



LEARNING OBJECTIVES

- To understand the risks related to the use of biohazardous materials
- To become familiar with risk groups and containment practices
- To learn good laboratory microbiological practices
- To learn spill clean up and contamination control measures
- To know what to do if accidental exposure occurs
- To be familiar with legislative requirements and regulations





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WHAT IS A BIOHAZARD

A living organism or a material produced by a living organism that has the potential to harm humans, animals or the environment

Examples:

- Viruses, bacteria, fungi or the toxins produced by one of these
- Human blood, bodily fluids or tissues
- Transformed cell lines





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POTENTIAL BIOHAZARDS IN OHIRC (Ottawa Heart Institute Research Centre)

- Bacteria (E.Coli, Salmonella Typhimurium)
- Viruses (Adenovirus, HIV Lentivirus)
- Unfixed human tissues (bloodborne pathogens)
- Animals (infected experimentally, zoonoses)
- Mammalian cell lines (Hela, HEK293, CHO, HUVEC ...)
- Human Stem Cells
- Microbial toxins (Diphtheria Toxin, Pertussis Toxin)



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WHAT IS BIOSAFETY?



The measures that must be followed when handling infectious material to prevent unintentional / accidental exposure to infectious material and biotoxins:

- containment principles – engineering controls
- appropriate laboratory practices and procedures
- laboratory facilities and safety equipment





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WHY IMPLEMENT A BIOSAFETY PROGRAM?

A biosafety program is put in place to:

- Protect your work / experiment / product from contamination
- Protect the environment by preventing an accidental release
- Protecting laboratory workers from laboratory acquired infections – protect the people in the lab from the agents that are in use



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Laboratory Biosecurity

- In recent years laboratory security has become as important as laboratory safety because of the potential dual use of laboratory materials. Dual Use refers to the inherent properties of materials that allows them to be used both for legitimate science, as well as, for criminal or terrorist activities;
- Laboratory Biosecurity refers to institutional and personal security measures designed to prevent loss, theft, misuse, diversion or intentional release of pathogens or toxins;
- Where laboratory biosafety protects people from (exposure to) the agent, laboratory biosecurity protects the agent from (the actions of) people;
- In OHIRC Biosecurity is part of a more encompassing Laboratory Security Plan.



OHIRC Laboratory Security Plan

- **Physical / Operational / Information Security**
 - Reducing the likelihood of unauthorized access to laboratories as well as to information and data that are in the laboratory
- **Personnel Reliability**
 - Only authorized persons allowed into laboratories – must have completed training
 - Visitors must be accompanied
- **Material Accountability** – All labs are required to keep accurate lists of all materials present
 - Biohazards / Pathogen inventory
 - Chemical Inventory
 - Radioactive Materials etc ...
- **(Bio)security Incident and Emergency Response**
 - Missing or lost inventory....
 - Internal investigations / report to Biosafety Officer / if not found a report made to security(12999)
- **Plan Update and Re-evaluation**
 - The Plan must be reviewed and updated on a regular basis



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Who is responsible for Biosafety?

Internal Stakeholders:

- Chief Scientific Officer
- Principal Investigator
- Biosafety Officer and Biosafety Committee
- YOU

External Stakeholders:

- Health Canada and the Public Health Agency of Canada (PHAC)
- Canadian Food Inspection Agency (CFIA)
- Transport Canada
- Ontario Ministry of Labour



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COMPLIANCE REQUIREMENTS

OHIRC Laboratories must comply with:

- The Human Pathogens and Toxins Act (PHAC)
 - Human pathogens importation regulations
 - Canadian Biosafety Standards
- The Health of Animals Act
- WHMIS 2015– Biohazardous Infectious Material
- The UOHI Biosafety Program



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UOHI Biosafety Program

- Certification - all laboratories where biohazards or infectious materials are used must have an unexpired Biohazardous Materials Use Certificate
- Laboratory Inspections
- Worker Training – Laboratory and Biosafety training is mandatory
- Operational Procedures – Good Microbiological Practices
- Accident Reporting and Investigation
- Occupational Health and Medical Surveillance
- Emergency Response – Decontamination procedures, Spill Response
- Movement of Pathogens
 - Purchase/Importation/Transfer/Gifts
- Waste Disposal



BIOLOGICAL RISK GROUPS

The Public Health Agency of Canada (PHAC) defines a pathogen as a microorganism, nucleic acid or protein capable of causing disease or infection in humans and / or animals.

- Microorganisms are categorized into risk groups based on the risk to public health, livestock and poultry
- The World Health Organization has identified 4
- Group 1 = lowest risk to Group 4 = highest risk
- Risk group is determined by:
 - Pathogenicity/virulence
 - mode of transmission
 - host range
 - Infectious dose
 - availability of effective preventive measures
 - availability of effective treatment



RISK GROUPS

Risk Group 1	Lowest risk of causing disease – to individuals and to the community	Baker's yeast
Risk Group 2	Can cause disease but unlikely to be a serious hazard - Moderate risk to individuals / low community risk	Adenovirus, salmonella
Risk Group 3	High individual risk / low community risk – causes serious human disease	HIV, Rabies, TB
Risk Group 4	Highest risk both to individuals and to the community – causes serious, often untreatable disease that is easily spread	Ebola, Small pox



OTHER CATEGORIES OF BIOHAZARDS

Blood Borne Pathogens	Could be present in primary specimen such as human blood, bodily fluids and tissue – HIV, Hep B, Hep C
Tissue Culture	Potential to contain pathogens – naturally, by contamination or by transformation
Recombinant DNA	Depends on factors such as the gene being transferred, viability of the vector and the containment level of the host
Animal Work	Can harbour infectious agents - naturally or introduced – some agents can also cause disease in humans
Unconventional Pathogens	Prions - resistant to destruction by methods that normally kill viruses
Biological toxins	A broad range of poisons of natural origins (e.g. bacterial toxins) that can cause serious incapacitation. They are the key virulence factors of pathogens but are not infectious themselves and cannot replicate
Autologous Cells or Tissues	Working with infected or transformed cells of laboratory personnel puts those individuals at risk as natural immunity to recognize and destroy foreign cells is now bypassed. Such work is prohibited.



HANDLING INFECTIOUS SUBSTANCES

- Individuals who work in a laboratory where infectious substances are handled are at risk of exposure
- Laboratory acquired infections (LAI) not uncommon - >5000 up to 1999 / 190 deaths
- Routes of Entry – ingestion, inhalation, contact with mucous membranes, contact with non-intact skin
- Accidental exposures – infectious aerosols, splashes, needlesticks
- WHO / PHAC etc established biosafety or containment levels



Biological Toxins

- Biological toxins may be produced by plants, animals and micro-organisms
- The HPTA only regulates microbial toxins
- Some are called “Prescribed toxins” or Security Sensitive Biological Agents (SSBA) ... because of additional biosecurity considerations .. They have the potential to be weaponized
- Facilities handling regulated toxins must operate at a minimum containment level 2 – aerosol generating procedures are to be carried out in a biosafety cabinet
- A designated fixed locked storage area for the toxin must be identified in the laboratory; if an SSBA is in use the storage area must be in a location where the public has no access
- Given the large variety of biological toxins and differences in physical properties a standardized decontamination procedure cannot be provided in this training – The PI must determine best decontamination plan – heat or chemical such as sodium hypochlorite
- A Standard Operating Procedure for Biotoxins can be found on Hearthub at the following link:

<https://hearthub.ottawaheart.ca/policies-and-forms/policies/policy/14141>



BIOLOGICAL CONTAINMENT LEVELS

- The Canadian Biosafety Standard identifies 4 – CL1 to CL4
- Containment levels relate to risk groups but are not necessarily equivalent
 - Labs are required to complete risk assessments
- Describe the minimum requirements at each level to handle organisms safely
 - Physical requirements – engineering controls
 - Operational requirements – how you work



CONTAINMENT LEVEL 1

- The minimum requirements of all laboratories where microbiological work is done
- No special design features
- Biosafety cabinets are not required – work can be done on an open bench
- The containment level 1 lab is the foundation of all other higher containment laboratories – standard microbiological practices



STANDARD MICROBIOLOGICAL PRACTICES

Biosafety Management	Access (to the laboratory)
Laboratory Safety Manual required	Only authorized personnel - Lab doors always kept closed
Personnel training mandatory	Children (under 16) never allowed in the laboratory working areas
A mechanism to report and investigate accidents promptly	
First aid and or medical attention provided	



STANDARD MICROBIOLOGICAL PRACTICES

Personal Protection:

- Lab coat – fastened all the way, sleeves rolled down
- Proper footwear – closed toes and heels
- Gloves as appropriate – when there is a risk of contact
- Wash hands after gloves are removed
- Safety goggles, face shields
- Remove protective clothing before entering non-lab areas
- Remove gloves before touching common objects and surfaces
- Do not eat, drink, apply cosmetics, insert contact lens in the lab
- Tie back long hair, confine loose clothing



STANDARD MICROBIOLOGICAL PRACTICES

Procedures:

- Oral pipetting is prohibited
- The use of needles and other sharps is minimized
- Sharps are disposed of in appropriate puncture proof containers
- Hands are always washed before experiments and after gloves are removed
- Closed leak proof containers and secondary containers are used to transport infectious material
- Efficacy monitoring of the autoclave is carried out using appropriate biological indicators



STANDARD MICROBIOLOGICAL PRACTICES

Procedures:

- All contaminated material is decontaminated before disposal or re-use
- Contaminated clothing must be decontaminated before placing in the hospital linen basket



STANDARD MICROBIOLOGICAL PRACTICES

Laboratory Working Areas:

- The lab is kept clean and tidy, bench tops uncluttered
- Paperwork stations and report writing areas are separate from wet work areas
- Work surfaces are cleaned and decontaminated with appropriate disinfectants at the end of every day and immediately after any spill
- An effective rodent and insect control program must be in place



CONTAINMENT LEVEL 2 (In addition to CL1 Requirements...)

OPERATIONAL PRACTICES	PHYSICAL REQUIREMENTS
<ul style="list-style-type: none">•Written emergency plan for spills, BSC failure etc•Housekeeping and Facilities staff must be made aware of the hazard•Appropriate signage outside the lab•Aerosol generating procedures carried out in a biosafety cabinet	<ul style="list-style-type: none">•Inward directional flow of air•Work surfaces are resistant to chemicals, physical damage•Lab located away from patient care areas, public areas•Doors to the containment lab must be lockable



CONTAINMENT LEVEL 3 AND 4

Containment Level 3

- Very specific physical requirements
- No level 3 labs at UOHI
- Level 3 labs at OHRI and U of O

Containment Level 4

- Requires an isolated unit and a sealed facility
- Only one in Canada in Winnipeg





CL2 / Level 3 Operational Practices

- Some specialized risk group 2 organisms or some viruses must be handled at an enhanced containment level – ex. Lentiviral vectors
- Includes all CL 1 and CL2 practices as well as some CL3
 - Requires enhanced PPE – solid front gown with tight fitting wrists, gloves and respirators
 - Requires pre-treatment of biohazard waste before placing in hospital waste stream – double bagged and autoclaved
- Section 3.6.3 of the Laboratory Safety and Procedures Manual
- PI must carry out a risk assessment to determine if this enhanced level is required
- Must consult the Biosafety Committee



Biological Risk Assessment

- The Canadian Biosafety Standards and Guidelines recommend that risk assessment be carried out in laboratories to identify all hazards associated with the microbial agent in use and the activities carried out
- The Principal Investigator must carry out a risk assessment to determine the appropriate containment for the organism in use
- The risk assessment must consider the following:
 - The properties of the agent – pathogen or not, attenuated strain, indigenous to Canada etc
 - Laboratory procedure hazards such as the use of needles, the potential for aerosol generation, hazards associated with animal use etc
 - Decontamination requirements
 - Personnel training requirements



Biological Safety Cabinets

- Most important safety device – second only to safe work practices
- A primary containment device that provides protection for the person, the environment and the product (for the cabinets that are in use in OHIRC laboratories)
- Highly efficient in reducing exposure by the use of directional airflow away from the breathing zone of the user
- HEPA Filtered 0.3um removes microbial agents before the air is exhausted
- Must be certified annually
- 3 classes of cabinets: I, II and III



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BSC Class II

Most common type of BSC
Good for Risk Groups 1,2 and 3
Protects the worker, the work and
the environment





BSC Safe Operational Procedures

Start up:

- Turn off UV light (UV light is used to sterilize the surfaces of the BSC however, exposure to UV light may cause skin or eye damage – never work with the UV light on) / turn on fluorescent light
- Turn on cabinet blower
- Ensure sash is in the appropriate position and the intake and exhaust are not blocked
- Check inward flow with a piece of tissue
- Disinfect the surfaces
- Load cabinet, segregating clean from dirty items taking care not to block the grilles



BSC Safe Operational Procedures

Working in the cabinet:

- Purge the cabinet for 5 minutes
- Don protective clothing and gloves
- Perform operations as far to the rear of the work area as possible
- Enter and exit the cabinet from straight on and avoid excessive movements of the hands and arms
- Do not work with open flames inside the cabinet
- Keep discarded, contaminated material to the rear of the cabinet. Do not discard them in containers outside of the cabinet



BSC Safe Operational Procedures

Completion of the work:

- Allow the blower to run for five minutes with no activity
- Close or cover open containers before removing them from the cabinet
- Surface disinfect objects before removal from the cabinet
- Remove contaminated gloves and dispose as appropriate; wash hands
- Don clean gloves and ensure all materials are placed into biohazard bags within the cabinet
- Disinfect interior surfaces of the cabinet including surface of the UV light
- Turn off fluorescent light and cabinet blower and turn on UV light (except if people are working close by)



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Biosafety Cabinet Use Practical Training

- Practical training on how to properly and safely work in the biosafety cabinet is mandatory – either in the laboratory by the principal investigator or lab manager OR by the Core Laboratory Technician:

Contact Vivian Franklin, for practical hands on training

14468 or vfranklin@ottawaheart.ca



SAFE USE OF CENTRIFUGES

Centrifuges may pose a hazard because of:

- Mechanical failure
- Tube failure – cracks and leaks which could lead to chemical or biological material leaks
- Creation of infectious aerosols
- Operator error





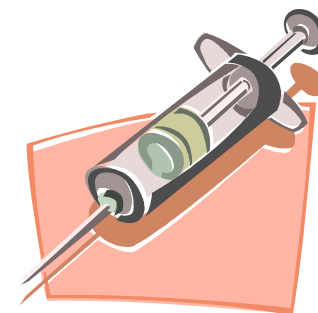
SAFE USE OF CENTRIFUGES

Before Use	After run
<ul style="list-style-type: none">•Check for cracks, chips, stress lines•Make sure caps / stoppers are properly in place•Make sure the tubes are balanced•And the rotor is locked to the spindle•Close lid during operation	<ul style="list-style-type: none">•Allow centrifuge to completely stop•Check for leaks or spills•Allow aerosols to settle (about 30 minutes) or open in a BSC•Disinfect weekly and always after a spill



SAFE USE OF NEEDLES AND SYRINGES

- Avoid use whenever possible or use safety engineered devices
- Fill syringes carefully
- Shield needles when withdrawing from stoppers
- Do not bend, shear or recap needles
- Dispose of all used needles and syringes in a yellow sharps container
- Report Needle Stick Injuries immediately to your supervisor





PIPETTES

The use of pipettes can present a risk of aerosol generation:

- Do not mix infectious liquid cultures by alternating suction and expulsion
- Discharge pipette as close to medium as possible allow discharge to run down the side of the container wall
- Never mouth pipette





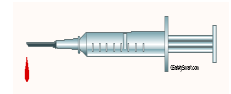
INNOCULATION LOOPS

- Sterilization in an open flame may create aerosols
- Recommend:
 - Shielded electric incinerator
 - Loops with shorter handles(minimizes vibration)
 - Disposable plastic loops



UNIVERSAL (Standard) PRECAUTIONS

- Based on the premise that all blood, body fluids, secretions, mucous membranes etc are potentially infectious
- Minimum standard of practice for preventing the transmission of Blood Borne Pathogens. Include:
 - Hand Hygiene - proper hand washing
 - Using appropriate personal protective equipment – gloves, gowns, lab coats, goggles etc
 - Following safe working procedures such as:
 - Use caution when handling needles and other sharps
 - Cover up broken skin when handling infectious material





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HAND WASHING

- **The simplest most effective means reducing the spread of infection when done properly and frequently**
- **Wash with soap and water**
 - After removing gloves
 - On completion of all work or procedures
 - Before leaving the laboratory
 - Before eating, drinking, after using the washroom
 - If hands are visibly contaminated soiled





DECONTAMINATION

- The destruction of microorganisms to a lower level such that it removes the
- Failure to decontaminate could result in Laboratory Acquired Infections or unintentional release of organisms into the environment
- Two types:
 - Sterilization: The complete destruction of all viable microorganisms and their spores
 - Disinfection: Will eliminate pathogenic organisms but not necessarily their spores
- Heavily soiled materials must be pre-cleaned to remove dirt and other organic matter



AUTOCLAVES

- Very effective and reliable method of sterilization (the only method available in OHIRC)
- Located in H1254
- Learn how to use the autoclave safely
 - The PI is responsible to ensure staff is trained either in house in the laboratory or with the the Core Laboratory Technician (14468 or vfranklin@ottawaheart.ca)
 - Use PPE
- Not everything can be autoclaved
 - Phenol, javex / bleach, corrosives, flammables
 - Tissue culture medium that has been chemically neutralized
 - certain types of plastics
 - materials contaminated with radioisotopes



DISINFECTION

- Chemical disinfectants - used for the decontamination of surfaces and equipment that cannot be autoclaved
- They eliminate pathogenic microorganisms, but not necessarily their spores
- Choice is dependant on several factors:
 - Resistance of the microorganism
 - Stability of the chemical disinfectant
 - Associated health hazards or toxicity of the chemical
- Selection of the appropriate disinfectant is the responsibility of the PI as part of the risk assessment
- Most commonly used is bleach



BIOHAZARD SPILL CONTROL

- All users must be familiar with spill clean up procedures – you spill it / you clean it
- Response is dependent on:
 - What is spilled
 - How much
 - Where
- Clean up should be immediate
- Always don appropriate personal protective equipment before cleaning a spill
- All spills must be reported (to the supervisor)





SPILLS – GENERAL CLEAN UP PROCEDURES

- Cover spill with absorbent paper (to reduce aerosol production)
- Flood with appropriate disinfectant working from the perimeter to the centre (to prevent further spread of the spill)
- Allow the disinfectant to contact the material for 30 minutes
- Cover with a second layer of absorbent paper and pick up
- Use forceps to pick up any broken glass
- Place all waste in an appropriate biohazard waste container
- Disinfect all areas contacted by the spill



SPILLS – SPECIAL CASES

Within the Centrifuge	Within the Biosafety Cabinet
<ul style="list-style-type: none">•Leave the lid closed and allow aerosols to settle for about an hour•Notify others in the lab that the centrifuge is out of use (identify with a tag or sign)•Wipe down inside of centrifuge including the lid with appropriate disinfectant•Wipe down rotor with disinfectant•Remove rotor from centrifuge and repeat disinfection•If bleach is used rinse the rotors with water	<ul style="list-style-type: none">•Leave the ventilation on during the clean up•Follow the general clean up procedures•Run the ventilation for 15 minutes after clean up is completed



HANDLING BIOLOGICAL WASTE

- All biological waste must be decontaminated prior to disposal – made non-infectious before it goes to landfill
- Follow the hospital procedures:
 - appropriate yellow pails / yellow bags and yellow sharps containers must be used
 - OR grey biotub lined with yellow bags
- Tissue culture and microbiological waste must be decontaminated prior to disposal by either autoclaving or chemical decontamination – NEVER autoclave tissue culture that has had bleach or other disinfectant added to it ...this could produce toxic vapours
- Quantities up to 250ml of human blood may be discarded to the sewer – recommend decontamination with 10% bleach for 30 minutes contact time



OCCUPATIONAL HEALTH

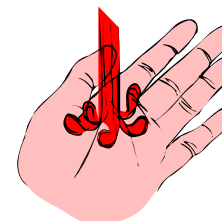
- Access to Occupational Health Services is mandatory with CL2 or risk Group 2
 - Immunization
 - Medical surveillance
 - Serum collection
 - Provide treatment and follow up
- For employees OHIRC has a service contract with Occupational Health at TOH
- For students – Health Services at the University



ACCIDENTAL EXPOSURES

Can be

- Needle sticks, cuts, abrasions
- Animal bites, scratches – may become infected
- Mucous membrane exposure to splash, splatter or spilled infectious materials
- Skin contamination through damaged gloves
- Exposure to infectious aerosols
- Indirect exposures through animal bedding, contaminated surfaces



* Report all exposures or accidents to your supervisor and complete an accident investigation form



NEEDLE STICK INJURIES

- Apply first aid immediately – bleed the wound if possible, wash the affected area with soap and water
- Report the accident to your PI or lab technician immediately
- * If the needle is contaminated with human blood/ tissue or bodily fluid, report immediately to the Emergency Department at the Ottawa Hospital – Needle sticks involving such exposures must be triaged within 90 minutes of the occurrence
- If the accident involves a dirty sharps incident other than human blood or tissue (such as mouse blood or tissue), a tetanus shot may be required



PHAC License

- A license from the Public Health Agency of Canada is required to carry out all “controlled activities” with pathogens (OHIRC has received a license from PHAC authorizing work with human and terrestrial animal pathogens)
- Controlled activities with pathogens are:
 - Possessing, handling, using ...
 - Producing a human pathogen or toxin
 - Storing ...
 - Permitting any person access to ...
 - Transferring ...
 - Importing or exporting ...
 - Releasing or otherwise abandoning ...
 - Disposing of ...
- Our licence is valid for 5 years at which time we will have to apply for a renewal



TRANSPORTATION and SHIPPING of INFECTIOUS MATERIAL

- Subject to strict regulations nationally and internationally
- Infectious Materials are a Class 6.2 Dangerous Good
- Must comply with the Transportation of Dangerous Goods Act and Regulations within Canada (TDG)
- If shipping internationally must comply with the requirements of the International Air Transport Association (IATA)
- Infectious materials must be packaged and transported according to tested and approved methods
- Any individual involved in shipping (shipper, carrier, receiver) be TDG trained
 - Classify, label and document shipments
- Never send infectious material by mail



TRANSPORTATION WITHIN THE BUILDING

- This would be between laboratories or back and forth from Animal care Services
 - Use a sealed primary container inside a secondary container
 - Use a lipped cart
 - Do not take the stairs – the freight elevator (Elevator #2) is preferred



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Summary

- Know the biohazard you are working
- Treat all materials as infectious – microorganisms, blood, tissues, tissue culture
- Use appropriate containment, PPE, disinfectants
- Follow regulations / procedures
- With proper knowledge, planning and care a biological exposure is avoidable



Let us keep that way!



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Now go to the related quiz The password is healthandsafety

<https://www.classmarker.com/online-test/start/?quiz=kne52e6c3277527b>