Submittal Form**
  - Determine Biological Safety Level (BSL) and containment levels
  - Establish practices and techniques
  - Determine status under NIH Guidelines **if using rDNA**
  - Update biological and chemical inventories
  - Verify personnel completion of this training and BBP training
  - Provide and document **lab-specific training** to staff
  - Maintain a **lab-specific biosafety manual** (if BSL2 or above)
V. NIH Guidelines and rDNA
• The IBC additionally reviews, approves and oversees research using rDNA to ensure compliance with the *NIH Guidelines*.

• The Committee is registered with - and accountable to - the NIH Office of Biotechnology Activities (OBA)

• The following slides outline the responsibilities each PI has to the IBC prior to the initiation of any research protocol.
During Conduct of Research

- PIs must:
  - Supervise the safety performance of the lab
  - Investigate and report any significant accident, incident or problem immediately. See Incident and Accident Reporting page.
    - Reporting incidents involving potential exposure to rDNA is critically important
    - Depending on the type of incident and the level of exposure, notification of the NIH may be required within 24 hours of the incident
    - BSO will make determination of necessity of reporting beyond LSUHSC
  
If you’re not sure, report it!

- Comply with all institutional policies

- Submit an annual IBC Update form
• Recombinant molecules are any molecules constructed outside of living cells by joining natural or synthetic DNA segments to DNA molecules that can replicate inside a living cell.

• Research with rDNA is overseen by the National Institutes of Health (NIH) Office of Biotechnology Activities (OBA).

• Compliance with OBA Guidelines applies to all rDNA projects and is a condition of NIH funding campus-wide.
Experiments involving rDNA call for the application of highly specific biological barriers.

Barriers should limit (i) the infectivity of a vector or vehicle – plasmid or virus – for specific hosts, (ii) its dissemination and survival in the environment.

Vectors can be genetically designed to decrease the probability of dissemination of rDNA outside of the lab.

rDNA should be a part of the comprehensive risk assessment for your lab, taking into account source, vector, polypeptide product, etc. and should complement consideration of microbial risk.
• All rDNA is biohazardous
• Handling, manipulation and disposal should be done in accordance with EHS Policy 300.2, 400.6 and SOPs for Routine Decontamination, Sharps Handling and Disposal, and Laboratory Waste
• Any incident involving rDNA must be reported immediately to the BSO at EH&S
• If EH&S is unavailable, notify University Police
VI. Biosafety Levels (BSLs)
• BSL determination should be informed by risk assessment

<table>
<thead>
<tr>
<th>A/BSL-1</th>
<th>A/BSL-2</th>
<th>A/BSL-3</th>
<th>A/BSL-4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low individual risk (non-infectious to healthy adults)</td>
<td>Moderate individual risk (Not generally severe, treatment usually available)</td>
<td>High individual risk (Treatment may or may not be available)</td>
<td>Severe individual risk (Treatment often not available)</td>
</tr>
<tr>
<td>Low risk to community</td>
<td>Low risk to community</td>
<td>Low risk to community</td>
<td>High risk to community</td>
</tr>
</tbody>
</table>

Examples

- **E. Coli** lab strains (e.g., DH5α, K12)
- Mice
- Rats
- Rabbits
- Human cells, fluids, tissues
- NHP cells, fluids, tissues
- Lentiviral vectors
- Rhesus Macaques
- Toxins with an LD50 >100 ng/mg
- Animals infected with some BSL2 agents
- *M. tuberculosis*
- West Nile virus
- *Francisella tularensis*
- Yellow fever virus
- Monkeypox virus
- Animals infected with BSL3 agents
- Ebola virus
- Lassa virus
- Marburg virus
- Animals infected with BSL4 agents
• PPE recommendation and guidance are available for each BSL

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<tr>
<td>Lab coats, gowns, etc.</td>
<td>Lab coats, gowns, etc.</td>
<td>Lab coats, gowns, etc.</td>
<td>All PPE indicated up</td>
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<tr>
<td>Eye protection</td>
<td>Eye protection</td>
<td>Eye protection</td>
<td>to and including</td>
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<td>Latex or nitrile gloves</td>
<td>Latex or nitrile gloves</td>
<td>Latex or nitrile gloves</td>
<td>BSL-3</td>
</tr>
<tr>
<td>• Change when</td>
<td>• Change when</td>
<td>• All manipulations</td>
<td></td>
</tr>
<tr>
<td>contaminated</td>
<td>contaminated</td>
<td>performed inside a</td>
<td></td>
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<tr>
<td>• Double glove when</td>
<td>• Double glove when</td>
<td>BSC</td>
<td></td>
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<tr>
<td>necessary</td>
<td>necessary</td>
<td>Full protective</td>
<td></td>
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<tr>
<td>• Remove gloves and</td>
<td>• Remove gloves and</td>
<td>clothing that must not</td>
<td></td>
</tr>
<tr>
<td>wash hands after</td>
<td>wash hands after</td>
<td>leave the lab</td>
<td></td>
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<tr>
<td>working</td>
<td>working</td>
<td>Eye protection</td>
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<tr>
<td>• Do not re-use gloves</td>
<td>• Do not re-use gloves</td>
<td>Latex or nitrile gloves</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>• BSL-3 work practices</td>
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<td></td>
<td></td>
<td>Appropriate</td>
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<tr>
<td></td>
<td></td>
<td>respiratory protection</td>
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</table>

• A risk assessment pertinent to your lab and your work should yield PPE and work practice directives specific to your workplace
• Laboratory has doors to limit traffic
• Hand washing sink is available
• Work surfaces are easy to disinfect
BSL-1 Work Practices

- Limit access when working
- No eating, drinking, applying cosmetics or handling contact lenses
- No mouth pipetting
- Gloves must be worn and lab coats and protective eyewear are recommended
- Minimize splashes and creation of aerosols
- Disinfect waste and work surfaces
- Biological waste should be placed in a biohazard disposal box, labeled, and placed outside for pickup when ¾ full
All BSL-1 requirements, plus:

- Autoclave is available
- Eyewash is present
- Signage is posted
- Biological waste stream is separate
All BSL-1 practices, plus:

- A supervisor must limit access to those who are trained and approved
- Policy for handling sharps must be implemented
- Laboratory equipment must be routinely decontaminated
- Protective lab coats or disposable gowns must be worn
- Laboratory-specific Biosafety manual must be available in the lab
All BSL-2 requirements, plus:

- Lab is separated from general traffic
- Negative air flow must be maintained
- Autoclave inside laboratory
- Enclosures for aerosol-generating equipment
- Sealed room penetrations
- Hands-free sink
- Enter and exit through ante-room
All BSL-2 practices, plus:

- All infectious materials must be placed in durable, leak-proof containers
- All surfaces and equipment should be regularly disinfected
- All work with infectious agents must be done inside the BSC
- Users must complete training and demonstrate proficiency with laboratory manager before being granted access
- It is recommended that personnel work in pairs
• At all biosafety levels, personnel must be apprised of potential hazards upon assignment of a task, or when a procedural change occurs

• All staff must be trained for the tasks to which they are assigned and demonstrate proficiency

• Training must be refreshed annually and documented

• Personnel should be given information regarding how personal health status can affect susceptibility
VII. Risk Assessment (RA)
• Work practices, use of containment equipment, PPE, training, etc. should be guided by a thorough risk assessment (RA)

• A risk assessment ensures protection of personnel, the environment, the community and the integrity of your experiments

• Your RA should be included in your lab-specific Biosafety Manual

• Risk assessment guidance can be found in the CDC *BMBL 5th Ed.* or at the American Biological Safety Association (ABSA) website
RAs are comprised of four steps:

1. Identification of health hazard
2. Quantification of the hazard
3. Exposure assessment
4. Determination of probability of disease

And should include consideration of:

- Virulence
- Pathogenicity
- Infectious dose
- Environmental stability
- Route of spread
- Communicability
- Operations and manipulation
- Quantity and availability of vaccine or treatment
VIII. General Laboratory Biosafety
Hand Washing

- All laboratories are required to have a sink available for hand washing
- Wash hands for 15 seconds using warm water and mild – preferably liquid – soap
- Rinse with warm running water
- Dry with disposable paper towel
Hand Washing

- Alcohol-based hand sanitizers are an alternative to hand washing
- Sanitizers are effective against common clinical microbes, but have not been tested against laboratory pathogens
- Hand washing is preferred
Gloves

- Latex or nitrile gloves should be used for all handling of biological materials
- Double gloves may be needed in some circumstances in order to avoid exposure or contamination
- The type of gloves necessary and the frequency of changing is specific to your work and should be indicated in lab-specific training
- Gloves must never be worn outside of the work area
Lab Coats and Gowns

- Lab coats or disposable gowns should be worn over street clothes any time you handle biological materials.
- The type of covering necessary and the frequency of changing is specific to your work and should be indicated in lab-specific training.
- Lab coats and gowns must never be worn outside of the work area.
Eye and Face Protection

• Wear protective eyewear when conducting procedures that have the potential to create splashes of microorganisms or other hazardous materials.
• People who wear contact lenses should also wear eye protection.
• At BSL2 and above, eye and face protection must be used for anticipated splashes and sprays of infectious materials when the microorganism is handled outside of a biosafety cabinet or other containment device.
Respiratory Protection

- Whether and what type of respiratory protection you need should be indicated in your lab-specific training and should be guided by your lab’s risk assessment
- Surgical masks do not provide respiratory protection
- If you need an N95, full- or half-face N100, PAPR, or any other kind of fitted respirator, please contact EH&S for a fit test
- Fit testing requires a medical evaluation
- See EH&S 200.08, Respiratory Protection Program for more information
Sharps precautions

- Sharps are any instrument that can puncture, cut or scrape.
- Use EXTREME caution when working with sharps.
- Whenever possible, alternatives to sharps – such as plasticware – should be used.
- When sharps are necessary, safety sharps should be selected whenever they are available.
• Examples of Safety Sharps
• Contact EH&S if you have questions about sharps alternatives
Sharps precautions

- Broken glassware must never be picked up by hand
- Pick up broken glass mechanically, using forceps, a brush and dustpan, tongs, etc
Sharps Disposal

- Used disposable needles must not be bent, altered, broken, recapped, removed from disposable syringes, or otherwise modified
- Always dispose of contaminated sharps in an approved, puncture-resistant sharps container
- Dispose of container when it is ¾ full by sealing the container and placing in a biological waste box
- An SOP for the safe use and disposal of sharps is available at the Biological Safety page of the EH&S website
Sharps Disposal

- Uncontaminated or decontaminated glass may be disposed of in a designated, labeled cardboard box
- Box should be sturdy and in good condition
- Take care not to overload the box – it should be kept to a reasonable weight, approximately 25 lbs.
Biological Waste Disposal

- To be used for all items contaminated with human or animal blood, fluid or tissue
- Also stocks, cultures or waste from infectious materials or microorganisms
- All materials that may be contaminated with recombinant molecules
- **Do not** place sharps in the biological waste box.
  - **Sealed sharps containers only** may be placed in the biological waste box
- When box is ¾ full or reaches 25 lbs.
  close and tie liner, securely close lid, label with PI name and room number and place in hallway for pickup
Disinfection and Decontamination

- *Disinfection* is the process of reducing a contaminant load
- Can be accomplished in the laboratory using a 70% solution of ethanol (EtOH) or a 10% solution of bleach (sodium hypochlorite)
- All works surfaces and materials should be disinfected before and after use
- SOPs for routine decontamination are available at the Biological Safety page of the EH&S website
Disinfection and Decontamination

- **Decontamination** is the process of removing biohazardous agents
- Can be accomplished by physical or chemical means
- Is typically done using an autoclave, utilizing high temperature and pressure
- Aqueous solutions such as blood, urine, or microbial cultures *must* be autoclaved prior to disposal
- An SOP for the safe use of autoclaves is available at the Biological Safety page of the EH&S website
Autoclave Decontamination

- Place items in a secondary container made of stainless steel or autoclavable plastic.
- Most pathogens and recombinant molecules are sensitive to temperatures above 121°C for after 20 or more minutes.
- Larger loads require more time and should be arranged in a way that allows for steam penetration (i.e. not too densely packed).
- Do not cap vessels or add excessive liquid to the load.
- **Use caution** when opening autoclave at the end of the cycle – steam is usually still in the chamber.
Aerosols

- Are less than 5µm in diameter, but contain infectious particles
- Are subject to Brownian motion and will suspend indefinitely in static air
- Can be produced by any of the procedures listed in this table, and many others
- At BSL2 and above, any procedure that may produce aerosols must be performed inside of a Biological Safety Cabinet

### Aerosols from Common Laboratory Procedures

<table>
<thead>
<tr>
<th>Technique</th>
<th>Average No. of Clumps of Organisms Recovered from Air During Operation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pipetting 10ml culture into 1,000 ml broth</td>
<td>2.4</td>
</tr>
<tr>
<td>Drop of culture falling 12 in. (30 cm) onto:</td>
<td></td>
</tr>
<tr>
<td>Stainless steel</td>
<td>49.0</td>
</tr>
<tr>
<td>Painted wood</td>
<td>43.0</td>
</tr>
<tr>
<td>Hand towel wet with 5 percent phenol</td>
<td>4.0</td>
</tr>
<tr>
<td>Re-suspending centrifuged cells with pipette</td>
<td>4.5</td>
</tr>
<tr>
<td>Blowing out last drop from pipette</td>
<td>3.8</td>
</tr>
<tr>
<td>Shattering tube during centrifuging</td>
<td>1183.0</td>
</tr>
<tr>
<td>Inserting hot loop into broth culture</td>
<td>8.7</td>
</tr>
<tr>
<td>Streaking agar plates</td>
<td>0.2</td>
</tr>
<tr>
<td>Withdrawing syringe and needle from vaccine bottle</td>
<td>16.0</td>
</tr>
<tr>
<td>Injecting ten guinea pigs</td>
<td>16.0</td>
</tr>
<tr>
<td>Making dilutions with syringe and needle</td>
<td>2.3</td>
</tr>
<tr>
<td>Using syringe/needle for intranasal inoculation of mice</td>
<td>27.0</td>
</tr>
<tr>
<td>Harvesting allantoic fluid from five eggs</td>
<td>5.6</td>
</tr>
</tbody>
</table>
Biological Safety Cabinets (BSCs)

- Uses High Efficiency Particulate Air (HEPA) filters
- *Does not* protect against vapor or fumes, which may damage HEPA filters
- Class I:
  - Inward airflow protects personnel
  - Exhausts to outside

Class II:
- Four different types
- Protects personnel, materials and environment with directional airflow and multiple HEPA filters, as pictured

Class III:
- Both inlet and exhaust air are HEPA filtered (pictured)
# Biological Safety Cabinets (BSCs)

<table>
<thead>
<tr>
<th>Type</th>
<th>Face Velocity (fpm)</th>
<th>Airflow Pattern</th>
<th>Radionuclides/Toxic Chemicals</th>
<th>Biosafety Level(s)</th>
<th>Product Protection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Class I</td>
<td>75</td>
<td>In at front, rear and top through HEPA filter</td>
<td>No</td>
<td>2, 3</td>
<td>No</td>
</tr>
<tr>
<td>Class II</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Type A1</td>
<td>75</td>
<td>70% recirculated through HEPA; exhaust through HEPA</td>
<td>No</td>
<td>2, 3</td>
<td>Yes</td>
</tr>
<tr>
<td>Class II</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Type A2</td>
<td>100</td>
<td>30% recirculated through HEPA; exhaust via HEPA and hard ducted</td>
<td>Yes (Low levels/volatility)</td>
<td>2, 3</td>
<td>Yes</td>
</tr>
<tr>
<td>Class II</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Type B1</td>
<td>100</td>
<td>No recirculation; total exhaust via HEPA and hard ducted</td>
<td>Yes</td>
<td>2, 3</td>
<td>Yes</td>
</tr>
<tr>
<td>Class II</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Type B2</td>
<td>100</td>
<td>Same as B1, but plena under negative pressure to room and exhaust air is ducted</td>
<td>Yes</td>
<td>2, 3</td>
<td>Yes</td>
</tr>
<tr>
<td>Class III</td>
<td>NA</td>
<td>Supply air inlets and exhaust through 2 HEPA filters</td>
<td>Yes</td>
<td>3, 4</td>
<td>Yes</td>
</tr>
</tbody>
</table>
Laminar Flow Hood

- Is *not a BSC* and *does not* provide personnel protection
- Typically used for nucleic acid manipulation or other procedures that are very sensitive to contamination, but that do not pose a risk to personnel
- Air flows out toward the user
- Not to be used for work with infectious or potentially infectious materials
Chemical Fume Hood

- **Is not a BSC but does**
  provide personnel protection from chemical fumes by external ventilation
- **Is not** HEPA filtered
- **Not to be used for work with infectious or potentially infectious materials**
- **Exhaust containing infectious materials creates an exposure risk for the immediate environment**
Safe Operation of BSCs

Working with Radiation

• Radiolabeling of biological samples must be done inside a BSC
• The BSC must be labeled with a Radiation Warning label
• Proper shielding must be in place inside the BSC
• For radiation work that does not involve biological materials, work may be done in a chemical fume hood with proper labeling and shielding

Working with Chemicals

• Some BSCs allow for work with non-volatile chemicals – some have restrictions on the quantity to be used
• Check the indications for your particular class and type of BSC
Safe Operation of BSCs – before use

- Disinfect cabinets before and after each use with 70% ethanol or 10% bleach solution.
- After disinfecting the cabinet, load supplies and allow the cabinet to run for 10-15 minutes before beginning work.
- Supplies should include a small autoclave bag, sharps container and beaker with disinfectant for liquid waste.
- Your BSC should have a current certification label.
Safe Operation of BSCs – during use

- Check inward airflow by holding a piece of tissue near the opened sash
- Segregate clean and dirty materials to avoid contamination
- Place materials 4” or more inside cabinet to avoid disrupting airflow
- When reaching into the cabinet to work, avoid abrupt or excessive movements to maintain airflow
Safe Operation of BSCs – after use

- When finished working, discard solid and liquid waste and sharps in the appropriate manner
- Disinfect the work surface
- Lower the sash
- Turn on UV light
Safe Handling of Liquids

- Liquid materials must be placed in a container with a lid to prevent leaks and spills during collection, handling, processing, storage, transport or shipping

- Cultures
- Tissues
- Blood or body fluids
Safe Handling of Liquids

- Aqueous biological materials such as blood, cell cultures or microbial cultures must be either:
  - Decontaminated with bleach by adding 1 part bleach to each 9 parts liquid waste. Let stand for 20-30 minutes
  OR
  - Decontaminate by autoclaving on liquid cycle
  - *Do not* autoclaved bleach-treated liquid waste
Safe Operation of Centrifuges

- Check tubes for cracks, leaks or chips
- Use matching sets of tubes and buckets to ensure that the centrifuge is properly balanced
- Check that tubes and cups are sealed and that the rotor is locked and buckets are properly seated
- Close lid firmly
- When the cycle is finished, allow the rotor to come to a complete stop before opening lid
IX. Bloodborne Pathogens
• This training *does not* fulfill the bloodborne pathogen (BBP) training requirement
• BBP training is a dedicated module in the Knowledge Delivery System (KDS)
• BBP training is based on job classification – high risk jobs require annual training, while low risk jobs require training every five years
• BBP training is required by the Louisiana State Office of Risk Management and is conducted according to Occupational Safety and Health Administration standards
• All research using human blood, body fluids, tissue, cell lines, and OPIM is carried out using BSL 2 practices and procedures, because it is unknown if these materials contain bloodborne diseases such as HIV, HBV, HCV
• If you are at high risk and have not had the *Hepatitis B vaccination*, contact your business manager to inquire about getting it
X. Biosurety
An exposure is contact with blood or other infectious or potentially infectious materials

- For example, needlesticks or scrapes and cuts with contaminated sharps
- If you’re not sure if you’ve had an exposure, check for punctures in your glove. If the glove is broken, assume an exposure has occurred
- Contact with broken skin through cuts or rashes
- Splashes to the eyes, nose or mouth
Accident Response

- If you have an exposure:
  1. Stop what you are doing
  2. Thoroughly wash the affected area with soap and warm water for 15 minutes using a massaging motion
  3. For eye splashes, go to the nearest eyewash station and rinse with plain water for 15 minutes

- If you need medical attention:
  - Call 911 and tell the operator your location, name, nature of the injury
  - Then call the University Police at 568-8999

- After receiving the needed medical attention:
  - Notify your supervisor
  - Contact the Department of Human Resource Management at 568-3916
Incident Response

- Biological Spill Response
  - Alert others in the area
  - Put on appropriate PPE, then
    1. Cover the spill with paper towel(s)
    2. Disinfect by pouring a disinfectant around the perimeter of the spill and allowing to stand for 20 minutes
    3. Clean by wiping up with paper towel(s)
    4. Disinfect by spraying and wiping down with disinfectant and paper towel
  - If you are unsure of what to do or uncomfortable performing the clean-up, or if the spill is larger than you can respond to, call the University Police at 568-8999
Biosecurity

In order to ensure the security of potentially harmful biological materials:

- Control access to areas where biological agents and toxins are stored
- Know who is in your work area
- Know what materials are being brought into and taken out of your laboratory
- Have a protocol in place for reporting incidents or suspicious activities or people
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