



Biosafety in Biomedical Laboratories



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Major Safety Concerns

- Safe Handling & Containment of Infectious Agents
- OSHA Bloodborne Pathogens Standard
- Management of Biohazard Waste Material

Other concerns not addressed in this presentation:

- Recombinant & Synthetic DNA (Dr. Sollars)
- Human Subject Protection (ORI, Dr. Winger)
- Radioisotope Use (RSO, Dr. McCumbee)
- Use of Animals in Research (Dir. ARF, Dr. Howard)
- Chemical Safety (Health & Safety, Nathan Douglas)

General Principles of Biosafety

Purpose of Containment:

- To protect lab staff from exposure to hazardous biological agents
 - Bacteria, viruses, fungi, parasites
 - Recombinant DNA
 - Potentially dangerous cell lines
 - Transgenic animals or plants
 - Biological toxins
- To guard against the release of these biohazard materials

General Principles of Biosafety

- Three elements of containment:
 - Facility design (Engineering)
 - Lab practice and techniques (Administrative)
 - Personal Protective Equipment (PPE)
- Extent of containment depends on
 - Level of risk
 - Nature of biological agent



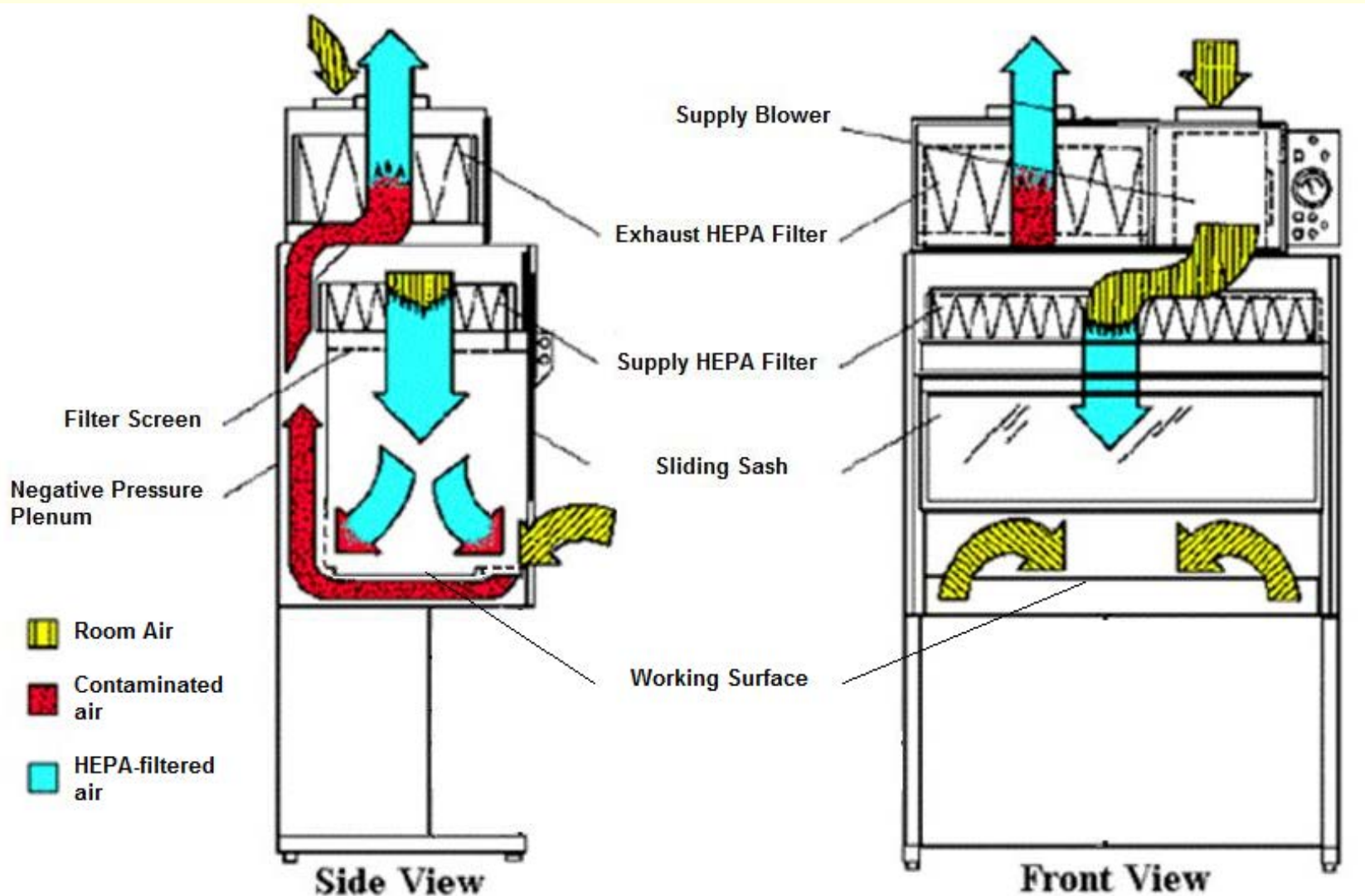
Primary Barriers - Safety Equipment

- Biological Safety Cabinet (Laminar Flow Hood)
 - protects you and the materials you are working with
- Chemical Fume Hood
- Centrifuge Safety Canister
- Sharps container & Broken glass box
- Biohazard waste container
- Personal Protective Equipment
 - gloves, lab coats, safety glasses, goggles, face shield, gowns, shoe covers

Biological Safety Cabinets (BSC)

- Class I and II BSCs provide an effective containment system for safe handling of moderate to high risk microorganisms
 - Biosafety Level (BSL) 2 and 3 agents
- Class II BSCs protect research material through high efficiency particulate air (HEPA) filtration.
- The Biotech building has shared facilities with Class II, Type A2 cabinets
 - Not suitable for use with radionuclides or toxic chemicals
 - 70% air recirculation & 30% exhaust to room

Biological Safety Cabinet Class II, Type 2A



Secondary Barriers - Facility Design and Construction

- Security measures and physical separation of lab work areas from areas of public access
 - Swipe card access to research hallways
 - Key access to research suites
 - Break areas outside of research areas
- NOTE: if fire is suspected, keep door closed to contain smoke
- Availability of decontamination stations
 - Handwashing and eye washing facilities
 - Emergency eyewash and shower stations in hall
 - Autoclave (only Room 119 for decon)
 - UV lights in Biosafety Cabinets – Use Caution
- Separate ventilation systems

Four Biosafety Levels (BSLs)

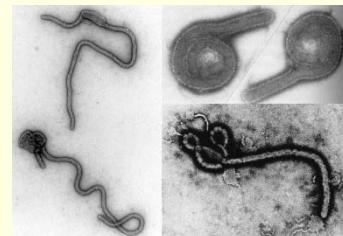
- BSL1-4 represent conditions under which the agent can be safely handled.
- Combinations of
 - lab facilities
 - lab practices
 - techniques
 - safety equipment
- Selected based on operations performed and routes of transmission of infectious agents and/or rDNA

Biosafety Levels 1-2

- BSL 1: appropriate for work with organisms known to not cause illness in healthy adult humans
 - *E. coli*, *Bacillus* spp.
 - Exempt categories of rDNA work
- BSL 2: indigenous, moderate risk agents
 - Hepatitis B, HIV, some *Salmonellae*
 - Human derived blood and blood products
 - Cell culture work and some rDNA
 - Primary Hazard: Skin break, mucous membrane exposure or ingestion

Biosafety Levels 3-4 (none in BBSC)

- BSL 3: indigenous or exotic agents with potential for respiratory transmission or lethal consequences
 - M. tuberculosis, St. Louis encephalitis virus (CNS complications and death)
 - Primary and Secondary barriers to protect personnel in contiguous areas
- BSL 4: lethal exotic agents where there is no vaccine or therapy
 - Ebola and Marburg viruses
 - Separate facility or HVAC isolated zone



Which Biosafety Level To Use

- Ask your lab head or primary investigator
- Read Centers for Disease Control's (CDC)
 - *"Biosafety in Microbiological and Biomedical Laboratories"*
- Go to American Biological Safety Association website for list of infectious agents and their BSL
<http://www.absa.org/resriskgroup.html>
- Read National Institutes of Health's (NIH)
 - *"Guidelines for Research Involving Recombinant DNA Molecules"*
- Contact Dr. Primerano by calling 304-696-7338, 304-208-3959 cell, or email primeran@marshall.edu
- All resources available on Marshall's Institutional Biosafety Committee web page: <http://musom.marshall.edu/biosafety>

Biosafety Level 1 Practices:

Known as “Standard Microbiological Practices”

- Access to laboratory is limited or restricted when experiments are in progress
- Persons wash hands after handling viable materials, after removing gloves, and before leaving lab
- Eating, drinking, smoking, handling contact lenses, and applying cosmetics are not permitted in the work areas. Food is stored outside the work area
- Mouth pipetting is prohibited; mechanical pipetting devices are used



Biosafety Level 1 Practices:

Known as “Standard Microbiological Practices”

- Policies for the safe handling of sharps are followed
- All procedures performed carefully to minimize the creation of splashes or aerosols
- Work surfaces decontaminated on completion of work or at end of the day, and after any spill or splash of viable material with disinfectant
- All cultures, stocks, and other regulated wastes are decontaminated before disposal. We use autoclave
- An insect and rodent control program is in effect
- Biohazard signage on outer lab doors

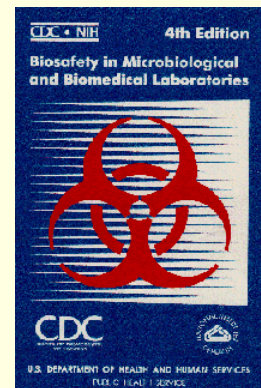
Biosafety Level 2 Practices:

Includes all practices from BSL-1, plus:

- Only persons who've been advised of the potential hazards and completed necessary immunizations may enter the lab
- Lab personnel get training on potential hazards associated with the work, precautions to prevent exposures, and exposure evaluation procedures
- Lab coats and eye/face protection are worn in the lab and are not worn outside of lab areas. Gloves are removed before exiting lab
- Extreme caution used with contaminated sharps to avoid autoinoculation and aerosol. Plasticware should be substituted for glassware when possible. Broken glass is not handled by hand, must be removed mechanically

General Biosafety Advice

- Discuss specific safety issues with your primary investigator or lab head
- Dr. Primerano is always available for questions at 304-696-7338, 304-208-3959 cell, and primeran@marshall.edu
- Labs should have written safety protocols that are lab/experiment specific
- Read “*Biosafety in Microbiological and Biomedical Laboratories*” as needed
- Review Animal Biosafety Levels when research involves infectious disease work with experimental animals



General Biosafety Advice

- Use a lab coat and appropriate safety equipment for all procedures
- Don't take lab coat home to wash it.
 - Using indelible ink, label the coat's tag with your initials and lab number
 - Place the coat in a labeled biohazard bag and give it to Julia Schreiber in room 119 or 121
- Don't wear open-toed shoes or sandals
- Keep hair short or tied back
- If you have glove allergies, vinyl or nitrile gloves may help

Work with Cell Lines and Cultures

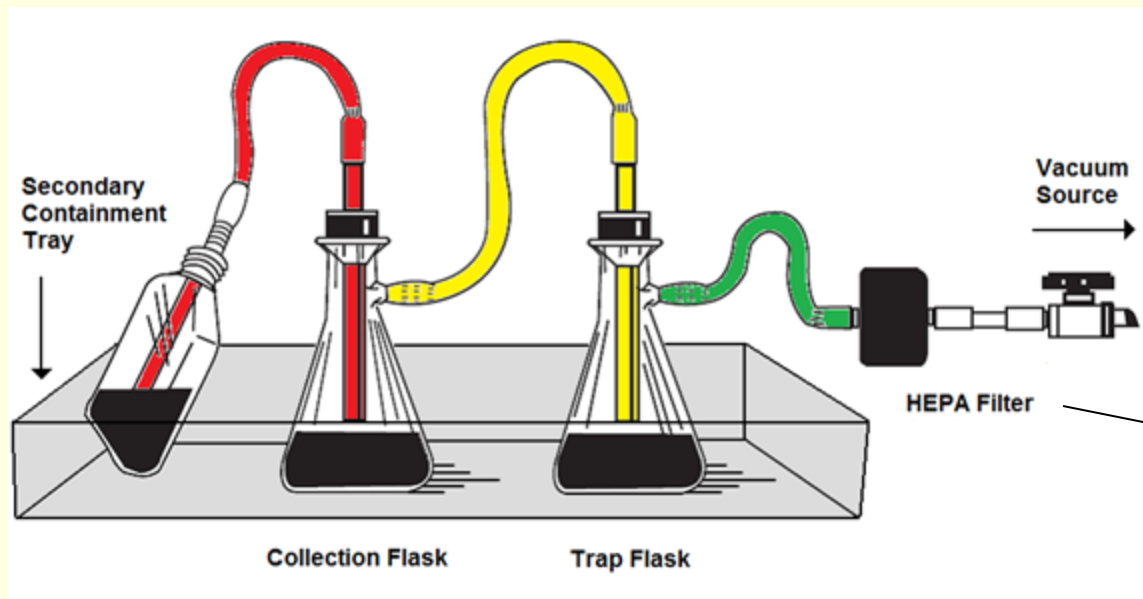
- Consider all cell lines and cultures infectious
- All work should be done in a biological safety cabinet
- Appropriate biosafety practices for handling cells known or suspected to contain a pathogenic virus or bacteria must be used when working with the cell culture
 - All primary and permanent cell lines must be handled using BSL-2 practices and containment
 - Any experiment involving transformed or cancer cell lines falls under BSL-2 practices

Work with Cell Lines and Cultures

- BSL-1 practices and containment may be used for cell lines that meet all of the following criteria. Cells must be:
 - non-primate
 - non-human
 - confirmed not to contain a primate virus, pathogenic bacteria, mycoplasma or fungi
 - well-established

Work with Cell Lines and Cultures

- Waste solutions collected properly



- All waste solutions and unused cells must be decontaminated for 24 hours before autoclaved
 - 10% bleach solution, at final concentration
 - bleach with 5.25% available sodium hypochlorite

Bloodborne Pathogens Standard

OSHA

Bloodborne Pathogen Standard

- A copy of the OSHA Bloodborne Pathogen Standard is available on the agency's web site

http://www.osha.gov/pls/oshaweb/owadisp.show_document?p_table=STANDARDS&p_id=10051

- Additionally, links to the BBP Standard are available on the following Marshall University web sites:
 - www.marshall.edu/safety/biological
 - musom.marshall.edu/biosafety

Bloodborne Pathogen Standard

Bloodborne pathogens are

- Pathogenic microorganisms present in human blood and can cause disease in humans
 - Includes, but are not limited to
 - hepatitis B virus (HBV) and
 - human immunodeficiency virus (HIV)
 - Others include:
 - hepatitis C, malaria, syphilis, babesiosis, brucellosis, leptospirosis, arboviral infections, relapsing fever, Creutzfeld-Jakob Disease, Human T-lymphotrophic Virus Type 1, and viral hemorrhagic fever

Bloodborne Pathogens

The following substances are specifically covered under the standard:

- Human blood,
- Blood components,
- Products made from human blood,
- Items contaminated with blood,
- Tissue and specimens

Bloodborne Pathogens

Other Potentially Infectious Materials (OPIM)

(1) The following human body fluids:

- cerebrospinal fluid, synovial fluid, pleural fluid, pericardial fluid, peritoneal fluid, amniotic fluid, semen, and vaginal secretions
- saliva in dental procedures,
- any body fluid that is visibly contaminated with blood
- all body fluids in situations where it is difficult or impossible to differentiate between body fluids

Bloodborne Pathogens

Other Potentially Infectious Materials (OPIM)

(2) Any unfixed tissue or organ (other than intact skin) from a human (living or dead)

(3) HIV-containing cell or tissue cultures, organ cultures,

- HIV- or HBV-containing culture medium or other solutions;
- blood, organs, or other tissues from experimental animals infected with HIV or HBV

Bloodborne Pathogens

- NOTE: While documented in sexual transmission, semen and vaginal secretions have not been implicated in occupational transmission
- Other body substances considered non-infectious include:
 - Feces, nasal secretions, saliva, sputum, sweat, tears, urine, and vomitus are not considered potentially infectious unless they contain blood
- The risk for transmission of HBV, HCV, and HIV infection from these substances is extremely low due to low quantities of viral particles

Exposure Pathways

- Percutaneous injury
 - cut or puncture from contaminated needles, broken glass, or other sharps
- Contact with non-intact skin
 - cut/abrasion, chapped, afflicted with dermatitis
- Contact with mucous membranes
 - splash in the eyes, nose, or mouth
- Inhalation of aerosols



Disease Transmission

Every exposure incident does not guarantee disease transmission. Several factors affect transmission:

- Infected Source - disease stage of the source
- Means of Entry - severity or depth of the puncture wound, broken skin, or direct contact with mucus membrane
- Infective Dose - the amount and type of fluid, as well as the amount of infectious agent in the fluid. Blood is the fluid of greatest concern
- Susceptible Host – immunocompromised at risk

Exposure Prevention

- The single most effective measure to control the transmission of Bloodborne Pathogens is:

Universal Precautions

- Treat all human blood and other potentially infectious materials like they are infectious for Hepatitis B and HIV



Exposure Prevention

Guidelines to reduce the risk of exposure:

- Frequent hand washing
 - Scrubbing with soap and warm water for 20 seconds (sing “Happy Birthday” twice through)
- Consistent use of PPE
- Vaccination against Hepatitis B virus
- Routine decontamination of work surfaces
 - Immediately after any spill/release
- Proper management of biohazard waste



Exposure Prevention - Aerosols

Guidelines to reduce the risk of exposure to aerosols during blending, grinding, sonicating, centrifuging, pipetting, etc.:

- All work should be conducted in biosafety cabinet where possible
- Allow aerosols to settle for 10 minutes before the container is opened
- Use safety blenders – no glass blender jars
- Place a disinfectant moistened paper towel on the blender during operation

Exposure Prevention - Aerosols

- Use plastic tubes instead of glass
- Sealed tubes and safety cups or buckets with an o-ring should be used for centrifuging
- After centrifuging, wait to open device for 5 minutes to allow aerosols to settle
- Open rotors & safety buckets in biosafety cabinet
- Collect all waste inside the biosafety cabinet
- A **shielded electric incinerator** or **hot bead sterilizer** should be used to minimize aerosol production, or use disposable loops

Hepatitis B Virus

- A DNA virus that primarily affects the liver
- Transmitted by exposures to infected blood and other potentially infectious material
- Initial infection may have no symptoms to flu-like symptoms, which include:
 - Fever, fatigue, nausea, abdominal pain, loss of appetite, jaundice, dark urine, and joint pain
- Incubation period avg. 90 days (60-150 days)
- Can develop into a chronic infection leading to cirrhosis, chronic active hepatitis, and liver cancer

Hepatitis B

- Prevalence rate of 0.45% in U.S.
 - Estimated 700,000 – 1.4 million chronic infection
- Statistically, the probability of being infected following an exposure to a known positive source is about 6-30%
- Hepatitis B is preventable through vaccination
 - a 3 shot series given over 6 months
 - Prevalence rate decrease in under 50 age group
- Hepatitis virus has been demonstrated to survive in dried blood at room temperature for at least 7 days, potentially up to 14 days

Hepatitis C

- An RNA virus, like HepB, primarily affects liver
- Prevalence rate of 0.96% in U.S.
 - At least 3 million US residents with chronic infection
 - Most common chronic bloodborne infection in the US
- Statistically, the probability of being infected following an exposure to a known positive source is about 1.8%
- No Hepatitis C vaccination
- Of greater concern due to
 - Larger number of chronically infected
 - Lack of vaccine prevention

HIV

- A retrovirus that can lead to AIDS
 - Damages CD4+ T cells that help the body fight diseases
 - AIDS is the late stage of HIV infection
 - Prior to AIDS, HIV can lead to many other diseases if untreated; medications can limit these problems
- Transmitted by exposures to infected blood and other potentially infectious material, frequently a needlestick
- Initial symptoms mirror many other illnesses, commonly flu-like symptoms
 - testing is the only proven way to establish transmission
- Incubation period is approximately 1 to 6 weeks

HIV

- Prevalence rate of 0.004% in US
 - Estimated 1.1 million actively infected
- The probability of being infected following an exposure to a known HIV positive source is about 0.3%
- HIV can only survive outside the body for several hours
- While the onset of AIDS may be delayed through drug therapy and opportunistic infections may be treatable, AIDS is at this time incurable and fatal.

Bloodborne Pathogen Standard

- Requires employers to provide Hepatitis B vaccination at no cost to employees
 - Available at Marshall Medical Center, Occupational Health & Wellness clinic
 - Diane Alcone, RN 304-691-1110, alcorn2@marshall.edu
 - Students conducting research should complete the vaccination series
- Personal Protective Equipment (lab coat, gloves, eye protection, etc.) must be available, **and used** when working with or around biohazard materials
 - Do not take lab coats home, they will be laundered at the Biotech or MEB

Bloodborne Pathogen Standard

- No eating, drinking, smoking, applying cosmetics or contact lenses in the labs
- No mouth pipetting; use pipetting device
- Proper disposal of biohazard materials
 - Liquids - 10% Bleach solution, then
 - Autoclave on-site
 - Items that cannot be autoclaved must be packaged for shipment to off-site treatment facility through a licensed transporter

Bloodborne Pathogen Standard

- OSHA also requires that the university have an Exposure Control Plan (on IBC web page)
- If you are working with human blood or OPIM, you must read and understand the Exposure Control Plan AND have met with Dr. Primerano
- Immediately report exposures to your supervisor and

Dr. Donald Primerano

304-696-7338 (w)

304-208-3959 (c)

Exposure Incident Response

All exposures must be properly addressed, regardless of severity

- Wash the exposed area with soap and water
 - Betadine soap is encouraged
- Remove any foreign material if present
- Disinfect with Betadine solution if available
- Flush splashes to eyes, nose, or mouth with cool running water for 15 minutes
- Report the exposure to supervisor and Dr. Primerano

Exposure Incident Response

After caring for your injury and notifying supervisor

- Go to Marshall Medical Center, Occupational Health clinic on 1st floor (304) 691-1187
- If after business hours, go to ER
 - Preferably Cabell-Huntington
- Tell medical staff that you've had a blood exposure
- Upon returning to work, fill out an exposure incident report
- Report all exposures, regardless of severity

Exposure Incident Response

- A confidential medical evaluation and follow-up will be made available to employees following an exposure incident.
 - Documenting route of exposure and circumstances of incident
 - Identifying and testing the source individual if feasible
 - Testing the exposed employee's blood if he/she consents
 - Providing post-exposure counseling and evaluation of reported illnesses

Management of Biohazard Waste Material

OSHA
and
WV Department of Health & Human
Resources

Infectious Medical Waste Rule

WV Legislative Rule 64 CSR 56

- Infectious Medical Waste is medical waste which is capable of producing an infectious disease.

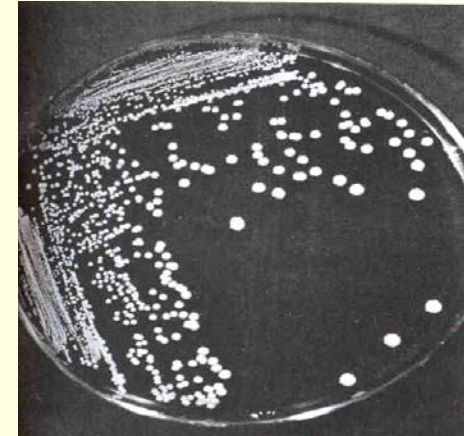
Medical waste shall be considered capable of producing an infectious disease if:

1. it has been, or is likely to have been, contaminated by an organism likely to be pathogenic to healthy humans,
2. if such organism is **NOT** routinely and freely available in the community, and
3. such organism has a significant probability of being present in sufficient quantities and with sufficient virulence to transmit disease.

Infectious Medical Waste Rule

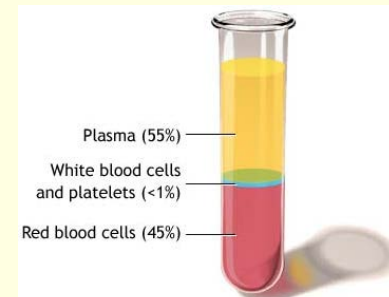
Infectious Medical Waste specifically includes :

- Cultures and stocks of microorganisms and biologicals
- Blood and blood products;
- Pathological wastes;
- Contaminated Sharps;
- Animal carcasses, body parts, bedding and related wastes;



Blood & Blood Products

- All human blood (wet or dried)
- Products from human blood (plasma)
- Does not include animal blood if not exposed to infectious agent
 - However, all blood will be managed and disposed in biohazard waste containers
 - This policy aides in visual checks for proper waste management



Sharps

- Any article that can puncture or cut
- Broken glass containers can be used for contaminated glassware if the box has a biohazard label on it
- Sharps boxes & contaminated glass containers will be autoclaved
- Do not bend, shear, or recap needles
- Do not overfill sharps containers
- Make sure lids are securely closed before the container is taken to the autoclave room



Pathological Waste

- Includes: tissues, organs, body parts, and containers of body fluids
- Unfixed pathological waste cannot be autoclaved and must be shipped off-site for treatment via incineration
- This waste must be packaged by someone trained as a US DOT Hazmat Employee
- Contact Connie Berk or Julia Schreiber (336R or 696-7341) if you need assistance with path waste disposal

Animal Waste

- Contaminated animal carcasses, body parts, animal bedding known to have been exposed to infectious agents during research
- These items, like pathological waste, must be shipped off-site for treatment
- Contact Connie Berk or Julia Schreiber (336R or 696-7341) for assistance



"Hey! This is the nonsmoking cage."

Infectious Medical Waste Management Plan

OBJECTIVES:

1. Provide a safe environment for faculty, staff, students and visitors
2. Properly manage infectious wastes in accordance with WV Legislative Rule 64 CSR 56, Infectious Medical Waste
 - Biotech & MEB Plan: Sterilize all bacterial, fungal, viral and parasitic organisms and cultured cells used in research.
 - If you have questions please contact Don Primerano (696-7338), Connie Berk (696-7341), or Nathan Douglas (696-3461)

IMW Packaging

- Orange biohazard bags must be used for infectious waste, clear and red bags are not acceptable
- Waste collection containers must be double-bagged
- Do not fill them more than 2/3 full
- Loosely gather the top of the bag and place a strip of autoclave tape around it, do not tie the bags closed. Steam must be able to enter the bag.
- Bags, flasks, and other containers of infectious material must be labeled with primary investigator's name, room number, phone number, and the contents.
 - Labels are available through Connie or Julia

IMW Packaging

- Labs must have separate containers for contaminated and uncontaminated glassware
- Broken glass boxes are acceptable, and must include a biohazard sticker when used for contaminated items
- Uncontaminated glass must go into a box labeled “Broken Glass”
- Contaminated pipette tips must be collected in a sharps box; or in a box with an orange bag, then placed in biohazard waste
- Contaminated Pasteur pipettes must go into a double-bagged, biohazard-labeled contaminated glassware box or a sharps box

Transporting Waste To Autoclave

- All waste must be transported on/in carts with secondary containment trays
- Infectious waste may not be sterilized in the satellite autoclaves on the floors
- Containers of liquid infectious waste are to be placed on the metal cart in the waste room
- Bags of solid infectious waste must be placed in the locked, grey bin in the waste collection room:

BBSC 119A, MEB 110, TGRI Autoclave Room

Ethidium Bromide

- EtBr contaminated waste is collected in waste receptacles in the following areas:
 - BBSC - 333
 - CEB - 214
 - TGRI - 272
- Place waste in a regular trash bag or other suitable container, not a biohazard bag, to prevent fluid leaks
- The packaged waste should be taken to the appropriate waste receptacle in the collection areas as noted above

Spill Response

- Notify Dr. Primerano, or Connie Berk, as soon as possible
 - Dr. Primerano: 696-7338 or 304-208-3959 (c)
 - Connie: 696-7341 or 304-429-7318 (h)
 - If you cannot reach either of them, contact
Nathan Douglas: 696-3461 or 304-208-7385 (c)
- Request assistance if needed or you are unsure what to do
- Spill kits are in the satellite autoclave rooms on every floor, and in rooms 119 and 121 of BBSC

Spill Response

- Attempt to prevent liquids from running free by laying paper towel or other absorbent material on top
- Do not use spill cleanup kit unless you are comfortable doing so, allow someone trained to clean the spill
- Either way, make sure you notify someone right away
- Complete a Biohazard Spill Occurrence form, available on IBC web page:

<http://musom.marshall.edu/biosafety>

Non-Infectious Items

- Paper towels, either from drying your hands or from disinfecting benches
- Wrappers from scalpel blades, needles, pipettes, etc.
- Benchkote or any other bench protector unless grossly contaminated
- Gloves and pipettes, unless contaminated
- Food or drink containers (not allowed in labs)
- Packaging materials such as cardboard, Styrofoam “peanuts”, etc.
- Paper (copy paper, etc.)
- Many items can be recycled in the blue bins in the middle hallway.

Lab Inspections

- Environmental Safety & Health conducts laboratory inspections to ensure compliance with all of the regulatory requirements
- Labs should conduct self-assessments routinely to ensure they are compliant
- State and Federal regulatory agencies have and will continue to inspect our labs as well
 - If your lab is visited by an inspector from a government agency:
 - Be polite, ask them to wait, and contact Safety if not already present

Review

- We care about biosafety to prevent the spread of bloodborne pathogens
- OSHA Standard covers HbV & HIV
- Everyone working with or around pathogens should have completed the 3-shot Hepatitis B vaccine series
- Gloves are used to protect us as we work
- Gloves must be removed before exiting laboratories, and hands should be washed.
- If items must be transported, use a cart.
- If you must carry something, use only 1 gloved hand, use the other to open doors.

QUESTIONS?

Contact Nathan Douglas: 696-3461,
douglas2@marshall.edu

Thank you for your time and attention.