Biosafety in Biomedical and Biological Laboratories

Marshall University

June 2, 2021

Please sign attendance roster

Biological Safety Officer

Vincent Sollars
Marshall University Dept of Biomedical Sciences
Biotech Center Room 336

(304) 696-7357
681.203.4119 (cell)
sollars@marshall.edu

Major Safety Concerns

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

- Other Safety Concerns:
- Recombinant & Synthetic DNA (Dr. Sollars)
- Radioisotope Use (RSO, Dr. McCumbee, June 8)
- Chemical Safety Video

General Principles of Biosafety

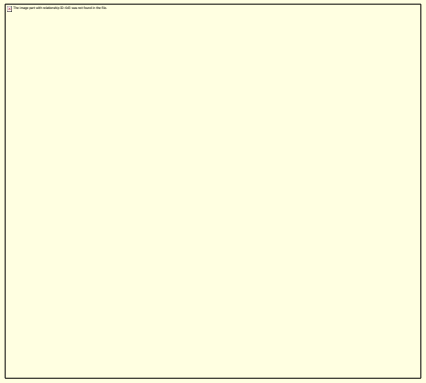
Purpose of Containment:

- To protect lab staff from **exposure** to hazardous biological agents
  - Bacteria, viruses, fungi, parasites
  - Recombinant DNA (rDNA)
  - 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 Practices)
  - Lab practice and techniques
  - Personal Protective Equipment (PPE)

- Extent of containment depends on
  - Level of risk of exposure or release
  - Nature of biological agent



Primary Barriers – Equipment + PPE

- Biological Safety Cabinet (Laminar Flow Hood)
  - protects you and the materials you are working with
- Centrifuge Safety Canisters
- Sharps containers and broken glass boxes
- Biohazardous waste container
- Personal Protective Equipment
  - gloves, lab coats, safety glasses, goggles, face shield, gowns, shoe covers

Biological Safety Cabinets (BSC)

- Class II BSCs provide an effective containment system for safe handling of moderate to high-risk microorganisms
- Class II BSCs protect research material through High Efficiency Particulate Air (HEPA) filtration.
- The Biotech Center has Class II, Type A2 cabinets in shared rooms and some individual labs
  - Designed to provide filtered air to the work area and protection against exposure to agents inside
  - Not suitable for use with radionuclides or toxic chemicals
  - 70% air recirculation & 30% exhaust to room
  - Some equipped with UV lights

BSC Use Videos

- https://www.youtube.com/watch?v=96-aZLom340
- https://www.nuaire.com/resources/working-in-your-biosafety-cabinet-video

Biological Safety Cabinet
Class II, Type 2A

Secondary Barriers - Facility Design and Construction

- Security measures/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
- Decontamination stations
  - Handwashing sinks in every lab
  - Emergency eyewash and shower stations in hall
  - Autoclaves (only Room 119 for decontamination)
  - UV lights in Biosafety Cabinets – Use Caution
- Separate ventilation systems

Four Biosafety Levels (BSLs)

- BSL1-4 represent lab designs and practices under which the agent can be safely handled.
- Combinations of
  - lab facilities
  - lab practices
  - techniques
  - safety equipment
- Procedures are selected based on lab methods and potential 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 disease-causing agents
  - Hepatitis B, HIV, some Salmonellae
  - **Human derived blood and blood products**
  - **Cell culture work and some rDNA molecules**
  - Primary Hazard: Skin break, mucous membrane exposure or ingestion

Biosafety Levels 3-4 (none at MU)

- BSL 3: indigenous or exotic agents with potential for respiratory transmission or lethal consequences
  - M. tuberculosis, St. Louis encephalitis virus (CNS complications and death), SARS-CoV-2*
  - Primary and Secondary barriers needed 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 Do I Use?

- **Ask your lab head or primary investigator.**
- Each lab has specific protocols for handling for infectious agents, blood, toxins and rDNA
- **In your spare time…**
  - Read Centers for Disease Control's (CDC) "*Biosafety in Microbiological and Biomedical Laboratories*" https://www.cdc.gov/labs/BMBL.html
  - Go to American Biological Safety Association website for list of infectious agents and their required BSL http://www.absa.org/resriskgroup.html
  - Read NIH "Guidelines for Research Involving Recombinant DNA Molecules"

Which Biosafety Level Do I Use?

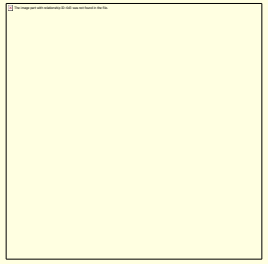
- Contact
  - Dr. Primerano at 304-696-7338 (primeran@marshall.edu) or Dr. Sollars at 304-696-7357 (Sollars@marshall.edu)

- All resources including this PowerPoint are available on Marshall's Institutional Biosafety Committee web page: https://jcesom.marshall.edu/biosafety

Biosafety Level 1 Practices:

Known as “Standard Microbiological Practices”

- Lab head provides training in lab methods and safe practices
- Access to laboratory is limited or restricted when experiments are in progress
- Persons wash hands after handling viable agents, 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 must be used



Biosafety Level 1 Practices (continued):

- PPE is provided to all lab workers
- Follow policies for the safe handling of sharps
- Follow procedures to minimize the creation of splashes or aerosols
- Decontaminate work surfaces on completion of work or at end of the day, and after any spill or splash of viable material with disinfectant
- All cultures, stocks, blood products must be decontaminated by autoclaving in most cases

- Biohazard signage on outer lab doors

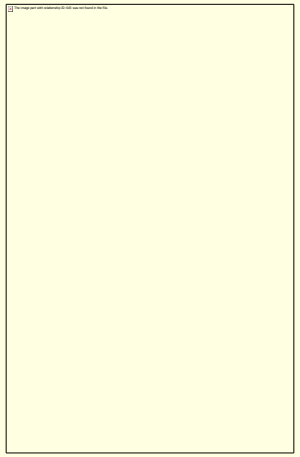
Biosafety Level 2 Practices:

Includes all practices from BSL-1, plus:

- **Lab specific safety manual** is provided to all lab members
- Only persons who have 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.
- Handwashing after work is complete and before leaving the 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 the lab head or designee
- Drs. Sollars and Primerano are always available for questions
- Labs should have written safety protocols that are lab or experiment specific
- Read "*Biosafety in Microbiological and Biomedical Laboratories*" (6th edition)
- Review Animal Biosafety Levels when
  - research involves infectious disease work with experimental animals



General Biosafety Advice

- Use a lab coat and additional safety equipment for all procedures
- Don’t take your 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

- **Treat tissue culture, cell lines and cultures as if they carry infectious agents**
- Most cell culture 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 mammalian primary and permanent cell lines must be handled using BSL-2 practices and containment. Must use Biosafety Cabinet!!**
  - **Any experiment involving transformed or cancer cell lines also 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

Bloodborne Pathogens Standard

Occupational Safety and Health Administration (OSHA)

Bloodborne Pathogen Standard

- OSHA's Bloodborne Pathogens Standard (29 CFR 1910.1030) is a federal regulation that prescribes safeguards to protect workers against health hazards associated with **human blood/ bloodborne** pathogens.
- The standard has provisions for **exposure control plans**, **engineering and work practice controls**, **hepatitis B vaccinations**, **hazard communication** and training, and recordkeeping.
- The standard imposes requirements on employers of workers who may be exposed to blood or other potentially infectious materials such as certain tissues and body fluids. BBP Exposure Control Plan

Bloodborne Pathogens

- Bloodborne pathogens are pathogenic microorganisms present in human blood that can cause disease in humans
  - Includes but are not limited to hepatitis B virus (HBV) human immunodeficiency virus (HIV), malaria, syphilis and others

Bloodborne Pathogen Standard

- A copy of the OSHA Bloodborne Pathogen Standard is available on the agency's web site: https://www.osha.gov/
- Links to the BBP Standard are available on the following Marshall University web sites:
  - www.marshall.edu/safety/biological
  - https://jcesom.marshall.edu/biosafety

Bloodborne Pathogens: Substances

Work with the following substances are specifically covered under the OSHA standard:

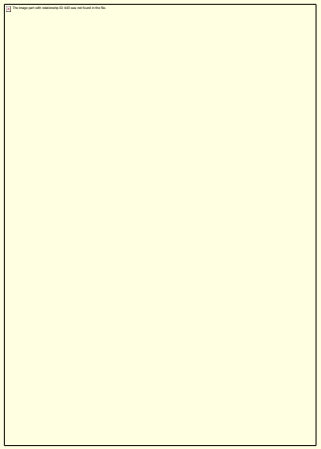
- Human blood,
- Human blood components,
- Products made from human blood,
- Items contaminated with blood,
- Human tissue and specimens

Bloodborne Pathogens

- Other Potentially Infectious Materials (OPIM)
  - Bodily fluids (including 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
  - Any unfixed tissue or organ (other than intact skin) from a human (living or dead)
  - HIV-containing cell or tissue cultures, organ cultures,

Exposure Pathways

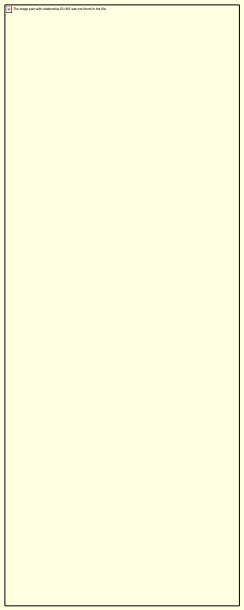
- Percutaneous injury
  - cut or puncture from contaminated needles, broken glass, or other sharps
- Contact with non-intact skin
  - cut/abrasion, chapped, afflicted with dermatitis
- Contact with mucous membranes
  - splash in the eyes, nose, or mouth
- Inhalation of aerosols



Exposure Prevention

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

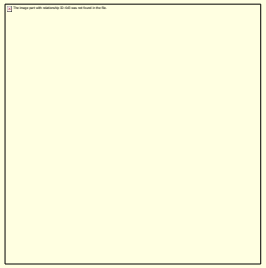
Universal Precautions



- Treat all human blood, blood products and tissues as if they harbor infectious agents (such as Hepatitis B and HIV)

Exposure Prevention Guidelines

- 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 when possible
- Allow aerosols to settle for 10 minutes before the container is opened
- Use blenders designed for the research lab

Exposure Prevention - Aerosols

- Use plastic tubes instead of glass when possible
- Sealed tubes and safety cups or buckets with an o-ring should used for centrifugation
- 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

Bloodborne Pathogen Standard

- Requires employers to provide Hepatitis B vaccination at no cost to employees
  - HepB shot is available at Marshall Medical Center (G. Ellis) or Cabell County Health Dept)
  - Students conducting research should complete the vaccination series
- **If haven't received the HepB vaccine or need other vaccinations for your work, please see me afterwards to arrange for the vaccinations**

Bloodborne Pathogen Standard: PPE Requirements

- Personal Protective Equipment (lab coat, gloves, eye protection, etc.) **must be available and used** when working with blood products or other biohazard materials
- Do not take lab coats home, they will be laundered at the Biotech or CEB.

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; same for most solids
  - Items that cannot be autoclaved must be packaged for shipment to off-site treatment facility through a licensed transporter

Bloodborne Pathogen Standard

- BBPS also requires that the university have an Exposure Control Plan (on IBC web page)
- **If you are working with human blood, human tissue or other blood products, 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.

- Immediately…
- 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 (eye wash stations)
- **Report the exposure to supervisor and Dr. Primerano**

Exposure Incident Response

After caring for your injury and notifying your supervisor or Collateral Duty Safety Officer

- Go to Marshall Medical Center, Occupational Health clinic on 1st floor (304) 691-1110 (1600 Hal Greer Blvd)
- If after business hours, go to ER
  - Preferably Cabell-Huntington or St. Mary's
- Tell medical staff that you've had a blood exposure
- Upon returning to work, fill out an exposure incident report available on the IBC website
- Report all exposures, regardless of severity

Post Exposure Follow-Up

- 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 Biohazardous Waste Material

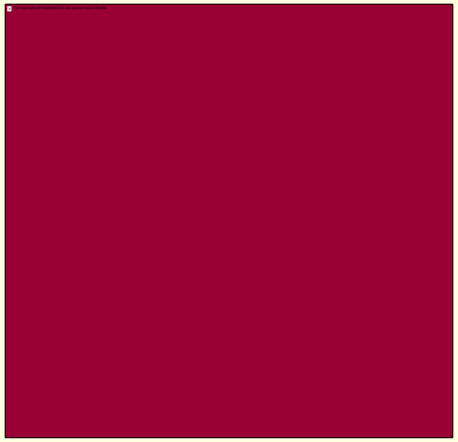
OSHA and WV Department
of Health and Human Resources

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

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

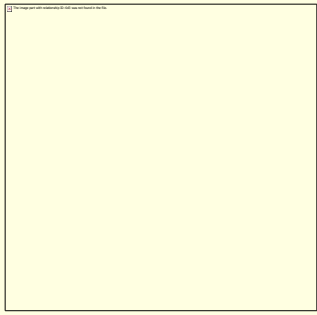


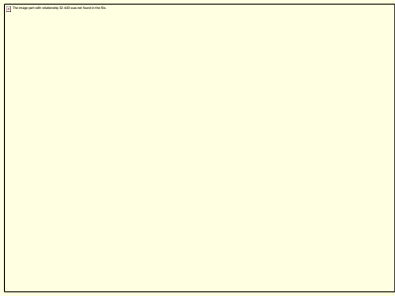
Blood & Blood Products

- 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 (needles, razors, scalpels..)

- Any tool 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





Pathological Waste

- Includes: human tissues, organs, body parts, and containers of body fluids
- Unfixed pathological waste and solid tissue cannot be autoclaved on site and must be shipped off-site for treatment via incineration
- This waste must be packaged by someone certified as a US DOT Hazmat Employee
- Contact Julia Schreiber (121 BBSC, 304-617-7922 (cell) or 696-3714 (w) or Margaret McFarland (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 or rDNA during research
- These items, like pathological waste, must be shipped off-site for incineration. (Stericycle)
- Deposit infected animals in the red plastic box in animal care facility cold room (116A)
- Contact Julia Schreiber for assistance

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

- Plans for the Biotech Center, Kopp Hall, WAEC and CEB Plan: Sterilize all bacterial, fungal, viral and parasitic organisms and cultured cells used in research.

Infectious Medical Waste 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 Julia Schreiber

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 2nd 3rd and 4th floors
- Containers of liquid infectious waste are to be placed on the metal cart in the waste room (119A BBSC)
- Bags of solid infectious waste must be placed in the locked, grey bin in the waste collection room (119A BBSC)
- BBSC 119A, CEB 214

Ethidium Bromide Waste

- EtBr contaminated waste is collected in waste receptacles in the following areas:
  BBSC – 333 (Satellite Autoclave Room)
  CEB - 214
- Place waste in a regular trash bag or other suitable container, not an orange biohazard bag, to prevent fluid leaks

Spill Response

- Notify Dr. Primerano, Vincent Sollars or Julia Schreiber as soon as possible
  - Dr. Primerano: 696-7338 (w) or 304-208-3959 (C)
  - Julia: 696-7341 or 304-617-7922 (c)
  - Vincent Sollars: 696-7357 or xxxx (c)
- Request assistance if needed or you are unsure what to do
- Spill kits are in the satellite autoclave rooms on every floor, and rooms 119, 121 and126 BBSC

Spill Response

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

  http://jcesom.marshall.edu/biosafety

Review

- We care about biosafety to prevent the spread of infectious agents and rDNA
- Make sure you receive initial training from your lab supervisor
- OSHA Standard covers Bloodborne pathogens
- Everyone working with or around pathogens should have completed the 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 from lab to lab, use a cart.
- If you must carry something, use only 1 gloved hand, use the other to open doors.

University Mask Guidelines
Updated MAY 26, 2021

- In accordance with new guidance from the U.S. Centers for Disease Control [**https://www.cdc.gov/coronavirus/2019-ncov/vaccines/fully-vaccinated.html**], Marshall University has updated its mask requirements for students and employees.
- Effective immediately, people who are fully vaccinated* are no longer required to wear a mask in university buildings, except in in-person instructional settings like classrooms, laboratories, studios, etc. For now, the mask requirement in those spaces remains in effect for everyone, regardless of vaccination status.
- Please note that if you have a condition or are taking medications that weaken your immune system, you may NOT be fully protected even if you are fully vaccinated. Talk to your health care provider. Even after vaccination, you may need to continue taking all precautions.

University Mask Guidelines Updated **MAY 26, 2021**

- Marshall University strongly encourages every member of the university community to get vaccinated. If you have not yet received the vaccine, visit the university's coronavirus website [**https://www.marshall.edu/coronavirus/return-to-campus-spring-2021/testing-and-vaccine-information/**] for clinic information.
- Students who plan to take classes this fall are required to complete the Student Vaccination Registry [**https://mubert.marshall.edu/vaccinerecord.php**].
- Please address any questions to **COVID19@marshall.edu**.

SARS-CoV-2

- CoV-2 vaccine is available and strongly recommended
- If you've completed the vaccination series, you don't need to wear masks in labs
- If you haven't completed vaccination series, you must wear a mask

QUESTIONS?

Contact Don Primerano at 304-696-3461, primeran@marshall.edu or Vincent Sollars at 304-696-7357 (sollars@marshall.edu)