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| BIOHAZARD CONTAINMENT |
| **Purpose** | This policy provides instructions for containing and assessing potential biohazard/infectious agents.Microbiologists/Virologists are subject to occupational risks associated with constant exposure to infectious agents. The greatest risk of infection is associated with the processing of primary specimens and manipulating the pathogens that are isolated. Four elements are necessary in order to initiate an infection, i.e., a susceptible host, an infectious agent, the concentration of the agent, and a route of transmission. Of these, the route of transmission is the least difficult to control through biosafety guidelines. The most common routes of transmission in the laboratory are oral, respiratory, percutaneous, and by direct contact with the skin and/or mucous membranes. The CDC and NIH have developed guidelines for the safe handling of microorganisms and have classified them into different biosafety levels (BSL). |
| **Policy Statements** | Children’s Microbiology/Virology Laboratory follows BSL 2 practices and uses class II biosafety cabinets. Microorganisms to be handled at BSL 2 are generally of moderate potential hazard to personnel and are usually associated with human disease, (e.g., hepatitis B virus, *Salmonella sp.,* and *Shigella sp.*) |
| **Responsibility** | All personnel that work in the microbiology and virology section are responsible for complying with this policy. |
| Principle | The biosafety risk management plan involves laboratory design, containment devices and administrative controls. **BSL 2 laboratory design and containment devices**1. Laboratory access should be limited to authorized personnel only. Doors are to be kept closed.
2. Sinks for hand washing are readily available. The sink should be foot or automatically operated and located near the laboratory exit.
3. An eyewash station is available in Microbiology.
4. A shower is available in Microbiology.
5. **ALL** specimen processing and any procedure producing spatters and /or aerosols should be performed in a BSC. All class II biosafety cabinets utilize HEPA laminar flow filters and are exhausted to the outside. BSC must be certified annually for proper functioning.
6. All sharps should be placed in puncture-resistant containers with biohazard labels.
7. All work surfaces should be decontaminated after a spill and on a routine daily basis.
8. PPE (personal protective equipment) such as lab coats and gloves, are available for use.
9. All biohazardous waste is to be placed in provided biohazardous containers and autoclaved or incinerated in conformity with local, state and federal regulations.
10. Media from cell culture refeeding is aspirated into 1-liter vacuum bottles containing 100ml of undiluted bleach. Liquid waste is treated with a gelling agent before placing in biohazard container for incineration.
11. Biohazard labels should be on all refrigerators, freezers, sharps containers, waste containers, and cupboards containing infectious materials.
12. Automatic pipetting devices are available.
13. A Stomacher is available for processing tissues.
14. Prion precautions for brain and spinal cord tissue:cover hood work surface with a disposable plastic backed pad, use disposable items for processing and discard all items into red trash for incineration. Immediately clean hood surface with 1:10 dilution of bleach. Rinse well with water.
15. A needle less system is available for processing blood cultures or any specimen that would require use of a needle for removal and/or inoculation.
16. Equipment should be cleaned and decontaminated on a regular basis and whenever necessary.
17. Vacuum lines are protected with 0.45μ filters and liquid disinfection traps.
18. Eating, drinking, smoking, and applying cosmetics are not permitted in the laboratory.
19. The laboratory should be free of clutter and physical hazards.
20. Personnel should wash their hands at frequent intervals during routine activities and before leaving the lab.

**Administrative controls**1. Children’s Hospitals and Clinics Infection Prevention Policies and the Laboratory Safety Policies are accessed electronically on the Children’s Star-net.
2. The Laboratory Safety Officer offers a continuing in-service education program for all laboratory personnel.
3. The E Learning Safety learning activity is to be completed annually by all employees.

**Risk assessment and exposure plan**

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|  | Exposure Risk |  | PPEb | Engineering Controlsc |
| Lab Section and Task | Blood and Body Fluids | BSL | Gloves | Goggles/face shield | N95 Mask | Lab Coat | BSC | Sharps Containers Availabled |
| General |  |  |
| Inventory: media and supplies | Low |  |  |  |  | Coat |  |  |
| Clerical: computer entry, telephones, records | Low |  |  |  |  | Coat |  |  |
| Instrument maintenance: parts contaminated with body fluids or blood | High | Variable | R |  |  | Coat |  |  |
| Instrument maintenance: parts not contaminated with body fluids or blood | Low | Variable | D |  |  | Coat |  |  |
| Surface decontamination | Low | Variable | R |  |  | Coat |  |  |
| Infectious-waste disposal | High | Variable | R |  |  | Coat |  |  |
| Bacteriology |  |  |
| Primary specimen processing | High | BSL 2 | R |  |  | Coat | Rh | Sharps |
| Cytocentrifuge slide prep | High | BSL2 | R |  |  | Coat | Rn | Sharps |
| Subculture blood culture bottles | High | BSL2 | R |  |  | Coat | Rh | Sharps |
| *Neisseria meningitidis*Possible agents of Bioterrorism: anthrax, brucellosis, botulism, tularemia, plague, smallpox | High | BSL 2e | R |  |  | Coat | Rh | Sharps |
| Subculture colonies or broth tubes | Low | BSL 2e |  |  |  | Coat | Rh | Sharps |
| --Identification tests and ASTDO NOT USE automated instrument (MALDI or Vitek 2) or microdilution (MicroScan) if suspect Bioterrorism organism | Low/High | BSL 2e | R | R |  | Coat | Rh | Sharps |
| Prepare smears and fix slides | Low | BSL 2e |  |  |  | Coat |  | Slides |
| Stain fixed slides and read | Low |  |  | R |  | Coat |  | Slides/ Coverslips |
|  | Mycobacteriology and Mycology |
| Primary specimen processing | High | BSL 2/3 | R |  |  | Coat | Rh | Sharps |
|  |  |  |  |  |  |  |  |  |
| Preparing lactophenol cotton blue preps | High | BSL 2/3 |  |  |  | Coat | R | Slides |
| Examine sealed fungal cultures | Low | BSL 2/3f |  |  |  | Coat |  |  |
| Stain fixed slides and read | Low |  |  | R |  | Coat |  | Slides/ Coverslips |
| Handling yeast cultures | Low | BSL 2 |  |  |  | Coat | D |  |
| Handling moulds and rapid growing Mycobacteria (MOTT)Plates must be shrink sealed | Low | BSL 2g |  |  |  | Coat | R | Sharps |
| Virology |  |  |
| Primary specimen processing | High | BSL 2 | R |  |  | Coat | Rh | Sharps |
| Processing specimens with possible high risk pathogens- MERS, SARS,Avian influenza | High | BSL2/3i | R |  | R | Coat | R | Sharps |
| Feed and manipulate uninoculated tubes | Low | BSL 2 |  |  |  | Coat | R | Pipettes |
| Read inoculated tubes or vials for CPE | Low | BSL 2 |  |  |  | Coat |  |  |
| Feed and manipulate inoculated cells | High | BSL 2 | R |  |  | Coat | R | Pipettes |
| Perform tests to identify viruses | High | BSL 2 | R |  |  | Coat | Dh | Sharps |
| Stain fixed slides and read | Low | BSL 2 |  |  |  | Coat |  | Slides/ Coverslips |
|  | Parasitology |
| Concentrate fecal specimens, smear and wet mounts | Low | BSL 2 | R | R |  | Coat |  | Pipettes/ sticks |
| Read fecal wet mounts | Low | BSL 2 | D |  |  | Coat |  | Slides/ Coverslips |
| Stain and read slides | Low | BSL 2 |  | R |  | Coat |  | Slides/Coverslip |
| Antigen detection/PCR/DNA Probes |  |  |
| Primary specimen processing | High | BSL 2 | R |  |  | Coat | Rh | Sharps |
| Processing specimens with possible high risk pathogens- MERS, SARS,Avian influenza | High | BSL2/3i | R |  | R | Coat | Rh | Sharps |
| Cultured microorganisms | Low | BSL 2 | D |  |  | Coat | R | Sharps |

Footnotes:a R:required; D: discretionary; AST: antimicrobial susceptibility testing; BSC: biological safety cabinet; BSL: biosafety level; CPE:cytopathic effectb Remove lab coats before leaving the laboratory. Hang in designated areas. Put contaminated coats in provided laundry bags.c Do not recap needles. Carry tubes in racks, or use plastic tubes. Plan each task to minimize known hazards. Wash hands before leaving the laboratory.d Sharps include scalpel blades, pipettes, plastic loops, sticks, needles, syringes, slides, and coverslips and glass tubes.**e Requires surveillance and action plan for occasional isolation of a BSL 3 organism (e.g., *Brucella sp., Francisella sp.,* and systemic fungi) especially if plates are held > 3 days.**f Requires a contingency plan for breakage of culture containers.g Mycobacteria other than tuberculosis (MOTT group) may be handled at BSL 2.**h Vortexing or other spatter-generating steps require use of a BSC.**i**Propagation requires BSL3. Refer viral culture request to MDH.** |
| References | 1. Isenberg, Henry D., *Essential Procedures for Clinical Microbiology*, 1998, ASM Press, Washington, D.C., Pg. 756-759.
2. Garcia, Lynne. *Clinical Microbiology Handbook*, 2010, 3rd edition, ASM Press, Washington, D.C.
3. Gerald A. Denys, Section editor, Biohazards and Safety, *Risk Assessment* 15.3.2 in *Clinical Microbiology Procedures Handbook*, Garcia, Lynne, editor, 2010, ASM Press, Washington, D.C.
4. Interim Laboratory Biosafety Guidelines for Handling and Processing Specimens Associated with Middle East Respiratory Syndrome Coronavirus (MERS-CoV) – Version 2, <http://www.cdc.gov/coronavirus/mers/guidelines-lab-biosafety.html>, Centers for Disease control, Atlanta GA,6/18/2015.
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| Training Plan/ Competency Assessment | 1. Employee must read the policy.
2. Complete E Learning Safety learning annually.
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| Historical Record |

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| Version | Written/Revised by: | Effective Date: | Summary of Revisions |
| 1 | Pat Ackerman | 9-13-99 | Initial Version |
| 1.1 | Pat Ackerman | 8-29-03 | Reformatted |
| 1.2 | Helen Stefan | 11-2-10 | Clarified class II BSC uses HEPA filter and is exhausted to the outside |
| 1.3 | Becky Carlson | 6/9/11 | Reformatted |
| 1.4 | Helen Stefan | 9/11/11 | Added A#10 handling of virology liquid waste before disposal |
| 2 | Becky CarlsonHelen Stefan | 4/3/2015 | Re-numbered MC 201Added face shield /goggle requirement in section C |
| 3 | Helen Stefan | 7/15/2015 | Added processing specimens with possible high risk pathogens (MERS,SARS, Avian influenza) |
| 4 | Susan DeMeyere | 11/1/2017 | Removed AFB smear from risk assessment. Changed CHEX to E Learning. Add MALDI to Identification tests.  |

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