Basics of Biosafety

Objectives

Course Objectives

- **This course on Biosafety is intended to increase your understanding of the following:**
  - Biosafety Definition
  - Mandates & Regulations
  - Impacts of non-compliance
  - JSC Biosafety Review Board
  - Risk Assessment
  - Containment - Lab practices and techniques, safety equipment, and facility design.
  - JSC Training Requirements

Definition

What is Biosafety?

Biosafety, according to the Centers for Disease Control and Prevention (CDC), is the “discipline addressing the safe handling and containment of infectious microorganisms and hazardous biological materials.”

Regulations

Mandates

- **JSC is mandated to comply with the regulations as established by:**
  - CDC: Centers for Disease Control and Prevention
  - BMBL: Biosafety in Microbiological and Biomedical Laboratories
  - DOT: Department of Transportation
  - ICAO: International Civil Aviation Organization
  - IATA: International Air Transport Association
- **The mandate can be found in NPR 1800.1**
  - NASA Procedural Requirements (NPR) 1800.1: NASA Occupational Health Program Procedures, Section 4.1.1 Biosafety

National Actions Subcommittee

- **National Actions Subcommittee on Oversight and Investigations**
  - **American Society for Microbiology**
    - Need to ensure adequate biosafety training
    - Strict Compliance with NIH Guidelines and BMBL (CDC/NIH)
  - **Government Accountability Office (GAO) Report**
    - Train lab staff in general biosafety
  - **National Institutes of Health (NIH) and Centers for Disease Control and Prevention (CDC) Testimony:**
    - Establishment of a Trans-Federal Task Force on Optimizing Biosafety Oversight, consisting of representatives of a broad range of Federal agencies concerned with biosafety risks
      - Biosafety Curriculum Development and Biosafety Training
      - Individuals with Certification
      - Institutions with Accreditation

Agencies Involved

ABSA:	American Biological Safety Association
CDC:	Centers for Disease Control and Prevention
BMBL:	Biosafety in Microbiological and Biomedical Laboratories
GAO:	Government Accountability Office
CAO:	International Civil Aviation Organization
DOT:	U.S. Department of Transportation
HHS:	Health and Human Services
IATA:	International Air Transport Association
ICAO:	International Civil Aviation Organization
NIH:	National Institutes of Health
OSHA:	Occupational Safety and Health Administration
USDA:	United States Department of Agriculture
WHO:	World Health Organization

Regulations

Responsibilities

CDC & NIH	NIH	OSHA	DOT, ICAO, and IATA
• Guidance for designing work areas and labs to the proper Biosafety level	• Research involving Genetically Modified Materials (i.e., Recombinant DNA)	• Bloodborne Pathogen Standards	• Transportation of Hazardous Materials • DOT regulates ALL shipments in the U.S. • ICAO/IATA regulates all international air shipments

CDC:	Centers for Disease Control and Prevention
DOT:	U.S. Department of Transportation
IATA:	International Air Transport Association
ICAO:	International Civil Aviation Organization
NIH:	National Institutes of Health
OSHA:	Occupational Safety and Health Administration

Use and Transfer of Select Agents

- Select Agents are microorganisms and toxins that have the potential to pose a severe threat to public health (humans, animals, and plants) and safety.
- Since September 11, 2001, two major pieces of legislation, the USA PATRIOT Act of 2001 and the "Public Health Security and Bioterrorism Preparedness Act of 2002" have changed the law governing the possession, use, and transport of etiologic agents.
- A current list of select agents and other updates may be found at the CDC Office of the Director Select Agent Program website, http://www.cdc.gov/od/sap/index.htm.
- Civil penalties for violating the regulations may amount to $250,000 for an individual.
- Criminal penalties include a fine or imprisonment up to five years for possession without registration or transfer of a select agent to an unregistered individual.
- Use and transfer of Select Agents is regulated by HHS and USDA.
  - HHS Select Agents and Toxins *(e.g. Yersinia pestis,* Ricin*)*
  - USDA Select Agents and Toxins *(e.g. Foot-and-mouth disease virus)*
  - USDA Plant Protection and Quarantine Select Agents and Toxins

HHS: Health and Human Services
USDA: United States Department of Agriculture

Genetically Modified Materials

- **NIH Guidelines specify practices for constructing and handling genetically modified materials:**
  - these are molecules constructed outside of living cells by joining natural or synthetic DNA segments to DNA molecules that can replicate in a living cell, or molecules that result from their replication.

More Information

- Area Specific Plans -
  - OMOHC Tuberculosis exposure control plan
  - Wyle BBP Program,
  - Wyle Chemical Hygiene Plan
  - Jacobs Chemical Hygiene Plan

- For links to the national and international guidelines and regulations - http://www.absa.org (Under resources and tools – Key topics and links)

Biosafety - Impacts your Career!

- **Career – Suspension of Work**
  - Texas A&M Incident

- Jul 3, 2007 (CIDRAP News) – The US Centers for Disease Control and Prevention (CDC) on Jun 30 ordered a biodefense research laboratory at Texas A&M University to stop all work on select agents and toxins while the CDC investigates reports of lab workers infected with the select agents *Brucella* sp. and *Coxiella burnetti*.

- In the Jun 30 letter, the CDC outlined the concerns it has about the lab, which include the adequacy of biosafety plans, security of the facility from unauthorized visitors, occupational safety protocols, authorization from the CDC to work with certain agents, and compliance with federal select agent regulations.

CIDRAP

BIOTERRORISM >> GENERAL BIOTERRORISM INFORMATION >> NEWS >>

CDC suspends work at Texas A&M biodefense lab

Lisa Schnirring • Staff Writer

Jul 3, 2007 (CIDRAP News) – The US Centers for Disease Control and Prevention (CDC) on Jun 30 ordered a biodefense research laboratory at Texas A&M University to stop all work on select agents and toxins while the CDC investigates reports of lab workers infected with the category B bioterror agents *Brucella* and *Coxiella burnetti*.

The alleged lab accidents, along with related alleged violations of federal law, were recently reported by the Sunshine Project, an Austin, Texas, nonprofit group that monitors biodefense research safety. The group used Texas freedom-of-information requests to obtain documents about the lab incidents.

In April, the Sunshine Project reported that a Texas A&M researcher had been infected with *Brucella* after a February 2006 aerosol chamber mishap and that the school did not immediately notify the CDC as required by federal law. Five days ago, the watchdog group reported that the exposure of three other Texas A&M workers to *C burnetti*, which causes Q fever, was confirmed in April 2006 but also was not reported to the CDC.

The Sunshine Project said its investigation of Texas A&M came about through its review of select-agent labs that are vying to host the federal government's planned new national biodefense facility, according to a Jun 27 report in the *Dallas Morning News*.

The laboratory is affiliated with the Foreign Animal and Zoonotic Disease Defense Center, a US Department of Homeland Security (DHS) center of excellence that focuses on the study of foot-and-mouth disease, avian influenza, and Rift Valley fever, according to the center's Web site. The center is funded by an $18 million DHS grant, according to an Associated Press report yesterday.

According to the Sunshine Project, Texas A&M notified the CDC about the *Brucella* infection in April 2007, a year after the worker's illness was confirmed.

Shortly after Texas A&M reported the *Brucella* infection, representatives from the CDC's Select Agents and Toxins Division inspected the laboratory and, in a Jun 30 memo, obtained and released by the Sunshine Project, ordered the lab to stop its work on all select agents and toxins until further notice.

Von Roebuck, a CDC spokesman, told CIDRAP News that the agency has never issued such a broad suspension order to a lab before. He said the Jun 30 order applies to every agent the lab works with. The list of affected agents is not public information because of security concerns, Roebuck said.

In the Jun 30 letter, the CDC outlined the concerns it has about the lab, which include the adequacy of biosafety plans, security of the facility from unauthorized visitors, occupational safety protocols, authorization from the CDC to work with certain agents, and compliance with federal select agent regulations.

Roebuck said CDC officials will visit the Texas laboratory again in the next few weeks to gather more information about its procedures and protocols. "Then the agency will move forward with any recommendations to get them under compliance," he said.

In a statement sent to Texas A&M faculty and staff yesterday, the school's interim president, Eddie J. Davis, said the laboratory incidents did not pose a threat to anyone on or off campus, but conceded that the university should have reported the worker's *Brucella* exposure in a more timely manner. (The university provided CIDRAP News with a copy of the

Impacts could be personal!

- **Personal – $$$, jail and infection**
  - Texas Tech Incident
    - Thomas Butler, an infectious disease specialist was arrested and jailed in 2003 for the “mis-handling” of Select Agents.
  - Elizabeth Griffin Research Foundation
    - In 1997, Beth Griffin, a research technician at Emory University, died after contracting B-virus as a result of inadequate prevention procedures and deficient treatment after exposure.

Biosafety at JSC

- The Biosafety Review Board (BRB) was established at JSC to identify, evaluate, control and prevent biological hazards in conformance with NASA, Federal and international health and safety regulations. http://hefd.jsc.nasa.gov/microbrb.htm

- The BRB includes a team of microbiologists, cell biologists, physicians, industrial hygienists, and occupational health professionals to assess the wide range of biohazardous materials encountered.

- Biohazardous materials may consist of bacteria, fungi, viruses, protozoa, cell cultures, and other infectious agents.

Functions of the JSC BRB

- **Operations** – provide assessments of flight operations regarding biosafety and potential exposure to crew-members
- **Research** - All biological materials for research use in JSC facilities are reviewed and approved prior to purchase.
- **Payloads** - All biological payload experiments are assessed and biohazardous materials identified are assigned a NASA biosafety level (BSL).

Additional Functions of the BRB

- Conduct annual inspection of laboratories at JSC that utilize biological materials.
- Train personnel in policies and procedures of transporting biological materials.
- Maintain inventory of biological materials that are used at JSC including genetically modified organisms (GMO)
- Investigate incidents that involved biological materials.
- Obtain Transportation Security Administration exemptions for hand-carrying of crew specimens.

BRB Members

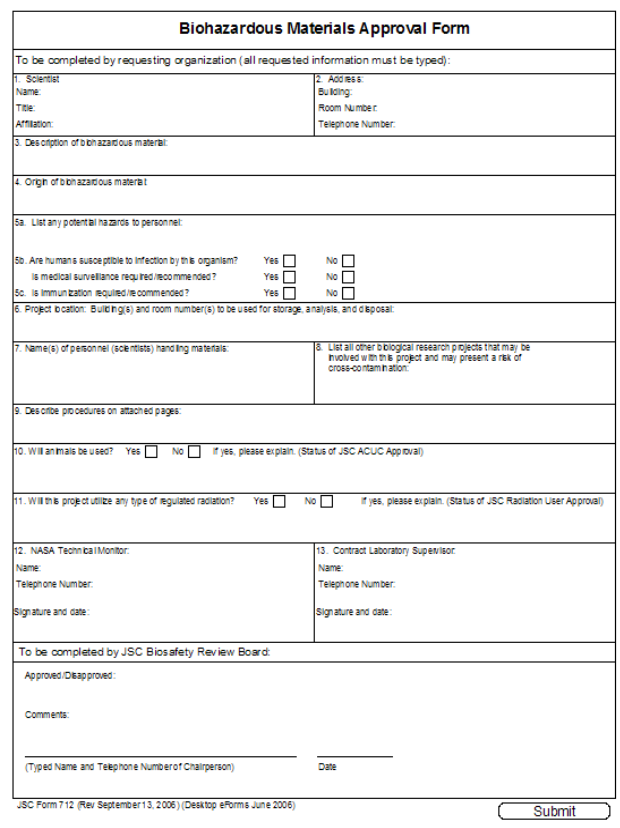
Microbiology	• D. L. Pierson - Chair • Mark Ott • Wing C. Wong
Occupational Health	• Sean Keprta • Robert Martel
Clinical	• Kathleen McMonigal • Reta Warren
Safety	• Tom Samson • Myra Smith
Cell Biology	• Neal Pellis • Tom Goodwin
Radiation	• Honglu Wu • Joan Robertson
Environmental Factors	• Bob Spann
Astromaterials	• Carl Allen
Biosafety	• Dee Zimmerman – UTMB • Debra Hunt – Duke University
Administrative Specialist	• Sharon Jackson

BRB Requirements

- This Biosafety Review Board Operations and Requirements Document establishes requirements for the information required by the BRB to identify and assess biohazardous materials utilized in payload or ground-based experiments. The document also defines the requirements for tracking biohazardous materials that are stored at JSC facilities and the requirements for inspecting JSC facilities utilizing biohazardous materials. This document supersedes all biological material references in JSC 27472 "Requirements for Submission of Data Needed for Toxicological Assessment of Chemicals to be Flown on Manned Spacecraft".

JSC BRB

BRB Forms



Biohazardous Materials Approval Form

To be completed by requesting organization (all requested information must be typed):

1. Scientist
Name:
Title:
Affiliation:

2. Address:
Building:
Room Number:
Telephone Number:

3. Description of biohazardous material:

4. Origin of biohazardous material:

5a. List any potential hazards to personnel:

5b. Are humans susceptible to infection by this organism? Yes ☐ No ☐
Is medical surveillance required/recommended? Yes ☐ No ☐
5c. Is immunization required/recommended? Yes ☐ No ☐

6. Project location: Building(s) and room number(s) to be used for storage, analysis, and disposal:

7. Name(s) of personnel (scientists) handling materials:

8. List all other biological research projects that may be involved with this project and may present a risk of cross-contamination:

9. Describe procedures on attached pages:

10. Will animals be used? Yes ☐ No ☐ If yes, please explain. (Status of JSC ACUC Approval)

11. Will this project utilize any type of regulated radiation? Yes ☐ No ☐ If yes, please explain. (Status of JSC Radiation User Approval)

12. NASA Technical Monitor:
Name:
Telephone Number:
Signature and date:

13. Contract Laboratory Supervisor:
Name:
Telephone Number:
Signature and date:

To be completed by JSC Biosafety Review Board:

Approved/Disapproved:

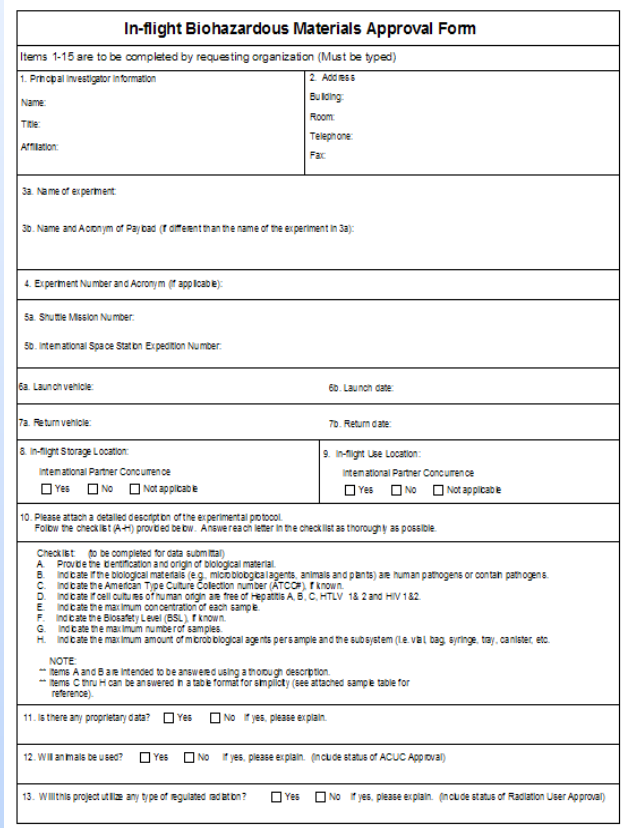
Comments:

(Typed Name and Telephone Number of Chairperson) Date

JSC Form 712 (Rev September 13, 2006) (Desktop eForms June 2006)

Submit

JSC Form 712



In-flight Biohazardous Materials Approval Form

Items 1-15 are to be completed by requesting organization (Must be typed)

1. Principal Investigator Information
Name:
Title:
Affiliation:

2. Address
Building:
Room:
Telephone:
Fax:

3a. Name of experiment:

3b. Name and Acronym of Payload (if different than the name of the experiment in 3a):

4. Experiment Number and Acronym (if applicable):

5a. Shuttle Mission Number:

5b. International Space Station Expedition Number:

6a. Launch vehicle: 6b. Launch date:

7a. Return vehicle: 7b. Return date:

8. In-flight Storage Location:
International Partner Concurrence
☐ Yes ☐ No ☐ Not applicable

9. In-flight Use Location:
International Partner Concurrence
☐ Yes ☐ No ☐ Not applicable

10. Please attach a detailed description of the experimental protocol.
Follow the checklist (A-H) provided below. Answer each letter in the checklist as thoroughly as possible.

Checklist: (to be completed for data submittal)
A. Provide the identification and origin of biological material.
B. Indicate if the biological materials (e.g., microbiological agents, animals and plants) are human pathogens or contain pathogens.
C. Indicate the American Type Culture Collection number (ATCC#), if known.
D. Indicate if cell cultures of human origin are free of Hepatitis A, B, C, HTLV 1& 2 and HIV 1&2.
E. Indicate the maximum concentration of each sample.
F. Indicate the Biosafety Level (BSL), if known.
G. Indicate the maximum number of samples.
H. Indicate the maximum amount of microbiological agents per sample and the subsystem (i.e. vial, bag, syringe, tray, canister, etc.

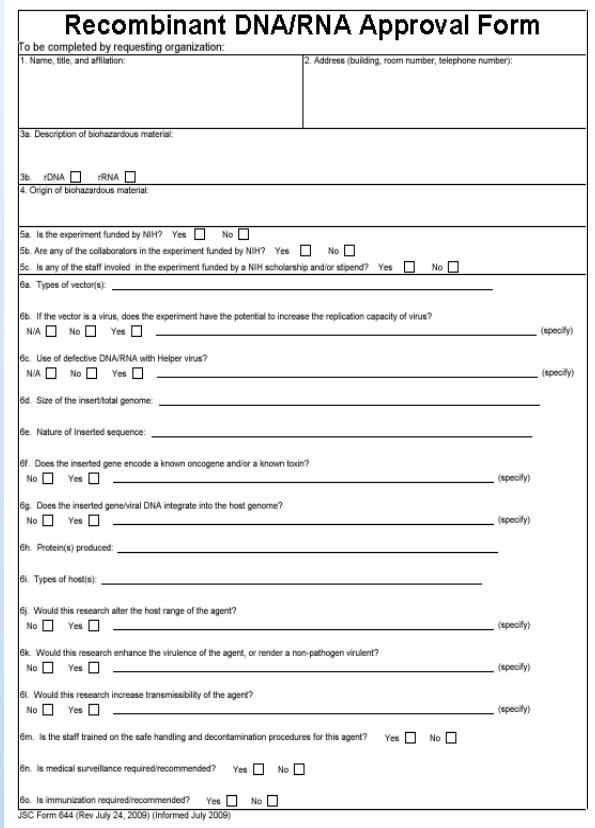
NOTE:
** Items A and B are intended to be answered using a thorough description.
** Items C thru H can be answered in a table format for simplicity (see attached sample table for reference).

11. Is there any proprietary data? ☐ Yes ☐ No If yes, please explain.

12. Will animals be used? ☐ Yes ☐ No If yes, please explain. (Include status of ACUC Approval)

13. Will this project utilize any type of regulated radiation? ☐ Yes ☐ No If yes, please explain. (Include status of Radiation User Approval)

JSC Form 713



Recombinant DNA/RNA Approval Form

To be completed by requesting organization:

1. Name, title, and affiliation:

2. Address (building, room number, telephone number):

3a. Description of biohazardous material:

3b. rDNA ☐ rRNA ☐

4. Origin of biohazardous material:

5a. Is the experiment funded by NIH? Yes ☐ No ☐
5b. Are any of the collaborators in the experiment funded by NIH? Yes ☐ No ☐
5c. Is any of the staff involved in the experiment funded by a NIH scholarship and/or stipend? Yes ☐ No ☐

6a. Types of vector(s):

6b. If the vector is a virus, does the experiment have the potential to increase the replication capacity of virus?
N/A ☐ No ☐ Yes ☐ (specify)

6c. Use of defective DNA/RNA with Helper virus?
N/A ☐ No ☐ Yes ☐ (specify)

6d. Size of the insert/total genome:

6e. Nature of inserted sequence:

6f. Does the inserted gene encode a known oncogene and/or a known toxin?
No ☐ Yes ☐ (specify)

6g. Does the inserted gene/viral DNA integrate into the host genome?
No ☐ Yes ☐ (specify)

6h. Protein(s) produced:

6i. Types of host(s):

6j. Would this research alter the host range of the agent?
No ☐ Yes ☐ (specify)

6k. Would this research enhance the virulence of the agent, or render a non-pathogen virulent?
No ☐ Yes ☐ (specify)

6l. Would this research increase transmissibility of the agent?
No ☐ Yes ☐ (specify)

6m. Is the staff trained on the safe handling and decontamination procedures for this agent? Yes ☐ No ☐

6n. Is medical surveillance required/recommended? Yes ☐ No ☐

6o. Is immunization required/recommended? Yes ☐ No ☐

JSC Form 644 (Rev July 24, 2009) (Informed July 2009)

JSC Form 644

BSL Facilities

BSL Facilities at JSC

- Astrobiology
- Baseline Data Collection Facility
- Biological Process Development Facility
- Biomedical Engineering for Exploration Space Technology
- Biosystems
- Cardiovascular
- Clinical
- HACO-Core
- Immunology
- Microbiology
- Muscle Research
- Nutritional Biochemistry
- Pharmacology
- Radiation Biophysics

Currently, the laboratories at JSC only work with BSL-1 and BSL-2 organisms/potentially infectious materials.

Risk Assessment

Biosafety – Assessing the Risk

- ☐ What are you working with? and how much?
- ☐ What Risk Group is it in?
- ☐ Is it a Select Agent? GMO?
- ☐ Do you have BRB approval for use?
- ☐ Do you have the proper facilities, PPE, disinfectants, training, vaccinations?

Risk Assessment

Biosafety – Assessing the Risk

- **Risk Groups (RG)**
  - A classification of infectious microorganisms based on the following factors:
    - Pathogenicity of the organism
    - Mode of transmission and host range
    - Availability of effective preventive measures (e.g., vaccines)
    - Availability of effective treatment (e.g., antibiotics)

Risk Assessment

Biosafety – Assessing the Risk

- Risk classification for World Health Organization, Australia, Canada, European Union, U.S.(NIH), and New Zealand:

Risk Group 1	Risk Group 2	Risk Group 3	Risk Group 4
Agents are not associated with disease in healthy adult humans. E.	Agents are associated with human disease which is rarely serious and for which preventive or therapeutic interventions are often available. *(i.e., S. typhimurium/ C. neoformans/Hep. B)*	Agents are associated with serious or lethal human disease for which preventive or therapeutic interventions may be available. *(i.e., Yersinia pestis/Histoplasma capsulatum)*	Agents are likely to cause serious or lethal human disease for which preventive or therapeutic interventions are not usually available. *(i.e., Ebola/Marburg/ Monkey B)*

Biosafety - Assessing the Risks

- **Biosafety Level (BSL)**
  - Biosafety Containment Level, handling practices, facility design; not directly correlated to RG.
  - Used by CDC/NIH in "Biosafety in Microbiological and Biomedical Laboratories (BMBL)"

BSL 1	BSL 2	BSL 3	BSL 4
BSL-1 is suitable for work involving well-characterized agents not known to cause disease in healthy adult humans, and of minimal potential hazard to laboratory personnel and the environment.	BSL-2 is suitable for work involving agents associated with human disease. Primary exposure routes include percutaneous exposure, ingestion, and mucous membrane exposure.	BSL-3 is for work associated with indigenous or exotic agents with potential for aerosol transmission. Disease may have serious health effects.	BSL-4 is required for work with dangerous and exotic agents which pose a high individual risk of aerosol transmitted laboratory infections and life threatening disease.

Currently, work at JSC is limited to BSL-1 and BSL-2 organisms.
For complete list of bacterial, viral and fungal agents and their corresponding biosafety levels and risk groups, please visit http://www.absa.org/resriskgroup.html

Risk Assessment

Microorganism Risk

Microorganism Risk ~ RG - Route of transmission, pathogenicity, ID

+

Microbiological Risk Assessment

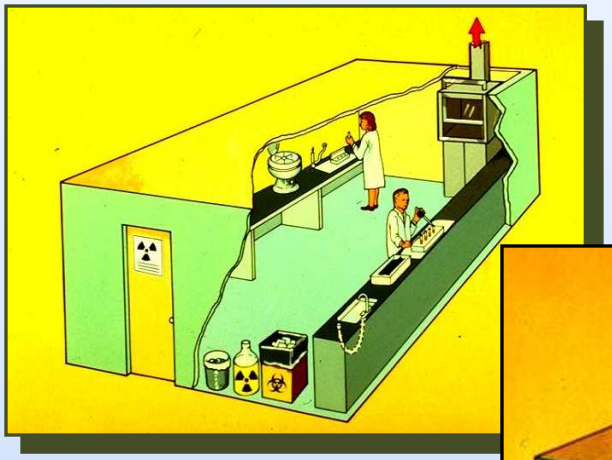
(Procedures, concentrations, Rx, host, etc.)

↓

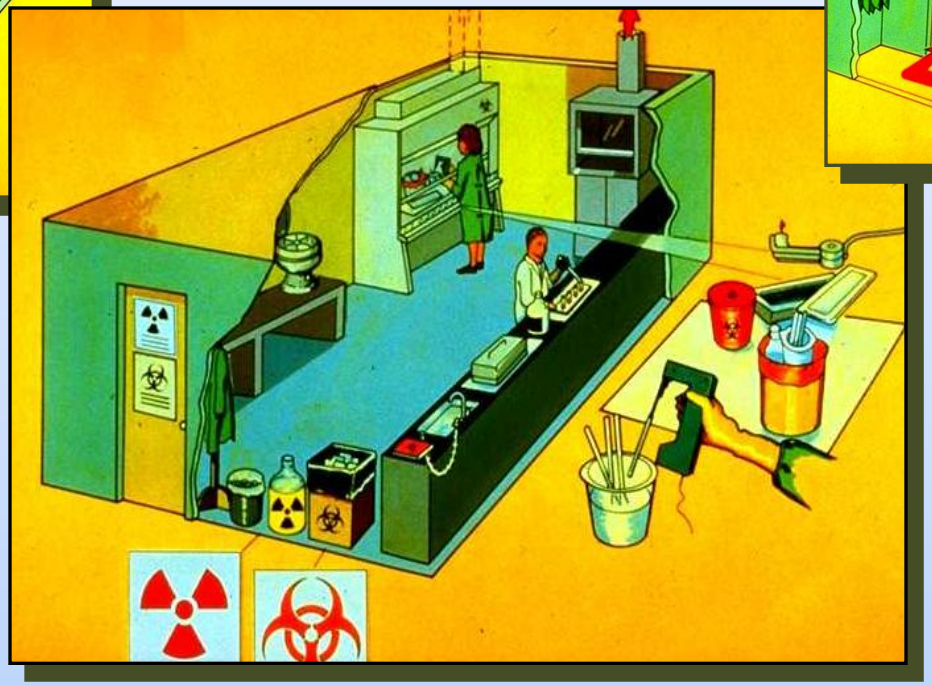
Biosafety Levels

1 2 3 4

Facility Design



BSL-1
Lower Risk



BSL-2 / Universal Precautions

BSL-3
Higher Risk

Risk Assessment

Biosafety – Assessing the Risk

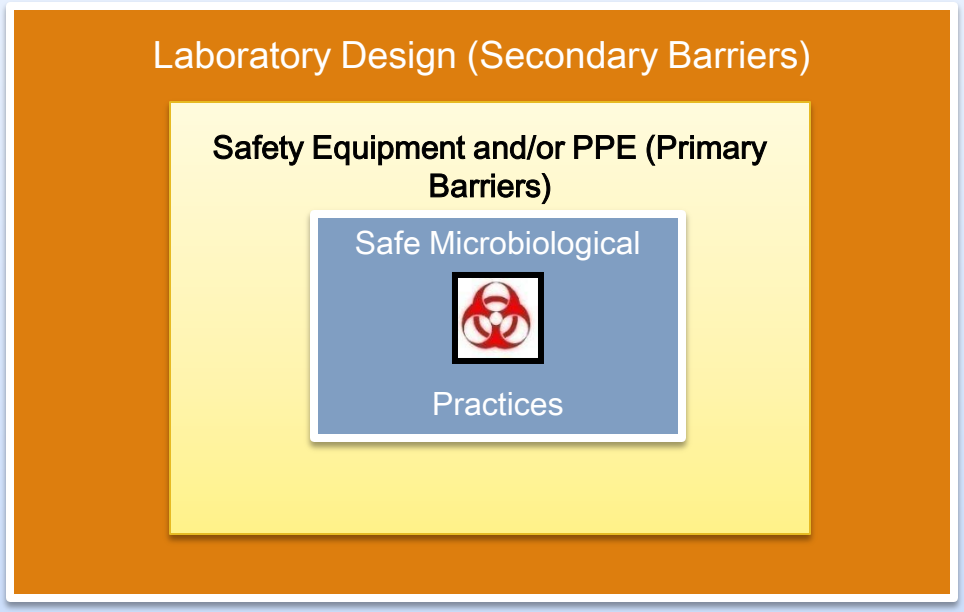
- **BSL-2 differs from BSL-1:**
  - laboratory personnel have specific training in handling pathogenic agents and are directed by scientists with advanced training;
  - access to the laboratory is limited when work is being conducted;
  - extreme precautions are taken with contaminated sharp items; and
  - certain procedures in which infectious aerosols or splashes may be created are conducted in biological safety cabinets or other physical containment equipment.

Reducing the Risk

- **Biological**
  - Use Attenuated or less virulent strains (*E. coli* K-12)
- **Physical**
  - Primary & secondary containment
    - Primary: physical separation of hazard from worker (i.e., personal protective equipment (PPE), biological safety cabinets, enclosed centrifuge containers)
    - Secondary: lab design, separation of hazard from others in facility and the environment, separate labs, unidirectional airflow
- **Risk Assessments/Safe Microbiological Practices/Training/Vaccinations**

Containment

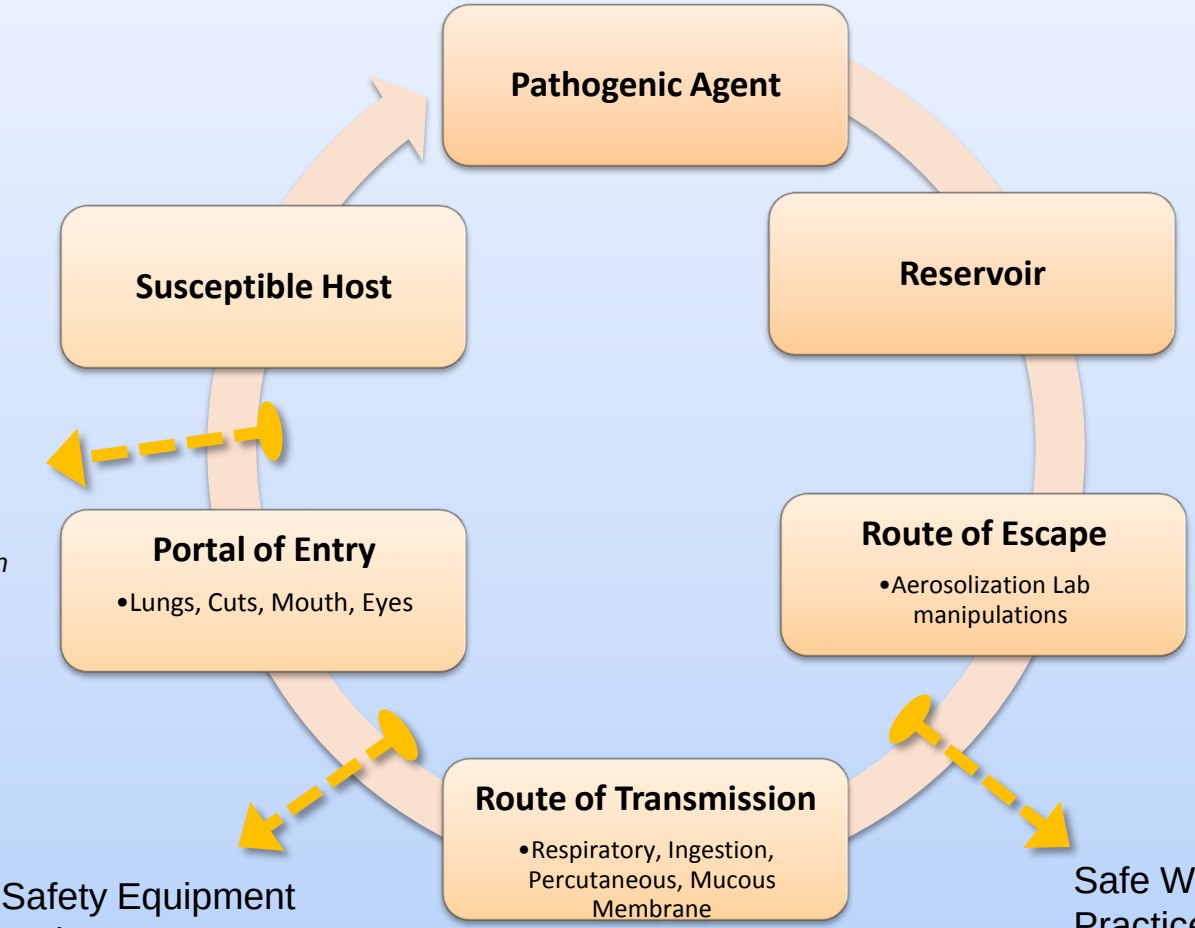
- Containment is used in describing safe methods for managing biological agents in the laboratory environment where they are being handled or maintained. The purpose of containment is to reduce or eliminate exposure of laboratory workers, persons outside the laboratory, and the environment to potentially hazardous agents.
- The three elements of containment include laboratory practice and technique, safety equipment, and facility design.
- The proper containment is determined by identification of the risk group and the appropriate biosafety level.
- The biosafety levels consist of combinations of laboratory practices and techniques, safety equipment, and laboratory facilities appropriate for the operations performed. They are also based on the potential hazards imposed by the agents used and for the laboratory function and activity.
- As a general rule, a biosafety level should be used that matches the highest Risk Group (RG) classification of the organism involved.



Essential Containment Components

Containment

Breaking the Chain of Infection



Vaccines*
Training, Rx*
**covered by Blood Borne pathogen training*

Safety Equipment and PPE

Safe Work Practices

Proper Biosafety Techniques

- Hands must be washed with soap and water frequently during the day after removing gloves, when gloves are torn or damaged, after spills, and before leaving the laboratory.
- All procedures are performed carefully to minimize the production of splashes or aerosols.
- Keep disinfectants and spill supplies close at hand.
- Work surfaces are decontaminated at the completion of work or at the end of the day and after any spills.
- When working with sharps extra-care should be taken and sharps should be disposed of in a hard-walled closeable container.
- Organize and plan work procedures with safety in mind and keep an uncluttered work space.
- Eating, drinking, smoking, applying cosmetics, and lip balm and handling contact lenses are prohibited in the laboratory.
- Closed-toed shoes are required in the laboratories.

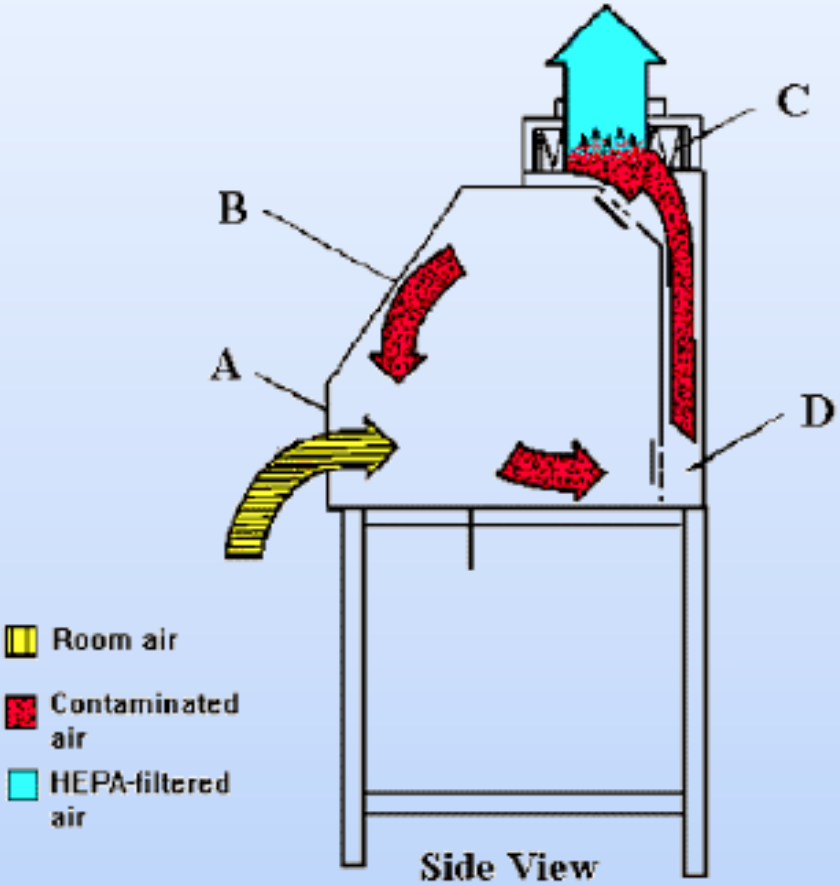
Biological Safety Cabinets (BSC)

- Biosafety cabinets are among the most effective and most commonly used primary containment devices when working with infectious agents.
- Biosafety cabinets complement careful work practices because aerosols can still escape the cabinet.
- All BSC have High-Efficiency Particulate Air (HEPA) filters.
- HEPA filters filter particulates down to size 0.3µm with an efficiency of ~ 99.97%
- As filters become loaded the pressure on the system should increase.
- If pressure is lower than the certification level, it could indicate a breach in the filter and the BSC should not be used.

Containment

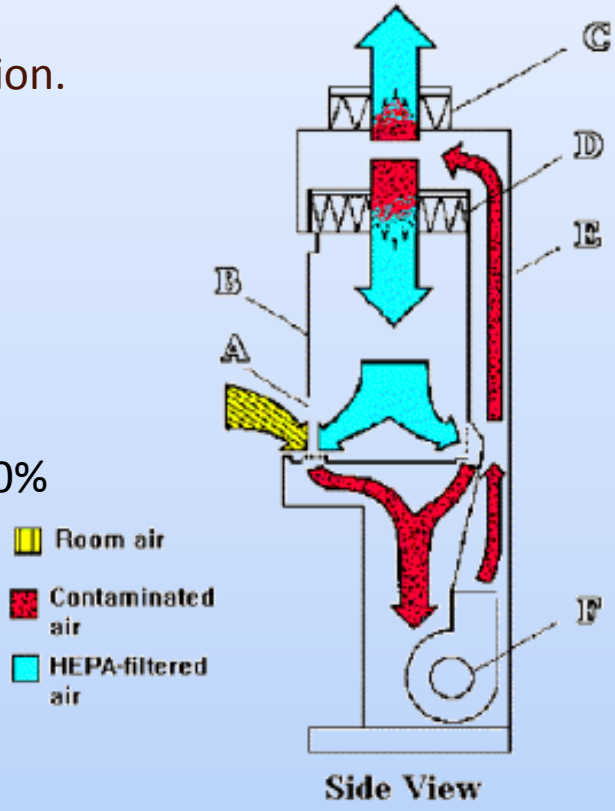
Biological Safety Cabinets (BSC)

- **Class I**
  - For BSL 1-3
  - Provides personnel and environmental protection, but no product protection.
  - It is similar in air movement to a chemical fume hood (+ HEPA filter)
  - All filtered air (100%) is exhausted into the lab or to the outside



Biological Safety Cabinets (BSC)

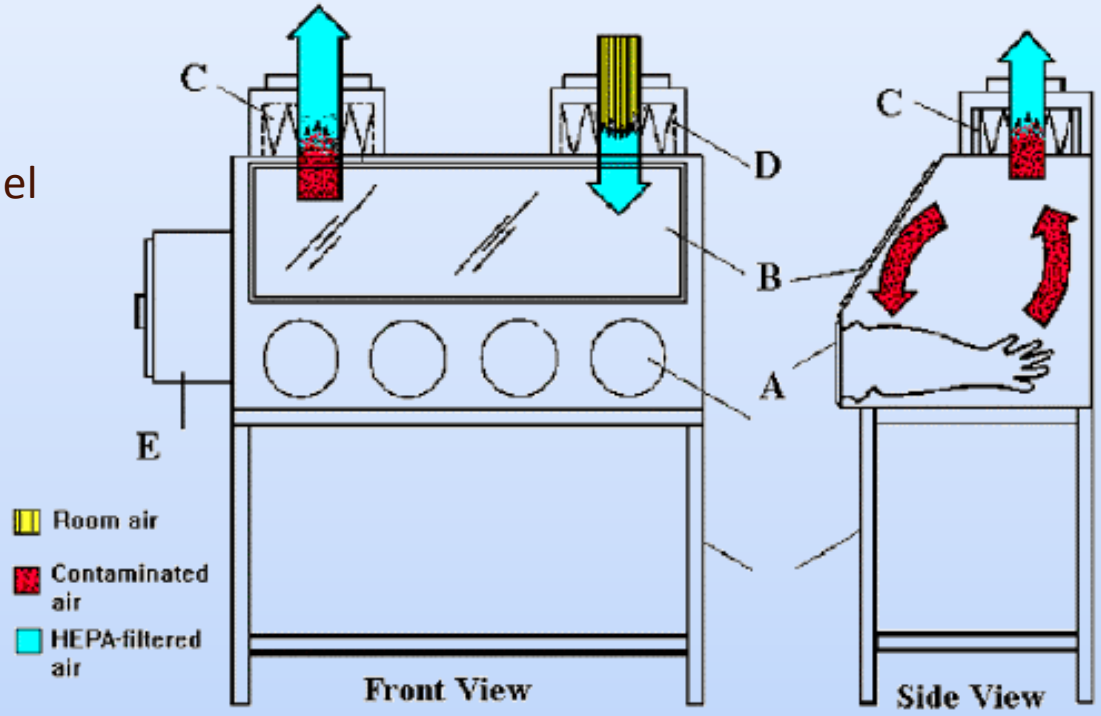
- **Class II Types A1, A2, B1 & B2**
  - For BSL 1-3.
  - Provides personnel, environmental, and product protection.
  - **NOT DUCTED** to Building Exhaust
    - Type A1 – 70% of air is recirculated
  - **DUCTED** to Building Exhaust
    - Type A2: 100% is exhausted
    - Type B1: Recirculates 30% of the air and exhausts 70%
    - Type B2: Exhausts 100% of the air



Containment

Biological Safety Cabinets (BSC)

- **Class III**
  - For BSL-4
  - Provides maximum personnel and environmental protection. Also provides product protection.
  - Negative pressure.
  - Double HEPA exhaust
  - Heavy duty rubber gloves



Biological Safety Cabinets (BSC)

- **Inward directional airflow is what protects you from the agents in the cabinet – Do not disturb it!**

- **Things that disrupt flow include –**
  - Sweeping sideways motion in the cabinet.
  - Repeated insertion and withdrawal of arms in and out
  - Opening and closing laboratory doors in the vicinity
  - Improper placement or operation of materials in the cabinet
  - Brisk walking past a BSC during use

Proper BSC Work Practices

- Check calibration and start-up BSC.
- Allow to run for 20 minutes to purge the air prior to working.
- Review procedure and plan your BSC layout – supplies, equipment, waste

Proper BSC Work Practices

- Disinfect cabinet and equipment going into cabinet.
- Do not place items over front grille, do not block back grille.
- Perform work 6 inches back of front intake grille. (use the middle of the cabinet)
- Use plastic backed towels on work surface to absorb spills.
- Minimize movement of contaminated items over clean items (work from clean to dirty).
- Clean items - front, contaminated - rear.
- Do not use a flame. Turbulence and filter damage may occur.
- Equipment that causes turbulence (centrifuge, blender, etc.) should be placed in back 1/3 of work surface. All other work in the cabinet should stop while apparatus is running.
- Waste disposal area – biohazard bags and disinfectant pans
- Remove contaminated items only after sealed or decontaminated.
- Decontaminate after work is complete with appropriate disinfectant.

BSC Certification

- New/at least annually
- When moved
- After maintenance
- Maintenance should be performed by NSF-certified personnel

Clean Bench is NOT a BSC

A clean bench is not a Biosafety Cabinet.

HEPA Filtered air blows into the employee's face protecting the samples – not the employee

Example of a Clean Bench

Ductless Fume Hood is NOT a BSC!

A ductless fume hood.

is not a BSC.

A ductless fume hood is used

for a variety of chemicals,

but not for Biosafety.

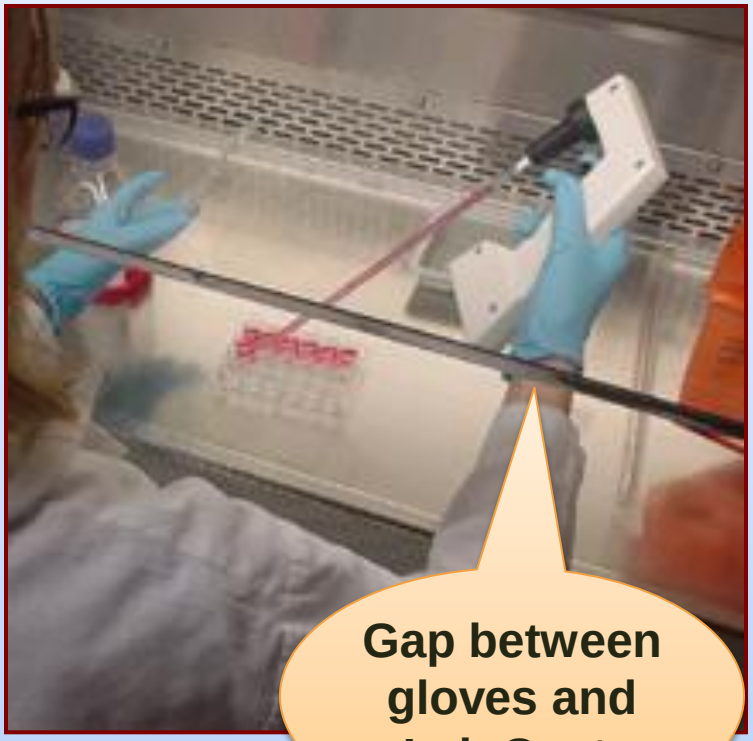
Example of a Ductless Fume Hood

Proper PPE Work Practices

- Minimum Personal Protective Equipment (PPE) requirements for BSL-2 are closed laboratory coat, one pair of gloves and closed-toed shoes
- Eye protection or splash guards in place for work that will produce aerosols.
- Wear PPE that is sized-properly.
- Don and doff gloves properly to prevent touching contaminated glove surfaces.

Proper PPE Work Practices – Gloves and Lab Coat

Incorrect



Correct

Facility Requirements for BSL-2

- Lockable doors (a must for restricted agents)
- Sink
- Bench tops/furniture impervious and easily cleaned
- Biological safety cabinet (if applicable)
- Eyewash
- Inward airflow (desirable)

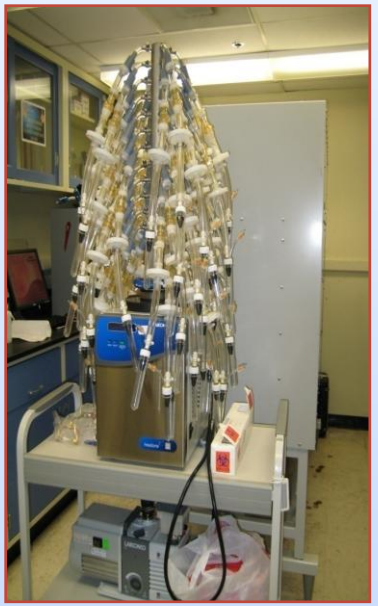
Aerosol Production

- Exposure to Aerosols can cause infection
- Aerosol-Producing Activities in the Lab
  - Centrifuges: *Use only sealed rotors or cups*
  - Vortexing
  - Rocking/shaking
  - Lyophilization
  - Sonification
  - Tissue Grinding
  - Pipetting: *Never blow out last drop in pipette, Use pipetting aids with filters, Discharge liquid down side of container, using tip-to-wall contact, Deliver as close as possible to contents*

Always wear eye protection when there is a chance for splashes!

Sealed Rotor

Unsealed rotor



Lyophilizer

Vacuum Line Protection

- Fill flask with disinfectant to ensure that when flask is 2/3 full disinfectant concentration is sufficient
- Empty flask when flask is 2/3 full
- Place disinfectant trap in a secondary container
- Ideal - plastic or plastic-coated flasks
- Label flasks with contents and NFPA diamond sticker

Decontamination

Autoclave

- An autoclave is a steam sterilizer that is used for biohazardous laboratory waste. In order to dispose of infectious laboratory waste (petri dishes, pipettes, culture tubes, glassware, etc.), the waste must be autoclaved for minimum of 15 minutes at 121 C (minimum 15 psi).
- The effectiveness of decontamination by steam autoclaving depends upon various loading factors that influence the temperature to which the material is subjected and the contact time.
- Particular attention must be given to packaging, including the size of containers and their distribution in the autoclave. Containers must be arranged in a manner that permits free circulation of steam.
- All processed items must have heat-sensitive tape, or other acceptable method (such as commercially available strips or vials of *Bacillus* species endospores or thermocouples), attached to confirm proper sterilization. Once the cycle has been completed, the waste can then be disposed of as medical waste.

Waste Disposal

- In lab - keep containers covered/closed!
- Use appropriate container
  - Plastic bags/cardboard
  - Red plastic bins are available for frozen items
- Do not over fill – use the 2/3 rule - must be able tie bags and close box
- Label box before putting in storage – Name, date and Room #

Labels and Signs

Labels and Signs

- Warning labels shall be affixed: to containers of regulated waste; incubators, refrigerators and freezers containing blood or other potentially infectious material; and other containers used to store, transport or ship blood or other potentially infectious materials.
- Biohazard signs must be posted at entrances to all biosafety laboratories. The labels shall be fluorescent orange or orange-red or predominately so, with lettering and symbols in a contrasting color.
- An Emergency Action Plan must also be placed in each laboratory.

Exposure / Spill Procedures

- Choose appropriate disinfectant and PPE
- Treat and allow to disinfect for specified time
- Dispose of all wastes including PPE in Biohazard
- Sharps Disposal - plastic or cardboard container

Further information on disinfectant selection can be found at http://www.cdc.gov/od/ohs/biosfty/bmbl5/BMBL_5th_Edition.pdf in Appendix B

Spill Cleanup

Spill Kits

- **Recommended kit contents–**
  1. Gloves (several pairs)
  2. Small disposable broom with dust pan, tongs, or forceps
  3. Red medical waste bags
  4. Disinfectant suitable for the biologically hazardous materials found in the lab (check expiration dates)
  5. Germicidal gelling agent (optional)
  6. Paper towels
  7. Diking material or spill pillows for large spills(optional)
  8. Instructions for spill clean-up

Reporting

Exposure

- **Needle sticks sharps incidence**
  - Notify supervisor
  - Report to JSC Clinic for treatment
- **If working with infectious agents, report any illnesses with a fever to your supervisor.**

Transportation

- Domestic and International Shipment: www.cdc.gov/od/ohs/biosfty/shipregs.htm
- Select Agent Transfer Tracking System: www.cdc.gov/od/ohs/lrsat.htm
- Import and Export permits for Biological Materials: www.cdc.gov/od/ohs/biosfty/imprtper.htm
- Organization specific protocols: examples: (JSC 290/Wyle SHE-WLS-319)
- IATA DGR training - specific to hazards being shipped

Biosecurity

Biosecurity at JSC

- **Biosecurity is a set of preventive measures designed to reduce the risk of loss and/or intentional removal (theft) of valuable biological material.**
- **Biosecurity at JSC is addressed at JSC by having the following safeguards in place.**
  - Risk and threat assessment;
  - facility security plans;
  - physical security;
  - security policies for personnel;
  - policies regarding accessing the laboratory and animal areas;
  - specimen accountability/inventory;
  - receipt of agents into the laboratory;
  - emergency response plans; and
  - reporting of incidents, unintentional injuries, and security breaches.

Recent Documented Exposures – *E.coli*

E.coli O157:H7, 2005, Spina et al, *J. Clin. Microbiology.*

- 4 lab workers in different diagnostic labs infected.
- Sudden increase in volume of specimens, low infectious dose, prolonged survival on stainless steel surfaces.
- Workers non-compliant with biosafety practices*, including no gloves, hands were not washed as needed, open lab coats*.

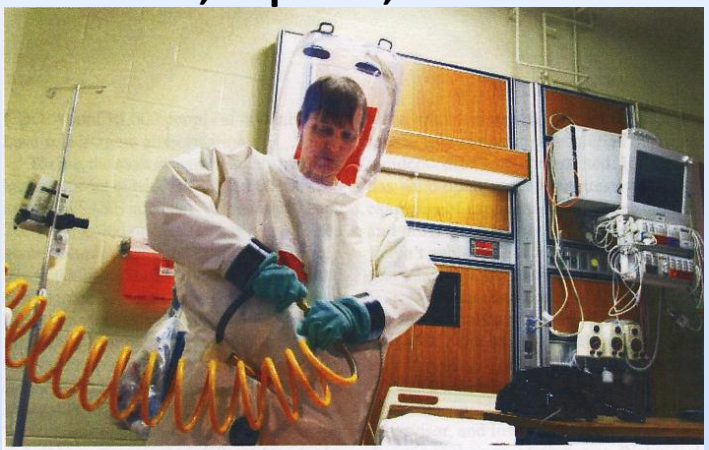
Recent Documented Exposures – Select Agent

- **Select Agent Exposures, 2006-7, Texas A&M**
  - lab worker infected with *Brucella melitensis*
    - Was not authorized to work with the agent (Select Agent Program)
    - Infected after reaching into the Madison Chamber to clean it after aerosol work with *B. melitensis*
    - Infection was not reported for over a year to CDC
    - 2 months after infection, 3 other lab workers exposed to *Coxiella burnetii* (Q fever), but were not reported.
    - July, 2007: CDC suspends research in SA lab

Exposures

Exposures

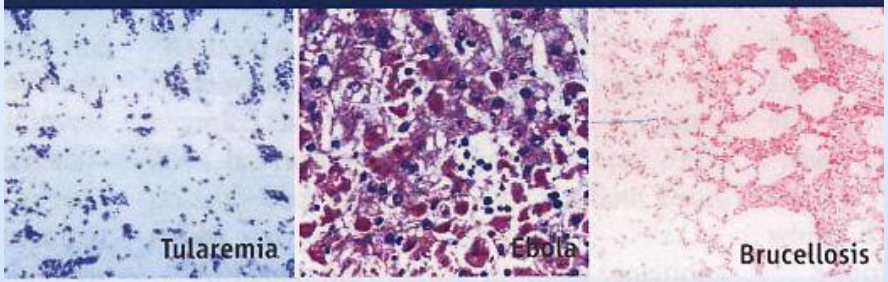
***Science*, Sept. 28, 2007**



BIOSAFETY BREACHES

Accidents Spur a Closer Look at Risks at Biodefense Labs

Some Recent Exposures in U.S. Biodefense Labs



2002, 2003: *E. coli* 0157:H7 infections in two USDA labs

2004: Three workers infected with tularemia, Boston University

2004: Ebola needle stick (no infection), USAMRIID

2004: Anthrax exposure (no infection), Children's Hospital, Oakland, CA

2004: Valley fever (*C. immitis*) infection, Medical College of Ohio

2005: Potential Q fever exposure, Rocky Mountain Labs, Hamilton, MT

2006: Brucellosis infection, Texas A&M

Biosafety Training Requirements

- Computer-based training every 2 years – all personnel handling BSL 1 and 2 materials

- Other related training-
  - One-time JSC PPE course
  - Annual BBP training – all personnel handling potentially infectious materials – JSC or Wyle
  - Annual Shipping of Biologicals training – personnel that ship Exempt Human Specimens – Wyle Bioastronautics
  - Shipping of dry ice – 2 years – personnel who ship samples on dry ice – Wyle Bioastronautics
  - Shipping of Infectious Substances – 2 years – personnel that ship infectious substances – Saf-T-Pak or other