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Biological Safety Training

October 1, 2014

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Contents

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I. What is a Biohazard

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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.

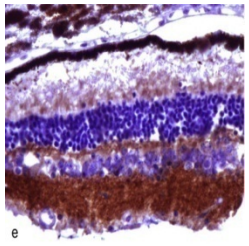
- Examples

Bacteria

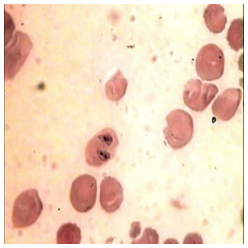
Fungi

Parasites

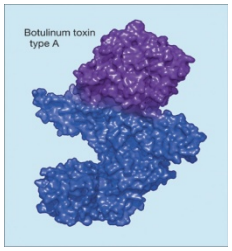
Prions



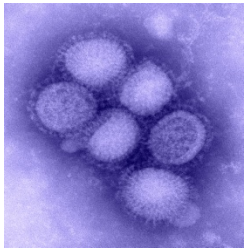
Rickettsials



Toxins



Viruses



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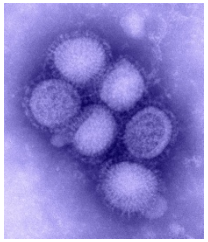
II. Who Should Receive Biosafety Training

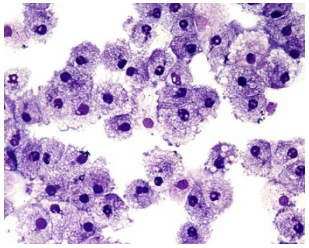
Biological Safety Training

- General biosafety training is required for all personnel who work with potentially viable biological materials, including (but not limited to):

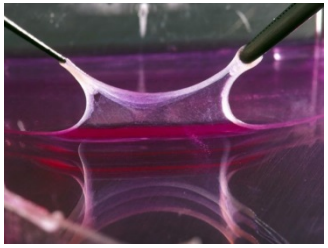
Microbes



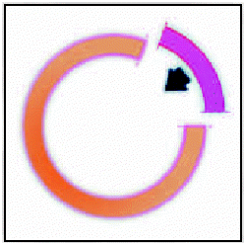
Cells



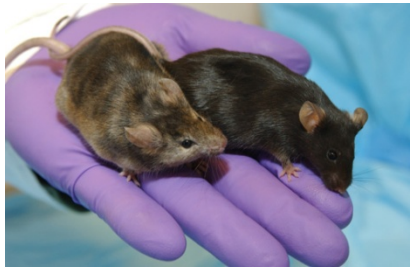
Tissue Cultures



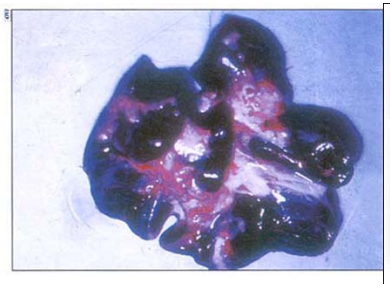
rDNA



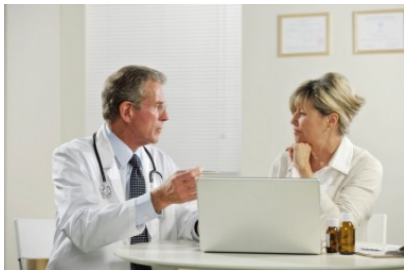
Animals



Animal Tissue or Fluid



Face-to-Face Contact



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III. History and Development of Biosafety Practices

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Origins of Biosafety

- 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

Development of Biosafety

- 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.

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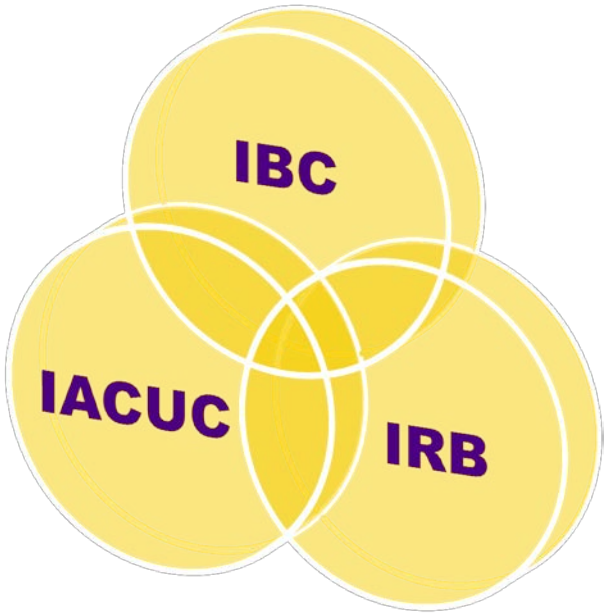
IV. Institutional Biosafety Committee (IBC)

Institutional Biosafety Committee

- 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



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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)

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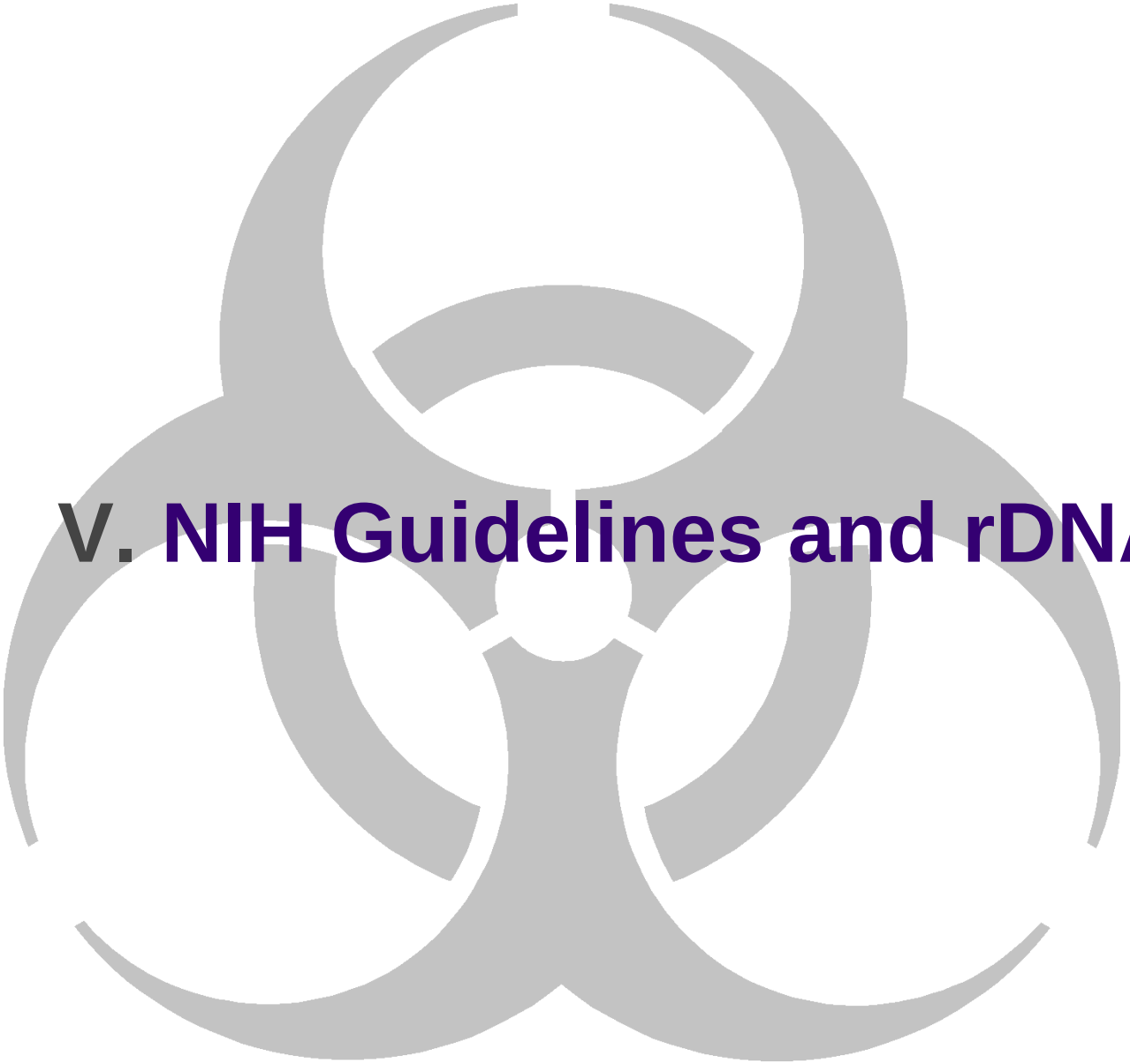

V. NIH Guidelines and rDNA

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NIH Guidelines

- 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

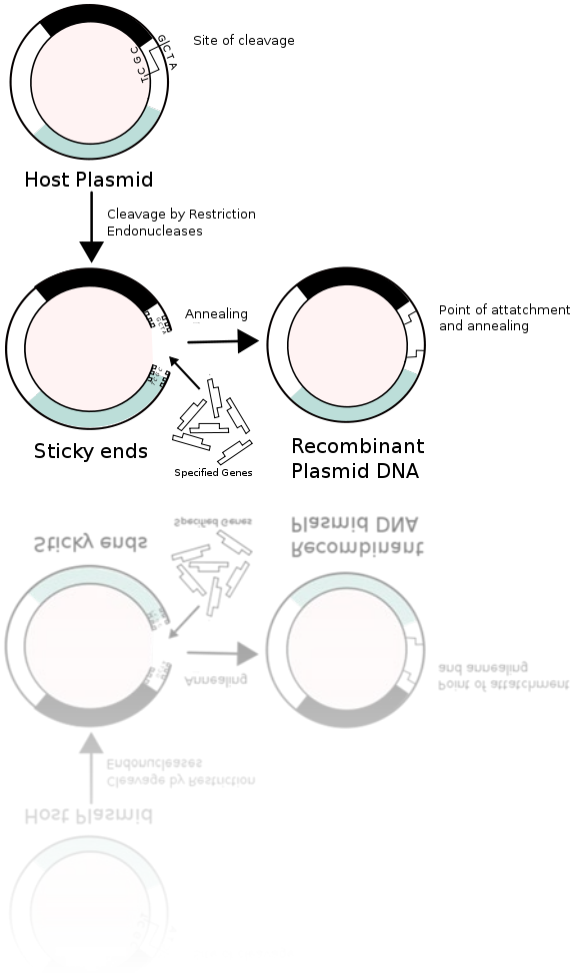
rDNA

- 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

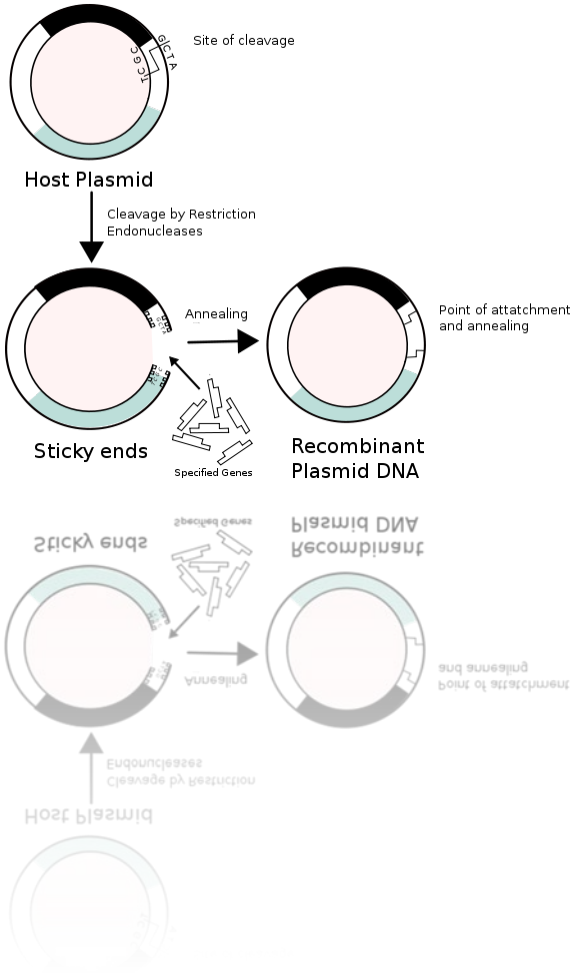
rDNA

- 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



rDNA Safety

- 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



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VI. Biosafety Levels (BSLs)

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Biosafety Level (BSL)

- BSL determination should be informed by risk assessment

A/BSL-1	A/BSL-2	A/BSL-3	A/BSL-4
Low individual risk (non-infectious to healthy adults)	**Moderate individual risk** (Not generally severe, treatment usually available)	**High individual risk** (Treatment may or may not be available)	**Severe individual risk** (Treatment often not available)
Low risk to community	**Low risk to community**	**Low risk to community**	**High risk to community**

Examples

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

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Biosafety Level (BSL)

- PPE recommendation and guidance are available for each BSL

A/BSL-1	A/BSL-2	A/BSL-3	A/BSL-4
Lab coats, gowns, etc. Eye protection Latex or nitrile gloves	Lab coats, gowns, etc. Eye protection Latex or nitrile gloves • Change when contaminated • Double glove when necessary • Remove gloves and wash hands after working • Do not re-use gloves	All manipulations performed inside a BSC Full protective clothing that must not leave the lab Eye protection Latex or nitrile gloves •BSL-3 work practices Appropriate respiratory protection	All PPE indicated up to and including BSL-3 Positive-pressure suit Special facility engineering features

- A risk assessment pertinent to *your* lab and *your* work should yield PPE and work practice directives specific to *your* workplace

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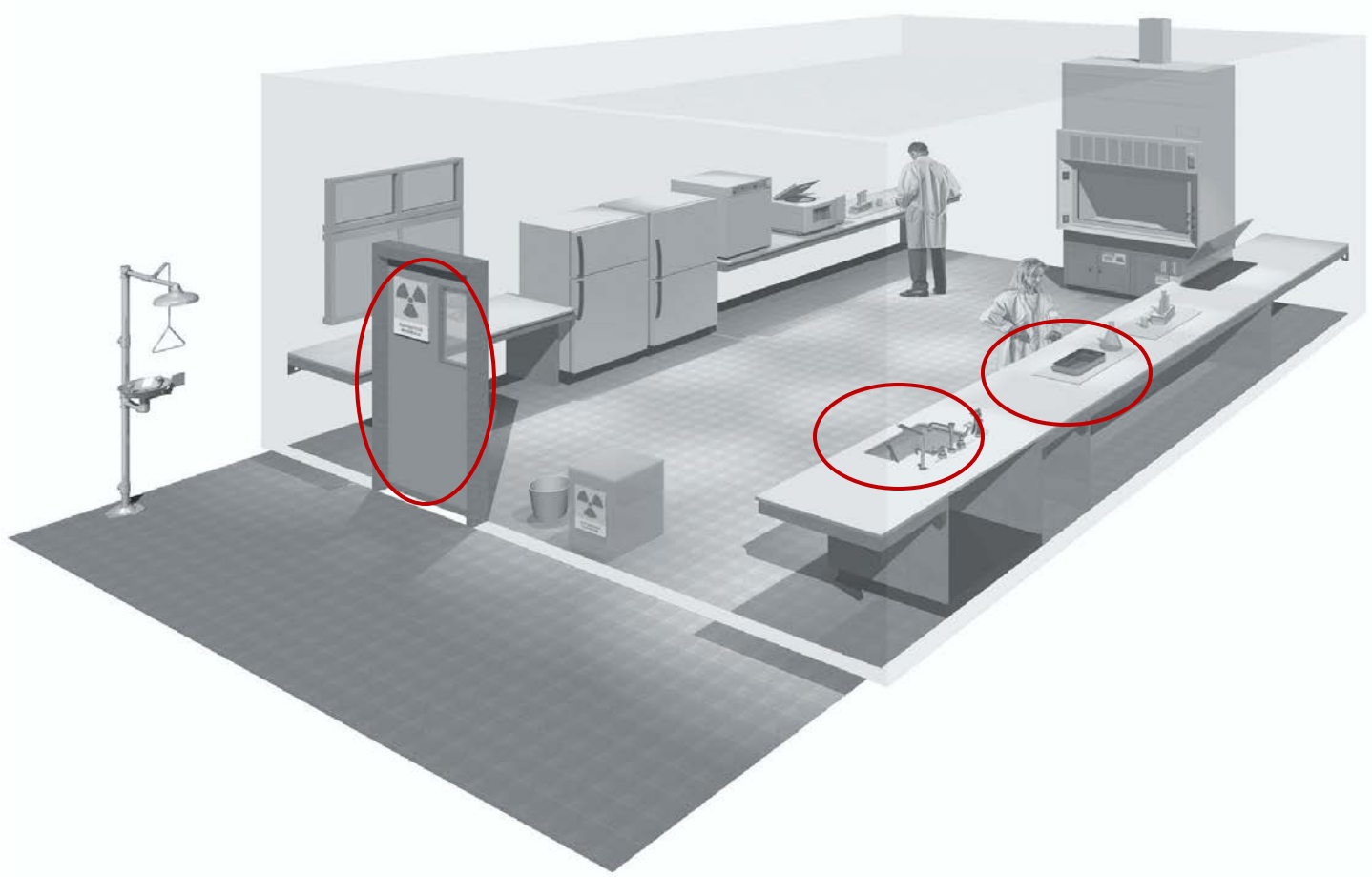
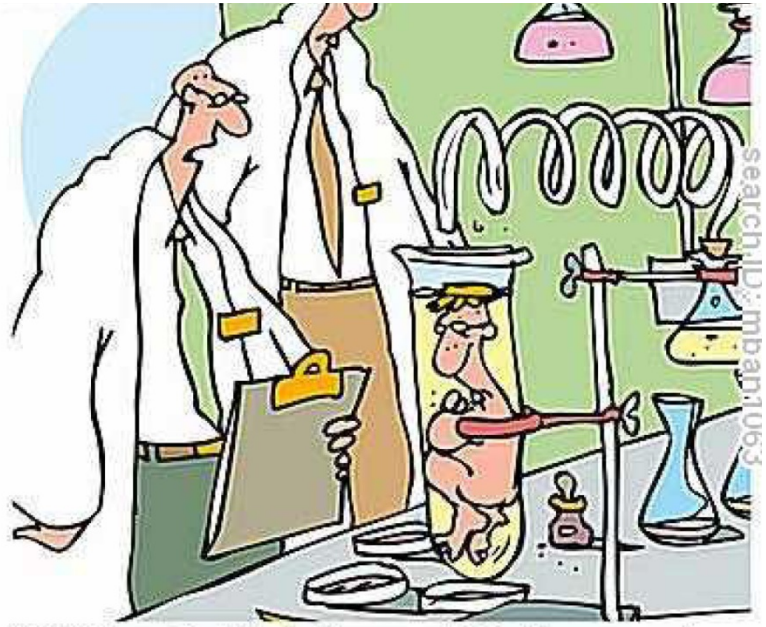
BSL-1 Facility Design

- 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



"Well, it certainly looks like your DNA. How many times have I told you to wear gloves before touching anything?"

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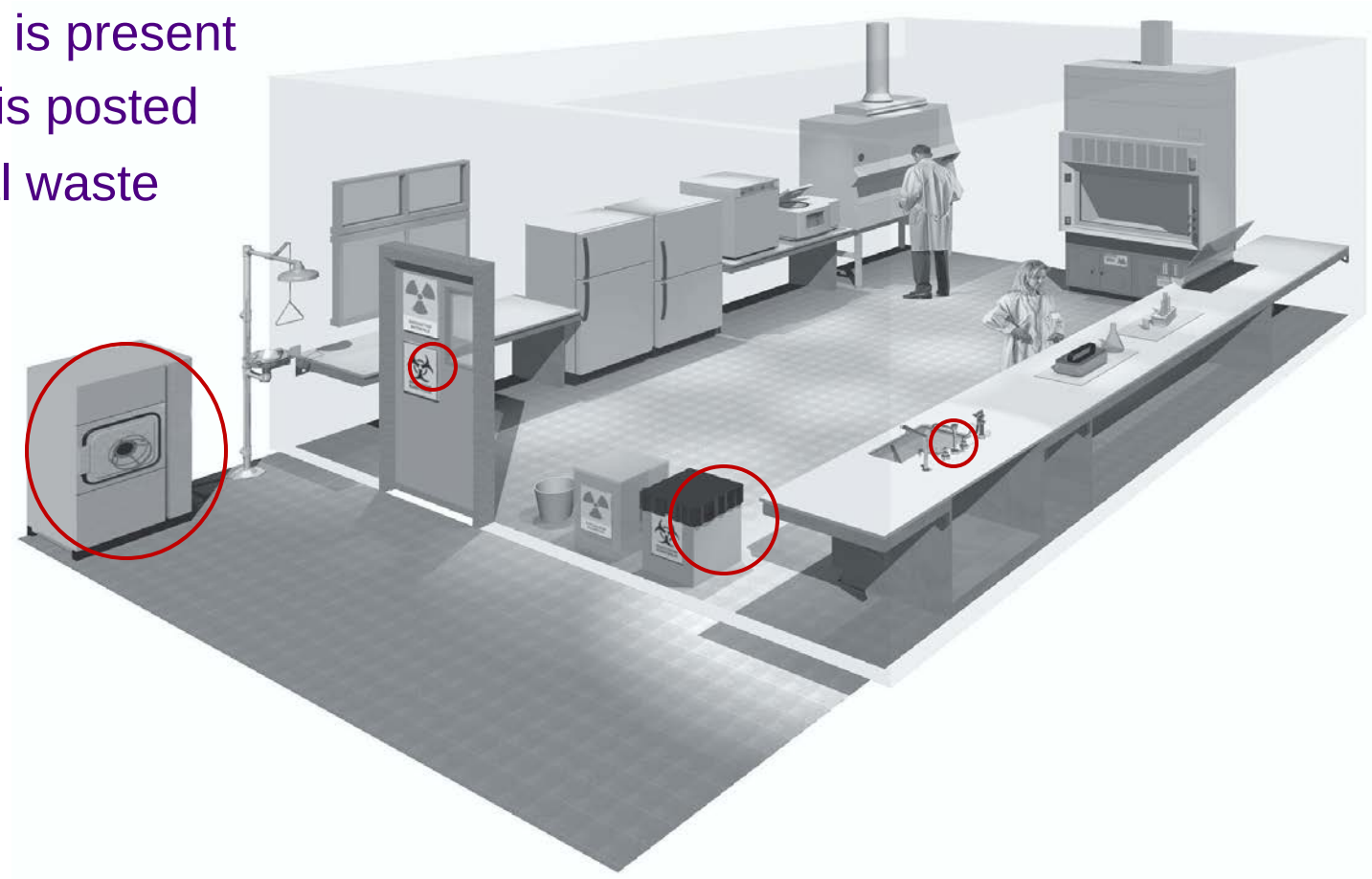
BSL-2 Facility Design

All BSL-1 requirements, plus:

- Autoclave is available
- Eyewash is present
- Signage is posted
- Biological waste stream is separate



BSL-2 Work Practices

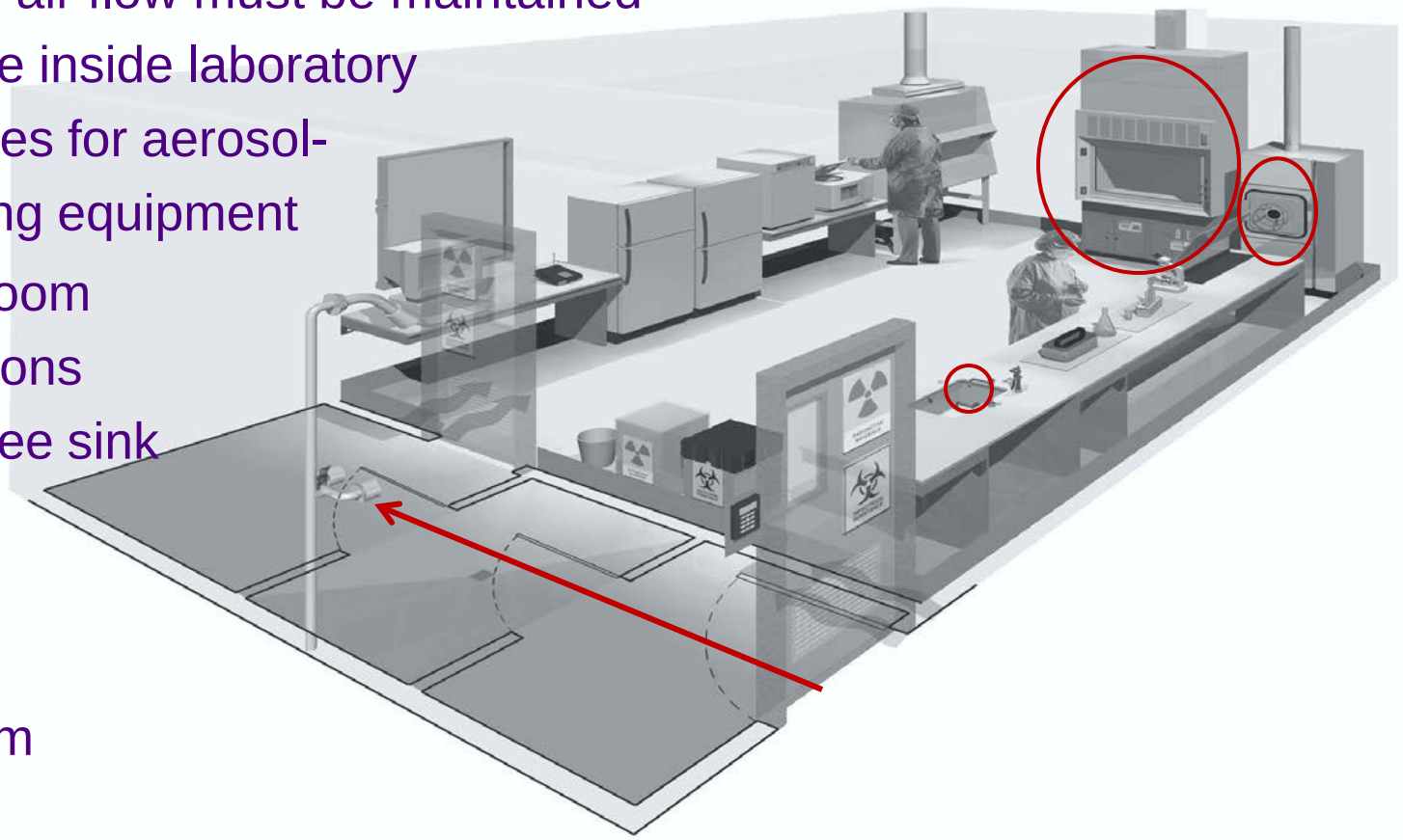
All BSL-1 practices, plus:

- 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

BSL-3 Facility Design

All BSL-2 requirements, plus:

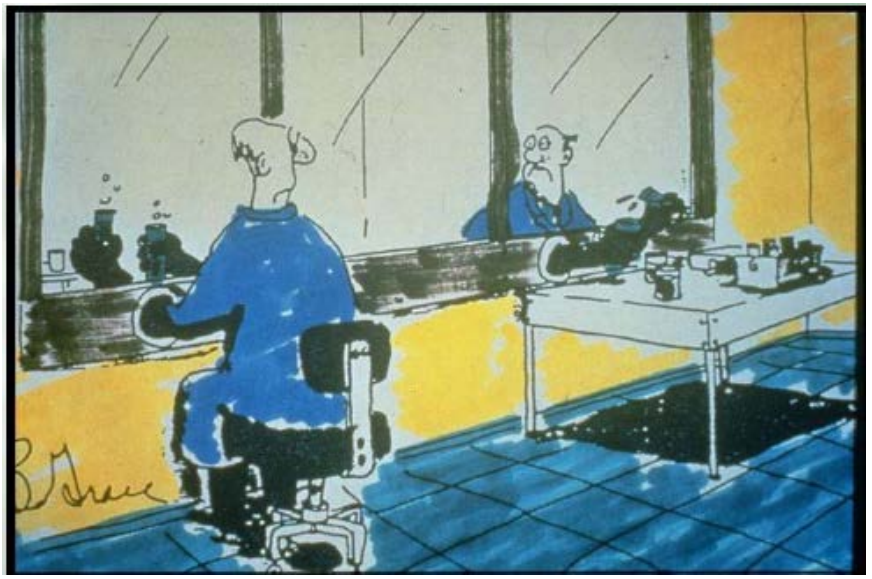
- Lab is separated from general traffic
- Negative air flow must be maintained
- Autoclave inside laboratory
- Enclosures for aerosol-generating equipment
- Sealed room penetrations
- Hands-free sink
- Enter and exit through ante-room



BSL-3 Work Practices

All BSL-2 practices, plus:

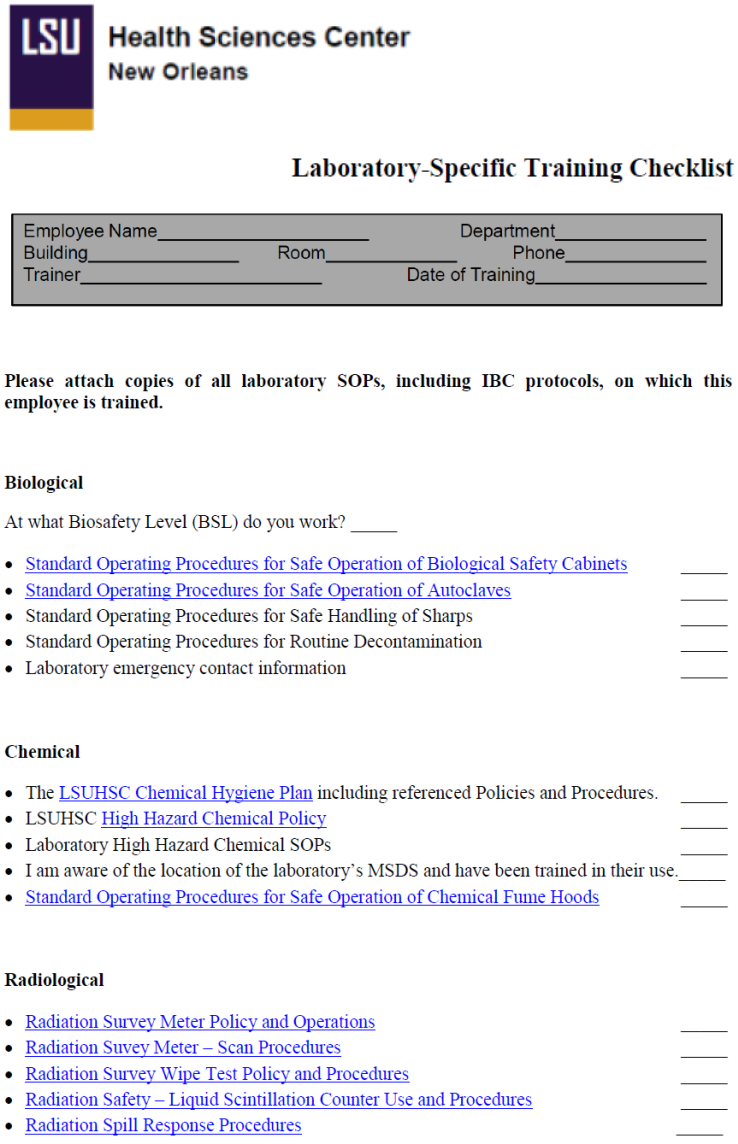
- All infectious materials *must* be placed in durable, leak-proof containers
- All surfaces and equipment should be regularly disinfected
- All work with infectious agents *must* be done inside the BSC



- Users *must* complete training and demonstrate proficiency with laboratory manager before being granted access
- It is recommended that personnel work in pairs

Training

- 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 sucseptibility



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New Orleans

Laboratory-Specific Training Checklist

Employee Name_____ Department_____
Building_____ Room_____ Phone_____
Trainer_____ Date of Training_____

Please attach copies of all laboratory SOPs, including IBC protocols, on which this employee is trained.

Biological

At what Biosafety Level (BSL) do you work? _____

- Standard Operating Procedures for Safe Operation of Biological Safety Cabinets _____
- Standard Operating Procedures for Safe Operation of Autoclaves _____
- Standard Operating Procedures for Safe Handling of Sharps _____
- Standard Operating Procedures for Routine Decontamination _____
- Laboratory emergency contact information _____

Chemical

- The LSUHSC Chemical Hygiene Plan including referenced Policies and Procedures. _____
- LSUHSC High Hazard Chemical Policy _____
- Laboratory High Hazard Chemical SOPs _____
- I am aware of the location of the laboratory's MSDS and have been trained in their use. _____
- Standard Operating Procedures for Safe Operation of Chemical Fume Hoods _____

Radiological

- Radiation Survey Meter Policy and Operations _____
- Radiation Suvey Meter – Scan Procedures _____
- Radiation Survey Wipe Test Policy and Procedures _____
- Radiation Safety – Liquid Scintillation Counter Use and Procedures _____
- Radiation Spill Response Procedures _____

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VII. Risk Assessment (RA)

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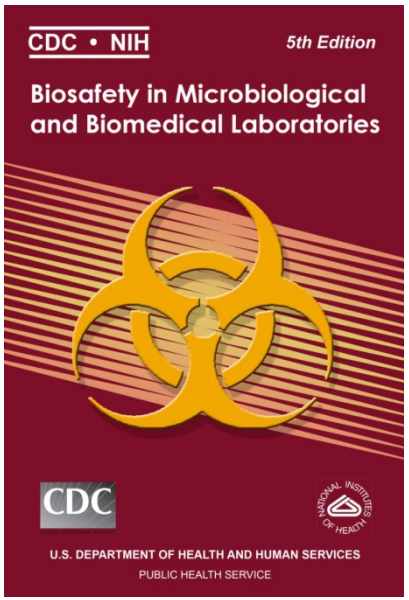
Risk Assessment

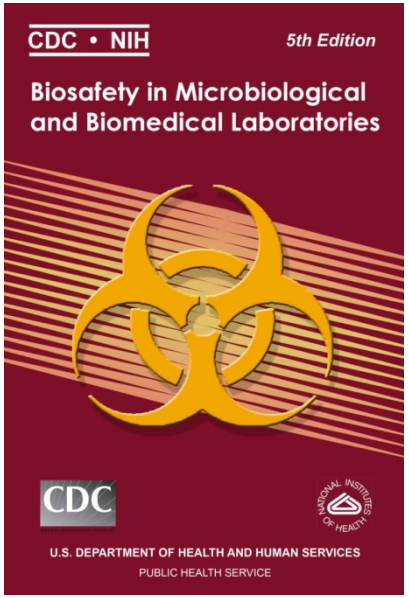
- Work practices, use of containment equipment, PPE, training, etc. should be guided by a thorough risk assessment (RA)
- A risk assessment ensures protection of personnel, the environment, the community and the integrity of *your* experiments
- Your RA should be included in your lab-specific Biosafety Manual
- Risk assessment guidance can be found in the CDC *BMBL 5th Ed.* or at the American Biological Safety Association (ABSA) website



Risk Assessment Process

- 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



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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
- They 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

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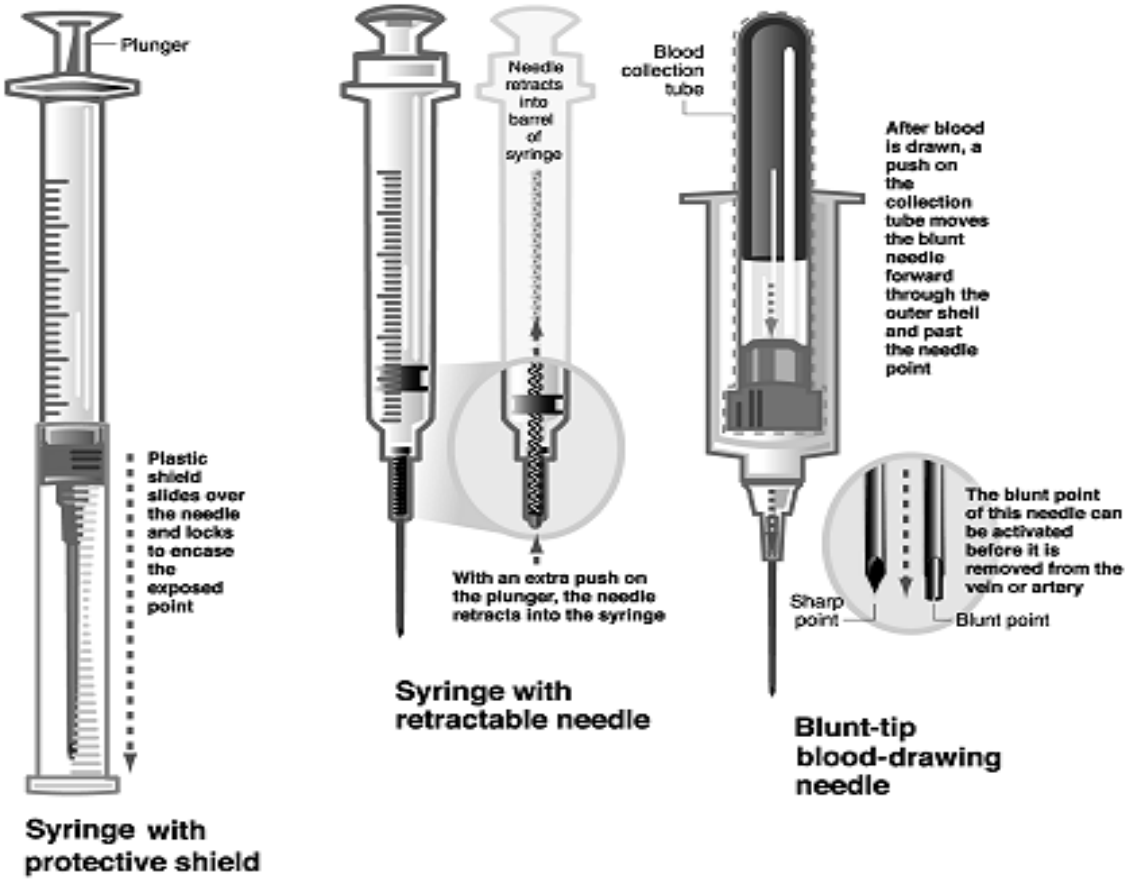
Work Practices

- 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

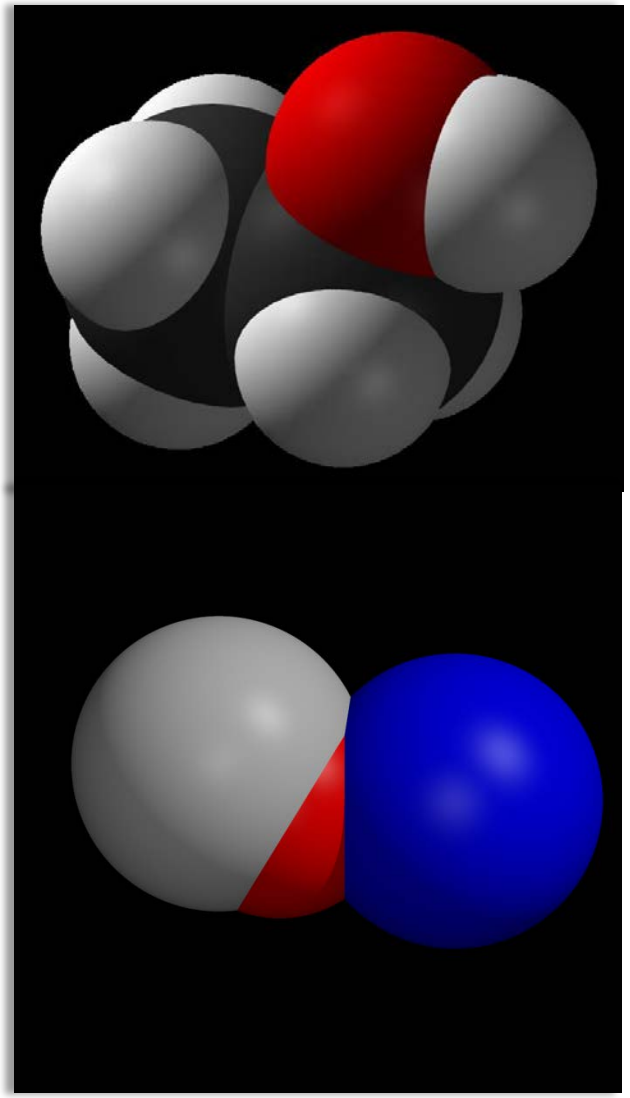
- Uncontaminated or decontaminated glass may be disposed of in a designated, labeled cardboard box
- Box should be sturdy and in good condition
- Take care not to overload the box – it should be kept to a reasonable weight, approximately 25 lbs.

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 *autoclaveable plastic*
- Most pathogens and recombinant molecules are sensitive to temperatures above 121ºC 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

Work Practices

Aerosols

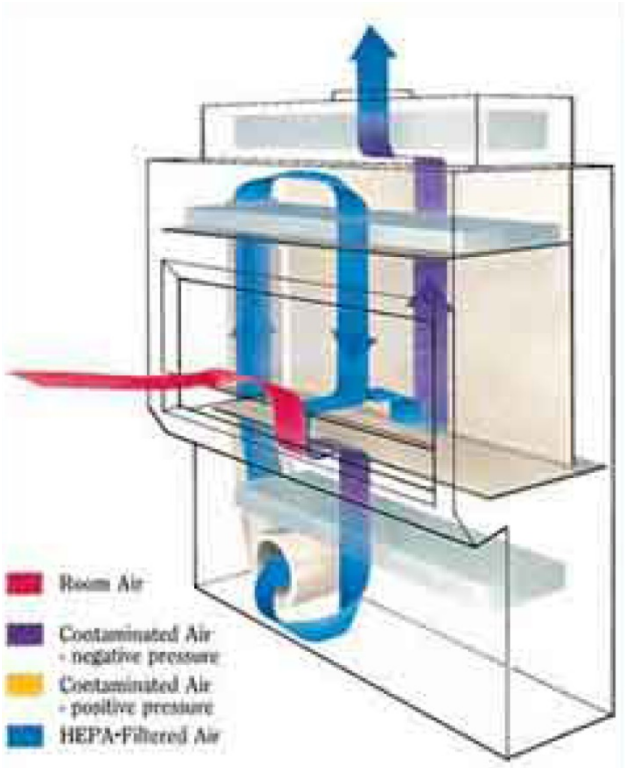
- Are less than 5µm in diameter, but contain infectious particles
- Are subject to Brownian motion and will suspend *indefinitely* in static air
- Can be produced by any of the procedures listed in this table, and many others
- At BSL2 and above, *any* procedure that *may* produce aerosols *must* be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

Aerosols from Common Laboratory Procedures

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

BSL-2 Primary Barriers

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)

BSL-2 Primary Barriers

Biological Safety Cabinets (BSCs)

Type	Face velocity (lfpm)	Airflow Pattern	Radionuclides/ Toxic Chemicals	Bio-safety Level(s)	Product Protection
Class I	75	In at front; rear and top through HEPA filter	No	2, 3	No
Class II Type A1	75	70% recirculated through HEPA; exhaust through HEPA	No	2, 3	Yes
Class II Type A2	100	30% recirculated through HEPA; exhaust via HEPA and hard ducted	Yes (Low levels/volatility)	2, 3	Yes
Class II Type B1	100	No recirculation; total exhaust via HEPA and hard ducted	Yes	2, 3	Yes
Class II Type B2	100	Same as B1, but plena under negative pressure to room and exhaust air is ducted	Yes	2, 3	Yes
Class III	NA	Supply air inlets and exhaust through 2 HEPA filters	Yes	3, 4	Yes

BSL-2 Primary Barriers

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

BSL-2 Primary Barriers

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

BSL-2 Primary Barriers

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

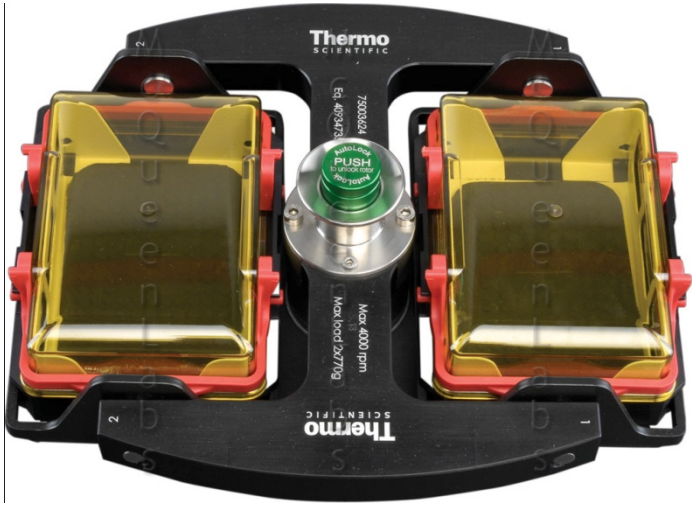
Safe Operation of BSCs – after use

- 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

BSL-2 Primary Barriers

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

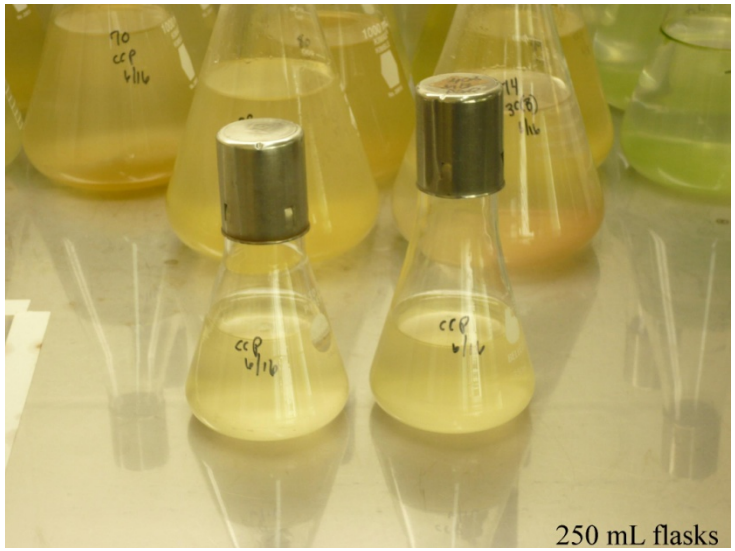


BSL-2 Primary Barriers

Safe Handling of Liquids

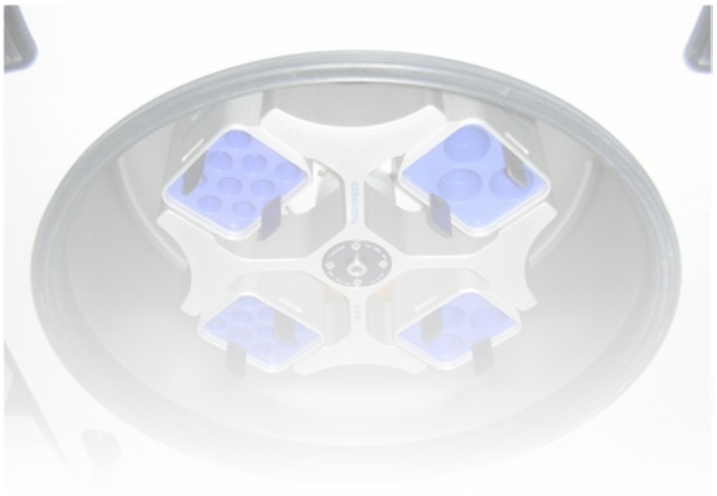
- Aqueous biological materials such as blood, cell cultures or microbial cultures must be either:
  - Decontaminated with bleach by adding 1 part bleach to each 9 parts liquid waste. Let stand for 20-30 minutes

  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



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IX. Bloodborne Pathogens

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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

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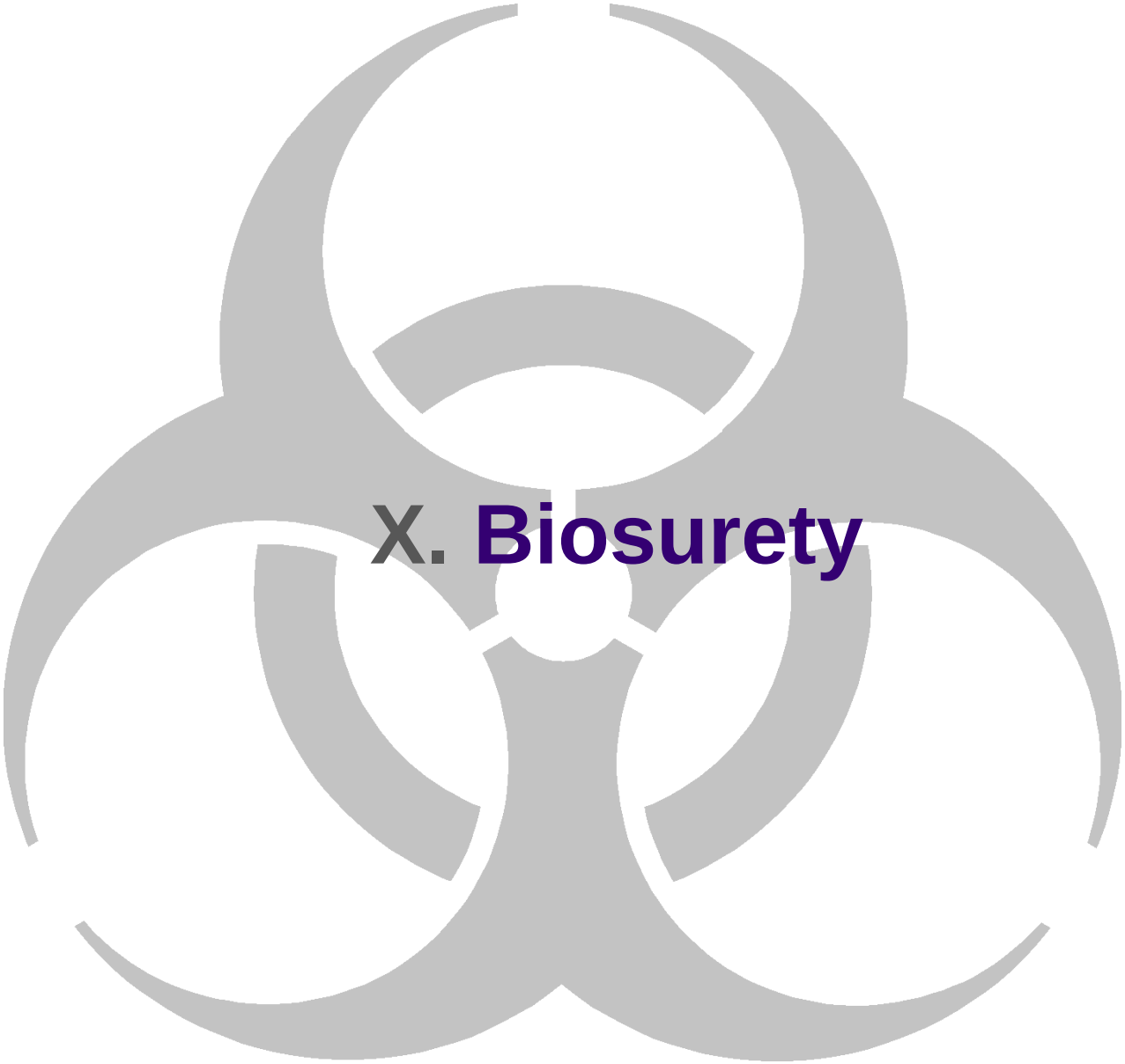

X. Biosurety

Accident Response

- 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

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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 911and 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 that 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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Contents Review

I. What is a Biohazard ✓

II. Who Should Recieve 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 ✓

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Contact Information

If you have any questions or comments, please contact the Biological Safety Officer, Taylor Kriete, at tkriet@lsuhsc.edu or (504)952-1337