BIOSAFETY

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1. Introduction

What is Biosafety?

- The combination of measures employed when handling biohazardous materials to avoid infecting oneself, others or the environment.

  Includes:
  - Engineering Controls
  - Administrative Controls
  - Practices and Procedures
  - Personal Protective Equipment

- The single most effective means of preventing exposures is adherence to good microbiological techniques.

What is a Biohazard?

- Any organism or its toxin, that is known or suspected to cause disease in animals or humans.
- Biohazardous or infectious materials fall under class d, division 3 of WHMIS.
- Includes microorganisms such as viruses, bacteria, fungi, and parasites and their toxins.
- Also includes blood and body fluids, as well as tissues from humans and animals.
- Transformed cell lines and certain types of nucleic acids also are considered as biohazardous.

Who's Responsible?

- Biological Safety has not as yet been regulated, other than through the Occupational Health and Safety Act, and WHMIS legislation.
- The University does however, acknowledge a responsibility to ensure that all those working with biohazardous material do so in a safe manner.
- EHSS ensures that all regulatory concerns are met or exceeded, provides information and instruction regarding hazardous materials, including biohazards, implements measures to ensure safety.
- University Biohazards Committee ensures that research and teaching utilizing biohazardous materials does so according to the Health Canada Laboratory Safety Guidelines.
- Deans, Directors, Chairpersons and Principal Investigators must show due diligence in the application of health and safety measures, which includes among others the requirement to provide information and instruction.
- Employees and Students must work in compliance with the provisions of the Occ. H. and S. Act , and follow all instructions provided.

University of Ottawa Initiatives

- Environmental Health and Safety Service
  - Biosafety Office
  - Training
  - Inspections
  - Annual Certification of Biosafety Cabinets

- Occupational Health and Safety Service
  - Medical Surveillance
  - Immunizations
  - Exposure follow-up

- Committees
  - Biohazard Committee
  - Animal Care Committee

- Biohazardous Use Materials Certificate*
- Risk Identification Questionnaire (Faculty of Medicine)*

Why are we concerned about safety in regards to biohazardous materials?

- One of the unfortunate consequences of working with infectious materials is the potential for acquiring a laboratory-associated infection (LAI)

- Most common routes of exposure are percutaneous inoculation (from a needle or a bite), inhalation of aerosols generated by accident or by work practices and procedures, contact of mucous membranes and contaminated material, and ingestion

- Literature reviews yield the following:
  - 25% needles
  - 27% spills and sprays
  - 16% sharp objects or broken glass
  - 13% aspiration through a pipette
  - 13.5% bites, scratches and contact with ectoparasites

Today, the most common laboratory acquired illnesses are those referred to as the "bloodborne pathogens".

2.0 Classification of Biohazards

- Conventional Agents
  - Risk Groups 1 to 4

- Unconventional Agents
  - Slow Viruses (Prions)

- Recombinant DNA

- Tissue Culture

- Animal Work

- Anatomical Specimens

Risk Group 1 Agents

- Referred to as low individual and community risk
- Includes those micro organisms, bacteria, fungi, viruses and parasites which are unlikely to cause disease in healthy workers or animals.
- Most of the biohazardous agents at the university are this level. Includes most of the work using *escherichia coli* (E. coli) for molecular biology work.
- Requires level 1 containment

Risk Group 2 Agents

- Referred to as moderate individual risk, limited community risk
- Includes pathogens that can cause human or animal disease, but under normal circumstances, are unlikely to be a serious hazard to healthy laboratory workers, the community, livestock or the environment. Laboratory exposures rarely cause infection leading to serious disease; effective treatment and preventative measures are available and the risk of spread is limited.
- Includes: **Bacteria** such as: *Salmonella enterica*, Escherichia coli strains such as 0157, and several Streptococcus species
  Viruses such as: Adenoviruses, Hepatitis A, Hepatitis B, Hepatitis C , Influenza viruses, most pox viruses, Rhinovirus, Measles, Mumps.
- Requires Level 2 Containment

Risk Group 3 Agents

- Referred to as high individual risk, low community risk agents
- Includes pathogens which usually cause serious human or animal disease, or which result in serious economic consequences, but do not ordinarily spread by casual contact from one individual to another, or that can be treated by antimicrobial or antiparasitic agents.
- Includes: Bacteria such as: *Bacillus anthracis*, *Mycobacterium tuberculosis*
  Viruses such as: Hantaan viruses, Yellow fever virus, Human immunodeficiency virus (HIV)
  Unconventional agents such as: Creutzfeldt-Jakob agent
- **NOTE**: Although HIV is listed as level 3, laboratories engaging in primary isolation and identification may perform these activities in Level 2 laboratories, using Level 3 operational requirements. All research and production activities require Level 3 physical and operational requirements.

Risk Group 4 Agents

- Referred to as high individual risk and high community risk agents.
- Includes pathogens that usually produce very serious human or animal disease, often untreatable, and may be readily transmitted from one individual to another, or from animal to human or vice-versa directly or indirectly, or by casual contact.
- Includes the following viruses: Lassa virus and Ebola virus.
- The requirement for Level 4 containment is absolute.
- **No Level 4 work is permitted at the University of Ottawa**

RISK GROUP 2 AGENTS: REQUIRING CONTAINMENT LEVEL 2

(moderate individual risk, limited community risk)

Bacteria, Chlamydia, Mycoplasma

Actinobacillus - all species
Actinomyces pyogenes (C. pyogenes)
Bacillus cereus
Bartonella bacillifonnis, B. henselae, B. quintana,B. elizabethae
Bordetella pertussis, B. parapertussis and B. bronchiseptica, Borrelia recurrentis and B. burgdorferi
Campylobacter spp. (C. coli, C. fetus, C. jejuni)
Chlamydia pneumoniae, C. psittaci (non-avian strains**),** *C. trachomatis*
Clostridium botulinum, Cl chauvoei, Cl difficile,Cl. haemolyticum
Cl. histolyticum, Cl novyi, Cl perfringens, Cl septicum,Cl sordellii, Cl tetani
Corynebacte-rium diphtheriae, C. haemolyticum,
C. pseudotuberculosis, C. pyogenes (A. pyogenes)
Edwardsiella tarda
Erysipelothrix rusiopathae (insidiosa)
Escherichia coli enterotoxigenic/invasive/hemorrhagic strains
Francisella tularensis Type B, (biovar palaearctica),*F novocida*
Fusobacterium necrophorum
Haemophilus influenzae, H. ducreyi
Helicobacter pylori
Legionella spp.
Leptospira interrogans: all serovars
Listeria monocytogenes
*Mycobacteria:*all species (except M. *tuberculosis, and M. bovis* (non-BCG strain), which are in Risk Group 3)
Mycoplasma pneumoniae, M. hominis
Neisseria gonorrhoeae, N. meningitis
Nocardia asteroides, N. brasiliensis
Pasteurella, all species (except P *multocida* type B in Level 3)
Pseudomonas aeruginosa
Salmonella enterica (S. choleraesuis)
Salmonella enterica serovar arizonae *(Arizona hinshawii)*
Salmonella enterica ser. gallinarum-pullorum *(S. gallinarumpullorum)*
Salmonella enterica ser. meleagridis *(S. meleagridis)*
Salmonella enterica ser. paratyphi B *(S. paratyphi B)* (Schottinulleri)
Salmonella enterica ser. typhi *(S. typhi)*
Salmonella enterica ser. typhimurium *(S. typhimurium)*
Shigella boydii, S. dysenteriae, S. flexneri, S. sonnei
Staphylococcus aureus
Streptobacillus moniliforinis
Streptococcus spp. (Lancefield Groups A, B, C, D, G)
Treponema carateum, T pallidum (including pertenue),T vincentii
Ureaplasma urealyticum
Vibrio cholerae (incl El Tor), V parahaemolyticus,V vulnificus
Yersinia enterocolitica, Y. pseudotuberculosis

Fungi

Cryptococcaceae
- *Candida albicans*
- *Cryptococcus neoformans*

Moniliaceae
- *Aspergillus flavus*
- *Aspergillus fumigatus*

Epidermophyton floccosum
Microsporum spp.
Sporothrix schenckii
Trichophyton spp.

Viruses

*Arthropod-bome viruses are identified with an asterisk. Only those viruses which may be associated with human or animal disease have been included in this list. Agents listed in this group may be present in blood, CSF, central nervous system and other tissues, and infected arthropods, depending on the agent and the stage of infection.

Adenoviridae
- Adenoviruses, all serotypes

Arenaviridae
- Lymphocytic choriomeningitis virus (laboratory-adapted strains)
- Tacaribe virus complex: Tamiami, Tacaribe, Pichinde

Bunyaviridae*
- Genus Bunyavirus
- Bunyamwera and related viruses
- Califomia encephalitis group**,** including LaCrosse,
- Lumbo and snowshoe hare

Genus Phlebovirus
- All species except Rift Valley fever virus

Caliciviridae: all isolates (including Hepatitis E & Norwalk)

Coronaviridae
- Human coronavirus, all strains
- Transmissible gastroenteritis virus of swine

Hemagglutinating encephalomyelitis virus of swine

Mouse hepatitis virus

Bovine coronavirus
- Feline infectious peritonitis virus
- Avian infectious bronchitis virus
- Canine, Rat and Rabbit coronaviruses

Flaviviridae*
- Yellow fever virus (17D vaccine strain)
- Dengue virus (serotypes 1,2,3,4)
- Kunjin virus

Hepadnaviridae
- Hepatitis B virus, includes Delta agent

Herpesviridae
- Alphaherpesvirinae
- Genus Simplexvirus: all isolates, including HHV 1 and HHV 2, except Herpes B virus which is in Risk Group 4
- Genus Varicellovirus: all isolates, including varicella/zoster virus (HHV 3) and pseudorabies virus

Betaherpesvirinae
- Genus Cytomegalovirus: all isolates including CMV (HHV 5)
- Genus Muromegalovirus: all isolates

Gammaherpesvirinae
- Genus Lymphocryptovirus: Epstein Barr Virus (HHV 4) and EB-like isolates
- Genus Rhadinovirus: all isolates (except *H. ateles and H.saimiri,* see Risk Group 3)
- Genus Thetalymphocryptovirus: all isolates

Unassigned Herpesviruses: includes HHV 6 (human Plymphotrophic virus), HHV 7, HHV 8, etc.

Orthomyxoviridae
- Genus Influenzavirus: Influenza virus type A: all isolates
  Influenza virus type B: all isolates
  Influenza virus type C: all isolates

- Papovaviridae
  - Genus Papillomavirus: all isolates
  - Genus Polyomavirus: all isolates
- Paramyxoviridae
  - Genus Paramyxovirus: all isolates
  - Genus Pneumovirus: all isolates
  - Genus Morbillivirus: all isolates except Rinderpest
- Parvoviridae
  - Genus Parvovirus: all isolates
- Picornaviridae
  - Genus Aphthovirus:
  - Genus Cardiovirus: all isolates
  - Genus Enterovirus: all isolates
  - Genus Hepatovirus: all isolates (Hepatitis A)
  - Genus Rhinovirus: all isolates
  - Poxviridae
    - Chordopoxvirinae (poxviruses of vertebrates)
    - Genus Capripoxvirus
    - Genus Molluscipoxv irus
    - Genus Yatapoxvirus
    - Genus Avipoxvirus: all isolates
    - Genus Leporipoxvirus: all isolates
    - Genus Orthopoxvirinae: all isolates (except Variola and Monkeypox in Level 4)
    - Genus Parapoxvirus: all isolates
    - Genus Suipoxvirus: Swinepox
  - All other ungrouped poxviruses of vertebrates
- Reoviridae
  - Genus Orbivirus: all isolates
  - Genus Orthoreovirus, types 1, 2 and 3
  - Genus Rotavirus: all isolates
- Retroviridae
  - Oncovirinae
  - Genus Oncomavirus C
    - Subgenus Oncomavirus C avian: all isolates
    - Subgenus Oncomavirus C mammalian: all isolates except HTLV-I, HTLV-II
    - Genus Oncomavirus B: all isolates
    - Lentivirinae:all isolates except HIV-I, HIV-II
    - Spumavirinae: all isolates
- Rhabdoviridae
  - Genus Vesiculovirus: (All laboratory-adapted strains)
  - Genus Lyssavirus: Rabies virus (Fixed Virus)
- Togaviridae
  - Genus Alphavirus*
    - Semliki forest virus
    - Sindbis
    - O'Nyong-Nyong
    - Ross river virus
  - Venezuelan equine encephalitis (Strain TC-83 only, no animal inoculation)
  - Genus Rubivirus
    - Rubella virus
  - Genus Pestivirus
    - Hepatitis C virus
    - Bovine diarrhoea virus
    - Border disease virus
  - Genus Arterivirus
    - Equine arteritis virus

Viruses (continued)

Unclassified viruses
- Toroviridae
- Other Hepatitis Viruses
- Borna disease virus
- Astro viruses
- Chronic infectious neuropathic agents (CHINAs): Scrapie, BSE (except Kuru, CJD, see Risk Group 3)

Parasites

Infective stages of the following parasites have caused laboratory infections by ingestion, skin or mucosal penetration or accidental injection. Preparations of these parasites known to be free of infective stages do not require this level of containment.

Protozoa

- *Babesia microti*
- *Babesia divergens*
- *Balantidium coli*
- *Cryptosporidium spp.*
- *Entamoeba histolytica*
- *Giardia spp*. (mammalian)
- *Leishmania* spp. (mammalian)
- *Naegleria fowleri*
- *Plasmodium* spp. (human or simian)
- *Pneumocystis carinii*
- *Toxoplasma gondii*
- *Trypanosoma brucei, T cruzi*

Helminths

Nematodes

- *Ancylostoma duodenale*
- *Angiostrongylus spp.*
- *Ascaris spp.*

Parasites (continued)

Nematodes (continued)

- *Brugia spp.*
- *Loa loa*
- *Necator americanus*
- *Onchocerca volvulus*
- *Strongyloides spp.*
- *Toxocara cants*
- *Trichinella spp.*
- *Trichuds trichiura*
- *Wuchereria bancrofti*

Cestodes

- *Echinococcus* (gravid segments)
- *Hymenolepis diminuta*
- *Hymenolepis nana* (human origin)
- *Taenia saginata*
- *Taenia solium*

Trematodes

Clonorchis sinensis
Fasciola hepatica
Opisthorchis spp.
Paragonimus westermani
Schistosoma haematobium
Schistosoma japonicum
Schistosoma mansoni

RISK GROUP 3 AGENTS: REQUIRING CONTAINMENT LEVEL 3

(high individual risk, low community risk)

Bacteria, Chlamydia, Rickettsia

Bacillus anthracis
Brucella: all species
Burkolderia (Pseudomonas) mallet; B. pseudomallei
Chlamydia psittaci: avian strains only
Coxiella burnetii
Francisella tularensis, type A (biovar tularensis)
Mycobacterium tuberculosis+
Mycobacterium bovis (non-BCG strains)
Pasteurella multocida, type B
Rickettsia: all species (also see Table 1)
Yersinia pestis

+Preparation of smears and primary culture of M. *tuberculosis* may be performed at Level 2 physical containment using containment Level 3 operational requirements. All other maniculations of M. *tuberculosis* require containment Level 3 physical and operational requirements.

Fungi

Moniliaceae
Ajellomyces dermatitidis (Blastomyces derinatitidis)
Coccidioides immitis
Ajellomyces capsulatum (Histoplasma capsulatum including var. *duboisii*)
Paracoccidioides brasiliensis

Viruses

*Arthropod-borne viruses are identified with an asterisk

Arenaviridae
- Lymphocytic choriomeningitis virus, neurotropic strain@

Bunyaviridae
- Unclassified Bunyavirus
  - Hantaan, Korean haemorrhagic fever and epidemic nephrosis viruses including virus responsible for Hantavirus pulmonary syndrome
- Rift Valley fever virus

Flaviviridae*
- Yellow fever virus (Wild type)
- St. Louis encephalitis virus
- Japanese encephalitis virus
- Murray Valley encephalitis virus

Powassan
Herpesviridae
Gammaherpesvirinae
Genus Rhadinovirus: *Herpesvirus ateles, Herpesvirus saimiri*
Retroviridae
Oncovirinae
Genus Oncornavirus C
Human T-cell leukemia/lymphoma virusl
Genus Oncomavirus D
Mason-Pfizer monkey virus
Viruses ftom non-human primates
Lentivirinae
Human immunodeficiency viruses (HIV all isolates)+
Rhabdoviridae
Genus Vesiculovirus (wild type strains)
Genus Lyssavirus
Rabies virus (Street virus)
Togaviridae
Genus Alphavirus*
Eastern equine encephalitis virus
Chikungunya
Venezuelan equine encephalitis (except Strain TC-83)
Western equine encephalitis
Unclassified Viruses
Chronic infectious neuropathic agents (CHINAs): Kuru, Creutzfeldt-Jakob agent (level of precautions depends on the nature of the manipulations and the amount of sera, bio/necropsv materials handled).

+laboratories engaging in pnmary isolation and identification of HTLV or HIV may perform these activities in containment Level 2 laboratories (physical requirements) using containment Level 3 operational requirements. All research and production activities require containment Level 3 physical and operational requirements.

Parasites
None

RISK GROUP 4 AGENTS: REQUIRING CONTAINMENT LEVEL 4

(high individual risk, high community risk)

Bacteria
None

Fungi
None

Viruses
*Arthropod-borne viruses are identified with an asterisk

Arenaviridae
Lassa, Junin, Machupo viruses, Sabia, Guanarito
Bunyaviridae*
Genus Nairovirus
Crimean-Congo hemorrhagic fever
Filoviridae
Marburg virus
Ebola virus
Flaviviridae*
Tick-bome encephalitis complex, including Russian Spring-Summer Encephalitis

Kyasanur forest virus
Omsk hemorrhagic fever virus
Herpesviridae
Alphaherpesvirinae
Genus Simplexvirus: Herpes B virus (Monkey virus)
Poxviridae
Genus Orthopoxvirinae
Variola
Monkeypox

Parasites
None

Unconventional Pathogens

Some progressive neurological diseases (spongiform encephalopathies) are caused by agents referred to as unconventional agents, slow viruses or prions (proteinaceous infectious particles). Examples of such diseases are: Creutzfeld-Jakob disease in humans, Mad Cow Disease and scrapie in sheep and goats).

These agents are resistant to destruction by chemical (10% formalin, gluteraldehyde, 70% ethanol, iodine) and physical (UV light, ionizing radiation, boiling) procedures that normally inactivate viruses.

While there have been no documented cases of laboratory acquired infections, the following precautions should be observed when handling neurological tissue from infected or potentially infected humans or animals.

- Handle tissue as Risk Group 2 or higher
- Handle formalin-fixed tissues and paraffin-embedded blocks as if still infectious
- Follow up-to-date disinfection protocols.

Recombinant DNA

Recombinant DNA technology or genetic engineering are terms used to describe the in vitro incorporation of segments of genetic material from one cell into another, from one organism to another.

In the United States and other countries, regulations have been introduced to closely monitor the experimentation involving manipulation of genetic material. In Canada no such regulations exist.

The Health Canada Laboratory Biosafety Guidelines indicate that in evaluating the level of risk for the manipulations, the source of the DNA being transferred, the vector and the host must be considered.

The University of Ottawa Biohazards Committee should be consulted, and will assist the investigator in this determination.

Tissue Culture

- All mammalian cell lines are to be considered infectious due to the possibility that they may contain or transmit infectious agents.
- Cells or primary cultures from animals and humans known or reasonably suspected to be infected should be in the risk group for the suspected agent.
- All primate cell lines, all cell lines exposed to or transformed by primate oncogenic virus and all mycoplasma-containing cell lines should be handled at Level 2 containment.
- Any cell lines containing mycoplasma should be likewise treated as Level 2
- All other tissue culture work should be considered as Level 1
- All waste generated from tissue culture must be autoclaved or disinfected by other means prior to disposal

Animal Work

- By definition, all work involving animals is considered biohazardous.
- Animals can harbour infectious organisms (naturally or through their use in research) which can be transmitted to humans.
- The University of Ottawa Animal Care Committee ensures that all work involving animals meets all the standards and regulations of the Canadian Council on Animal Care, and the Health of Animals Act.
- All work involving animal use must receive prior approval from the University of Ottawa Animal Care Committee

Anatomical Specimens

- Anatomical specimens are of particular concern for 2 reasons:
- The possibility of contracting a life threatening disease such as Hepatitis B or Aids from blood and blood products,
- And the possibility of encountering unconventional viruses (such as Creutzfeld-Jacob agent) which are resistant to conventional disinfection in human tissue samples.
- All specimens should be considered potentially infectious and level 2 containment should be utilized

3.0 General Laboratory Safety Guidelines

(Laboratory Biosafety Guidelines)

- All personnel must understand the hazards, and be trained.
- A laboratory safety manual must be prepared.
- The laboratory must be kept neat, orderly and clean.
- Appropriate protective equipment must be worn. It must not be worn in non-laboratory areas. This includes gloves, which must be worn for all procedures involving direct skin contact; safety eyewear, which must be worn to protect against splashes; and lab clothing.
- Eating and drinking, inserting or removal of contact lenses, application of cosmetics are not permitted.
- Oral pipetting is prohibited.
- Long hair must be tied back.
- Hands must be washed after gloves are removed, and before leaving the laboratory.
- Work surfaces must be cleaned and decontaminated daily.
- Aerosol production is to be minimized.
- All contaminated or infectious materials are to be decontaminated.
- Access to laboratories should be restricted.
- Hazard warning signs must be posted.
- The use of needles, syringes and other sharp objects should be limited.
- All spills, accidents and overt or potential exposures must be reported in writing.
- Baseline serum should be collected.
- Laboratory workers should be protected by immunization.
- A biological safety officer (BSO) and or biological safety committee should be established.

Bloodborne Pathogens

- In the United States, OSHA (Occupational Safety and Health Association) has introduced powerful legislation to ensure that appropriate precautions are taken when dealing with blood and blood products.
- In Canada, we have no such legislation, however Health Canada has released several documents including the Laboratory Biosafety Guidelines which **strongly recommend** certain practices
- Universal Precautions state simply that when handling any blood, blood products or body fluids, assume the material is infectious, and handle accordingly.

Precautions to reduce risk of Exposure to Bloodborne Pathogens

- Recognition of concept of **universal precautions**
- Correct and frequent handwashing
- Immune prophylaxis
- Wearing of gloves
- The wearing of appropriate protective clothing: closed front gown (face shield or safety glasses and mask to be added if potential for splattering)
- Personal hygiene (nothing by mouth, no eating or drinking)
- No mouth pipetting, **ever**
- Proper handling of blood tubes (outsides may be contaminated)
- Needles and syringes: use disposable, no recapping, disposal into labelled leak and puncture-proof containers, no bending or breaking of needles
- Appropriate disposal of specimens
- Disinfection of laboratory surfaces when work finished, tubes in appropriate racks not directly on bench
- Establishing a biosafety officer
- Reporting of accidents
- Written protocols

HIV and Hepatitis Facts

Hepatitis

There are 5 known types of Hepatitis virus at this time:

NAME	GENETIC MATERIAL	TRANSMISSION	INFECTIVITY	LABORATORY CONCERN
Hep A (Infectious Hepatitis)	ss RNA	Fecal Oral		for animal handlers esp. Chimpanzees
Hep B (Serum hepatitis)	DsDNA	**Bloodborne**	Very high titer	**LAI**
Hep C (Non A non B hepatitis)	SsRNA	**Bloodborne**	lower titer	**LAI**
Hep D	SsRNA	Bloodborne		only in individuals with Hep B
Hep E	SsRNA	Fecal Oral		

From the above table, the agents of most concern to laboratory workers at the University of Ottawa are those which may be transmitted as Bloodborne pathogens.

Most frequent routes of entry:

- Direct percutaneous inoculation
- Percutaneous transfer by overt mechanism ie: minute scratches, abrasions
- Contamination of mucosal membranes

Lab incidents other than the above:

- Indirect contact: through contamination of common laboratory surfaces (Hep B) and transferral to mucosal surfaces
- Splashing and centrifuge accidents

To protect against exposure, immunization is available (not all cases):

(As indicated below, immunization may be pre- or post-exposure. For further information, please contact the Occupational Health Nurse at extension 1472.)

Hep A:
Pre exposure: not necessary for lab workers, except if duties involve handling of primates
Post exposure: IG
Hep B:
Pre exposure: **Hepatitis B vaccine, strongly recommended** for all those handling blood or blood products, or human tissues
Post exposure: Hepatitis B vaccine, and/or HBIG within 7 days PE

Hep C: none available

Hep D:As for Hep B

Hep E: none available

NOTE: Hep B vaccine is 80-95% effective in preventing Hep B, and exhibits minimal side effects.

Disinfection, sterilization and decontamination:

Hepatitis B virus is capable of surviving drying and storage at 25 deg and 42% relative humidity for 7 days. It is inactivated by several disinfectants including gluteraldehyde, 500 ppm sodium hypochlorite, isopropyl and ethyl alcohols, phenolic and quaternary ammonium germicides
Sterilization may be accomplished by either steam sterilization or ethylene oxide. At the University of Ottawa, the former is the method employed.

In the event of a blood spill: wearing gloves and face shield, ensure spill site is cleaned of all visible blood (organic material will inhibit the action of the sodium hypochlorite). The area is then wiped down with clean towels soaked in appropriate disinfectant (1/100 bleach or 0.05% sodium hypochlorite). If the area where the spill occurred is porous, use a more concentrated solution (0.5%)

HIV or Human Immunodeficiency Virus

HIV-1 belongs to the group of RNA viruses known as retroviruses. These viruses are able to exist in a latent phase for prolonged periods before disease develops. The approximate incubation time between HIV-1 infection and the onset of disease is 8 years (for infants is approximately 2 years) .

Viability:

Various studies have shown that survival rates for dried HIV-1 virus can be as high as 3 to 7 days. Viral infectivity decreases by 1 log every 8 to 9 hours. Thus a blood spill containning 3 logs of virus per milliliter could still contain viable virus for over 1 day if allowed to dry. **Thus the necessity to promptly clean spills**. The virus is stable at room temperature.

The HIV virus is heat labile. It may be inactivated at 56 deg for 10-20 min (certain studies indicate it could require up to 5 hours at 56 to ensure inactivation). Inactivating to 56 deg may produce false positives and as such **is not recommended as a safety measure**. It is however used in the preparation of safe therapeutic blood products.

The virus does not appear to be inactivated by routine UV irradiation in biosafety cabinets. Sonication does not inactivate the virus, but exposure to high pH over 13 or below 1 will result in inactivatation.

Although it has been demonstrated to survive in liquid cultures for 2 weeks and in dried form for more than 3 days, disinfectants are very good . (Note: although most disinfectants work well against liquid virus, they are not as effective against dried HIV-1: ie dried HIV-1 was not inactivated by 10 min of 70% ETOH)

Occupational exposure:

Health Canada has established protocols for determining if exposure is deemed to have occurred based on the following criteria:

Type or extent of injury
Body fluid involved
Dose of the inoculum
Environmental factors
Recipient susceptibility

Route or extent of exposure:

Parenteral: 87% associated with needlesticks, or cuts with cntaminated objects
Mucous membrane exposures: splattering with blood
Direct contact transmission: not found but NIH feel potential is there
Aerosol producing procedures: not found either but potential there.

Viral concentration: the concentration of virus is dependant on the stage of the patient's illness, and the antiviral treatment. Viral concentration has been found to be very low in asymptomatic patients (HIV positive), low in Aids Related Complex patients and high in AIDS patients. Titers varied from 10^0 to 10^8 TCID/ml

Other factors: Virulence of the strain
Post exposure first aid
Immunological status of exposed worker
Inflammation around exposure site

- CDC estimates that **12,000 health care workers** will become infected with **HBV** every year whereas the total number of **HIV infected workers is 120** (TOTAL)
- Risk of HBV infection varies from 6 to 30% after parenteral exposure
- Risk of HIV 0.25%, due to lower concentrations of virus in the blood
- Incidence of a fatality with HBV is 1-2%
- Incidence of fatality with HIV is ultimately 100%
- The risk of mortality from parenteral exposure to bth viruses is essentially the same (0.06 to 0.3%)

Other bloodborne pathogens:

HTLV-1 Human T lymphotropic Virus Type 1: associated with adult T cell leukemia lymphoma..
HTLV-2 Variant of the first virus
HIV-2 second retrovirus capable of causing AIDS, closely related to simian immunodeficiency viruses.

The most efficient way to avoid exposure to Bloodborne Pathogens is to follow Universal Precautions.

4.0 Containment

- Primary Containment
- Secondary Containment
- Practices and Techniques
- Personal Protective Equipment
- Levels of Containment

- The most important element of containment is strict adherence to standard microbiological practices and

techniques.

- People working with infectious agents or potentially infected materials must be aware of potential hazards and must be trained and proficient in the practices and techniques required to handle the material safely.

Primary Containment

- The first line of defence.
- A protective envelope that effectively encapsulates the infectious agent or animal, or conversely the envelope may encapsulate the worker.
- Ensures the protection of personnel and the immediate laboratory environment from exposure to the infectious agent.
- Includes biological safety cabinets, glove boxes and animal caging equipment. Also includes a stoppered bottle!

Secondary Containment

- Protects the environment external to the laboratory from exposure.
- Includes facility design and operational practices.

Personal Protection

- Effective use of vaccines is often an overlooked form of protection against exposures.
- Although the preferred method of reducing exposure is at the source, to protect against failure of the primary containment, PPE can become an important line of defence.
- Two criteria for consideration:
  - degree of protection offered
  - ease of use
- Once the PPE has been identified, (as per the Occupational Health and Safety Act, and U of O policy 77 on Occupational Health and Safety) it is the **responsibility of both** the user and the supervisor to ensure that PPE is worn
- Each type of containment level (1,2,3 and 4) requires specific protective laboratory clothing.
- For each of your sectors, a standard has been established. Please confirm with your supervisor or with EHSS for further information.

Personal Protective Equipment

- **Laboratory Coats or gowns**

  The laboratory coat has 2 uses: to protect street clothing from biological or chemical spills, and to offer some additional body protection.
  Level 2: lab coat, gown, smock or uniform recommended that it be of 100% cotton due to flame retardancy and resistance to a number of chemicals
  Level 3: solid front or wrap around gowns. Some institutions recommend a 2 piece scrub suit as mandatory under the solid front gown

- **Head coverings**

  Generally not required in most biological areas, unless a complete change of clothing is required for access, and where product protection is required. A clear example of this is the Animal Care and Veterinary Services protocols for entering animal rooms, particularly those animals which must be disease free (SCID mice)

- **Shoes and Shoe coverings**

  Any area where there is a significant risk of dropping heavy objects should require the use of industrial safety shoes.

For general biological use, comfortable shoes such as tennis shoes or nurses shoes are recommended. **Sandals are not allowed** in laboratories using biohazards due to the potential exposure to infectious agents. A change from street shoes is strongly recommended for those working in Level 3 facilities and for those working with infected animals in animal rooms.. Alternately, shoe coverings may be used in Level 2 and Level 3 when a complete change of shoes and a dedicated pair of shoes are not required.

- **Gloves**

Gloves are the most widely used form of PPE. They are used for a wide variety of hazards including protection from heat, cold, solvents, caustics, toxins, infectious microorganisms, radioisotopes, cuts and animal bites. Unfortunately, there is no ideal glove that will protect against all hazards.
Gloves are made from a variety of materials including, rubber (latex), neoprene, neoprene-latex, nitrile, polyurethane, PVC, etc. Selection should depend on the hazard encountered.. If biological work will include the use of chemical solvents such as toluene, benzene or carbon tetrachloride, rubber, neoprene and PVC will be degraded. A full listing of glove resistances is obtainable from most glove suppliers, alternatively, contact the EHSS office for information.

In microbiological laboratories, surgical gloves of latex, rubber or vinyl are generally the preferred choice. They offer a high level of dexterity and a higher level of sensitivity, unfortunately they offer very little if any protection against needlesticks, sharps or animal bites. Remember the gloves are always the weakest component of the PPE. Some studies have shown however, that double layers of latex gloves or the addition of a pair of neoprene glove will increase the layer of protection form the normal 0.008-0.01 inch of latex to 0.03, resulting in a lower exposure dose in the event of needlestick injury.
Gloves should overwrap the cuff and lower sleeve of the laboratory clothing. When handling infected tissues during necropsies, often stainless steel mesh gloves are worn to protect against accidental cuts.

- **Respiratory protection**

Two types of respiratory protection exist. Those which supply clean air or those which remove the hazardous particulates.
A respirator such as a full face or half face cartridge respirator, require quantitative fit testing according to OSHA 29 (of the US). These types of respirators would be worn in atmospheres that pose an infectious or toxic hazard such as an animal room where infectious agents could be excreted in urine.
Single use paper dust masks are not classified as true respirators. They do not offer adequate respiratory protection in infected animal rooms or other areas where infectious aerosols may be present, because one cannot be ensured of an adequate fit. They are acceptable for use during necropsies and other surgical procedures in order to maintain a sterile surgical field The second type of respirator which supply clean air, include hoods, helmets and full suits, or self contained breathing apparatus. These are more bulky and ease of movement is often restricted.

- **Eye or Face Protection**

Eye or face protection is important because biological work may often use concentrated alkalis, and acids, concentrated disinfectants, including phenolics and quaternary ammonium compounds which can cause severe eye damage and blindness if splashed. Infection can also occur through the conjunctiva if certain pathogenic microorganisms are splattered into the eye. Full face respirators or half face respirators plus splash goggles are often recommended when respirable aerosols or droplets may be produced.
Safety glasses are intended to provide impact protection, but should not be used to protect against splashes. For these hazards, safety goggles or face shields should be used. Ordinary glasses offer better splash protection than nothing at all, however, they do not replace the need for approved safety eyewear.
Contact lenses? Most authors agree that contact lenses cannot replace proper safety equipment, but studies are divided over whether lenses on the cornea offer more protection to the wearer in the case of a spill or offer more of a hazard. In a biological laboratory, the use of contact lenses is discouraged because in the event of an accident where the lens needs to be removed, it is probable that hands would not be completely decontaminated prior to removal of the lens, and hence be at increased risk for infection.

Biosafety containment levels

- Although the containment levels follow the risk levels of the agents, the University of Ottawa Biohazards Committee will evaluate the research proposals and ensure that adequate levels of containment are available.

- **Level 1**:
  - Basic laboratory
  - Requires no special design features
  - Biosafety cabinets are not required and work may be performed on the open bench.

- **Level 2**:
  - Clinical and diagnostic facilities, as well as research and teaching laboratories with level 2 agents
  - Requires a class I or class II biological safety cabinet if any potential for aerosol or splash exists
  - An emergency plan for handling spills must be developed
  - All personnel who enter the facility must be informed of the hazards present. The biohazard sign with appropriate information must be posted.

- **Level 3:**
  - Specialized design and construction, with emphasis not only on primary barriers to protect the individual, but secondary barriers to protect the environment.
  - Must undergo annual performance, testing and verification
  - All work must be performed in type II or type III biosafety cabinets. All centrifugation must be performed in closed trunnion cup centrifuges
  - Staff must receive specific training for the agents employed, above the general hazard information
  - PPE is very specific, including solid front clothing and dedicated footwear, for use only within the facility
  - Written protocols must be provided and posted
  - A medical surveillance program must be in effect
  - A reporting system for accidents must be in place

- **Level 4:**
  - The highest level of containment available
  - All manipulations pose a high risk of exposure and infection
  - The worker must be completely isolated from the infectious material
  - Design specifications are extremely stringent
  - Entry and exits are through airlocks
  - Showers are mandatory
  - All work is performed in class III cabinets or class I or II cabinets with a positive pressure suit.

Biological Safety Cabinets

- The single most important safety device in the microbiology laboratory and second in importance only to **safe work practices**.

- There are 3 classes of cabinets: class I, II, III.

- All cabinets types serve to minimize contact between the operator and the infectious agent by the use of directional airflows.
- All biological safety cabinets contain HEPA filters to ensure the exhaust from the cabinets is free of infectious material.
- Biosafety Cabinets should be located away from doors, drafts, convection currents, diffusers and high traffic areas.
- They differ in design principles.
- Note: A **"**Clean Air Bench" is not a biological safety cabinet, it is used to protect a process, rather than the worker. It is **not for use with biological agents**.

- **Class I**:Is open fronted
  Provides flow of air into the front opening, across the work surface and out through a decontamination device, and an exhaust blower
  Similar in design to a fume hood
  Provides good protection of the worker
  Allows the use of small burners and other small equipment without degrading the containment field
  The inlet air velocity is in the range of 75-125 linear feet per minute
  Supply air is not HEPA filtered
  Does not ensure protection of the work from the worker or the environment.

- **Class II**:Is open fronted
  Referred to as vertical laminar flow
  Air flow is inwards through the front opening, then drawn (downwards) away from the work surface, to HEPA filter the air prior to crossing the work surface
  Provides good protection to the worker, as well as to the work being performed
  There are two general subtypes of class II cabinets IIA and IIB. These differ in their intake velocity (minimum 75 linear foot/min for IIA, with 100 lfpm for IIB) and the amount of air recirculated over the work surface (70% for IIA) whereas type IIB only recirculates a fraction of this, the remainder being exhausted. Another difference is that type IIB cabinets are hard ducted to a dedicated external exhaust that discharges directly outside.
  If **radioisotopes, toxic chemicals and carcinogens** are routinely used during the course of work, the type **IIB** is recommended, otherwise, they are identical in their protection of the worker from biological hazards. But if a type IIB is selected, sufficient exhaust capability must be present, as the exhaust is to the outside and not into the room.

- **Class III**: Totally sealed cabinet system suitable for extremely hazardous work.
  Cabinets are gastight and maintained at negative pressure
  All operations within the cabinet are conducted through arm-length rubber gloves.
  All supply air into the cabinet is HEPA filtered. Two HEPA filters ensure protection from exhaust.

5.0 SPECIFIC TECHNIQUES

Good Microbiological Technique (GMT)

- Summarizes a code of practice for working within a laboratory handling infectious material, as well as a series of specific technical procedures for the most common manipulations

Includes:

- Receipt and opening of new specimens
- Use of pipettes and pipetting aids
- Use of transfer loops
- Separating serum
- Techniques for use of homogenizers, shakers and sonicators
- Refrigerators and freezers
- Opening of ampoules

The following pages will cover the more common ones such as the use of syringes, centrifuges, handwashing and safe use of Biosafety cabinets.

How to work Safely in a Biological Safety Cabinet

- **Before using the cabinet**:
  Turn off UV lamp; turn on fluorescent lamp
  Disinfect work surfaces with **appropriate disinfectant**
  Place essential items inside cabinet
  Allow the blower to run for 5-10 min before work

- **After completion of work**:
  Leave blower on at least 5 minutes to purge cabinet
  Remove and decontaminate equipment and materials
  Disinfect cabinet surfaces
  Turn off blower and fluorescent lamp, turn on UV lamp

- **Maintenance**:
  Twice daily: Work surfaces wiped down
  Weekly: UV lamp should be wiped clean (dust decreases the intensity of lamp)
  Monthly: All vertical surfaces wiped down
  Annually: UV lamp intensity verified (intensity decreases with time)
  Annually: Decontamination with formaldehyde gas and certification

Safe use of Centrifuges

- Check centrifuge tubes for stress lines prior to use
- Avoid overfilling
- Ensure caps or stoppers are properly in place
- Use sealed buckets or rotors which can be loaded and unloaded in biological safety cabinets
- Ensure all buckets are properly balanced
- Ensure centrifuge achieves run conditions before leaving
- Ensure centrifuge completely stopped before opening lid
- Check immediately for spills or leaks prior to removing samples
- Clean all spills promptly and completely

Needles and Syringes

- Avoid the use of needles and syringes whenever possible
- Perform all operations within a biological safety cabinet
- Fill syringes carefully, avoid frothing or introduction of bubbles
- Shield needles with disinfectant-soaked cotton when withdrawing from stoppers
- Do not bend, shear or recap needles.
- If needles must be recapped, use a one-handed scoop method or holder
- All used needles and syringes are to be disposed of in the yellow puncture resistant containers

Handwashing

- One of the best defences to prevent exposure
- In Level 1 lab a non-antiseptic soap can be used
- Level 2 require antiseptic handwashing solutions. Most of the common antiseptic solutions contain chlorhexine gluconate or trichlosan. An alternative is to use alcoholic hand rubs. They contain an emollient to counteract the drying action of alcohol.
- Liquid dispensers should be used rather than bars
- When to wash?
  Before starting any manipulations
  Before leaving the lab
  When hands are obviously soiled
  Before and after completing any task in a biosafety cabinet
  Every time gloves are removed

Before contact with one's face or mouth
At the end of the day

Protocol for handwashing

- Turn on faucets and wet hands with tepid water
- Dispense non antiseptic soap or antiseptic compound into a cupped hand
- Spread soap or compound around both hands and between fingers
- Wash (lather) hands for **at least** 10 sec. Vigorously rub both sides of hands starting a few inches above the wrist, extending downwards between the fingers and around and under the fingernails.
- Rinse thoroughly under the tepid running water. Rinsing should start above the wrist area and proceed to the tips of the fingers. Note: if faucets are not knee- or foot-operated, do not turn off water yet.
- Dry hands thoroughly with paper towels. If hand operated, turn faucets off once hands dry, using a paper towel to protect hands.

6.0 Decontamination, Disinfection and Sterilization

- **Definitions:**
  - **Decontamination**:
    Destruction or removal of microorganisms to a lower level, such that there is no danger of infection to unprotected individuals.
  - **Sterilization** :
    Use of physical or chemical means to bring about the total destruction of all viable microorganisms.

  - **Disinfection**:
    Use of physical or chemical agents to destroy pathogens and potential pathogens on inanimate objects

- **Physical methods**

  - **Heat:**
    Autoclaving: most practical and recommended
    Incineration: for disposal of sharps and tissues

  - **Irradiation**:
    UV light: wavelength of 253 nm is germicidal
    Gamma : disrupts DNA and RNA

  - **Filtration**: HEPA: biological safety cabinets, ventilation
    0.2 micron physically removes particulates

- **Chemical methods**

  - Generally for disinfection rather than sterilization
  - Choice depends on many factors (number and nature of organism, type of item to be disinfected, purpose of treatment, other chemicals, contact time required for disinfection, toxicity, cost)
  - Most common are chlorine compounds and alcohols

Disinfection: What to use for my organism?

Vegetative bacteria (E.coli, ect.)	1% domestic bleach 75% Ethanol Quaternary ammonia 6% formulated Hydrogen peroxide
Mycobacteria and fungi	1% domestic bleach Phenolic compounds 75% Ethanol 6% formulated Hydrogen peroxide
Spore forming bacteria (Bacillus) (Time extended for several Hours to ensure sporicidal)	10% domestic bleach Gluteraldehyde Formaldehyde 6% formulated Hydrogen peroxide
Enveloped viruses (HIV, Herpes)	1% domestic bleach 75% Ethanol Quaternary ammonia 6% formulated Hydrogen peroxide
Non enveloped viruses (Hepatitis, Adenovirus)	10% domestic bleach 6% formulated Hydrogen peroxide Gluteraldehyde Formaldehyde

- **Disinfection for Prions**

  Autoclave at 132-136°C for 60 minutes, or
  Autoclave at 121°C for 4.5 hours, or
  Soak in 1N sodium hydroxide for 1 hour, then autoclave at 121° for 1.5 hours, or
  Soak in 3% SDS for 1 hour, then autoclave at 121° for 1 hour
  Treat work surfaces with 10 % sodium hypochlorite (industrial strength bleach) for at least 30 minutes.
  2N sodium hydroxide may also be used to treat surfaces.
  If skin becomes contaminated, treat for 5-10 minutes with 1N sodium hydroxide followed by extensive washing with water.

Chemical Decontamination Methods Halogen releasing chemical germicides

Chlorine componunds	**Type**	**Effective concentration; contact times**	**Advantages**	**Disadvantage**s	**Examples of Uses**
	Sodium Hypochlorite solution (domestic bleach)	0.1-1% free chlorine 10-60 minutes	Broad spectrum Inexpensive Widely available Bactericidal at low temperatures	Toxic, corrosive to skin and metals Unstable at optimum pH of 6 Inactivated by organic matter Deteriorates under light and heat; shelf life of dilutions: 1 week	General disinfectant Waste liquids Surface decontamination Emergency spill clean up Instrument disinfection *
	Calcium hypochlorite (granules, powder, tablets)	As for liquid bleach	As for liquid bleach but more stable	As for liquid bleach, but shelf life is longer	As for liquid bleach
	Sodium dichloisocyanurate (NaDCC) (tablets, powder)	As for liquid bleach	Stable at pH 6. More stable than hypochlorites. No strong smell.	Toxic, corrosive Inactivated by organic matter	As for liquid bleach
	Chloramine-T (Sodium tosylchloramide) (powder)	As for liquid bleach	More stable, less affected by organic matter than hypochlorites Longer activity than hypochlorites	Deteriorates under humidity, light and heat. Leaves powdery residue.	As for liquid bleach
	Chlorine dioxide	Demand release of chlorine in situ	Longer activity than chlorine compounds Less corrosive, toxic than other chlorine compounds Effective at pH 6-10	Aqueous solutions decompose under light	Instrument disinfection Gas sterilization of germ-free animal chambers
Iodine Preparations	Iodophors	0.003-.1% free iodine 10-30 minutes	Broad spectrum Germicidal over a wide pH range Less toxic and less irritating than aqueous or alcoholic iodine solutions	Not consistently sporocidal Readily neutralized by organic matter May allow growth of Pseudomonas species and other bacteria	Germicidal soap and antiseptics Surface decontamination of skin prior to procedures

Household sodium hypochlorite (Javex) is recommended for most applications. A 10% dilution for most disinfection purposes, with full strength used for spills.

Heat Decontamination methods

Type	Principle/Conditions	Advantages	Disadvantages	Uses
Dry Heat	**Thermal inactivation: destroys by oxidation**	**Non-corrosive Simple design and principle**	**Less effective than moist heat; requires longer times and/or higher temperatures**	**Materials that are damaged by, or are impenetrable to, moist heat**
Hot Air Oven	160-180°C for 2-4 hours	Penetrates water-insoluble materials (e.g.,grease and oil) Less corrosive to metals and sharp instruments than steam	Slow diffusion, penetration. Loading, packing critical to performance. Not suitable for reusable plastics	Anhydrous materials, such as oils, greases and powders Laboratory glassware,instruments Closed containers
Red-heat Flame	Oxidation to ashes (burning)	Rapid	Initial contact with flame can produce a viable aerosol Possibility of accidental fire	Inoculating loops, needles
Incineration	Oxidation to ashes (burning) 1-60 minutes: temperatures may exceed 1000°C	Reduces volume of waste by up to 95%	Improper use may lead to emission of pathogens in smoke Requires transport of infectious waste. Excess plastic (>20%) content reduces combustibility	For decontamination of waste items prior to disposal in landfill
Moist Heat	**Irreversible coagulation of microbial proteins**	**More rapid and more effective than dry heat**		
Pasteurization	Heating to below boiling point (normally 60-70°C) for up to 30 minutes	Can be used on heat sensitive liquids and medical devices Low cost	Not reliably sporicidal	Milk and dairy products Some heat-sensitive medical equipment
Tyndallization	Heating to 80-100°C for 30 min on 3 consecutive days with incubation periods in between	Resistant spores germinate and are killed on the second and third day	Time consuming Not reliably sporicidal	Heat sensitive materials such as bacteriological media, solutions of chemicals, biological materials
Boiling	Maximum temperature obtainable is 100°C at sea level. Minimum 10 minutes	Minimal equipment required	Cumbersome: not practical for everyday use Not reliably sporicidal	Small Instruments and equipment
Autoclaving	Steam under pressure 121°C/15 psi for 15-90 min	Minimal time required Most dependable sterilant for lab use	Loading and packing critical to performance Shielding dirt must first be removed Maintenance and quality control essential Damages heat sensitive items	Preparation of sterile glassware, media and instruments Decontamination of reusable material Decontamination of infectious waste

At the University of Ottawa, autoclaving is the method of choice for decontamination.
Sharps as well as animal and human tissue are disposed of by incineration.
Contact your Faculty Environmental Health and Safety Officer for further information.

Chemical Decontamination Methods
Non Halogen chemical germicides

Type	Effective concentration; contact times	Advantages	Disadvantages	Examples of uses
Alcohols	70-85% ethanol 60-95% isopropanol 3-30 minutes	Low toxicity Low residue Non corrosive	Rapid evaporation, reduces contact time Flammable, skin dessicant Non sporicidal, ineffective with unconventional agents	Skin disinfectant Surface decontamination Benchtop, cabinet wipedown
Phenolic compounds	0.04-5% 10-30 minutes	Leaves an active residue Biodegradable	Pungent odour, corrosive , toxic Non sporicidal, limited activity against viruses	Disinfection of floors and other surfaces Antiseptic soaps
Quaternary Ammonium compounds	0.05-1.5% 10-30 minutes	Has combined detergent and germicidal activity Stable Working dilutions have low toxicity	Not sporicidal, limited against viruses, mycobacteria Not readily biodegradable	Surface decontamination Equipment wipedown antiseptic
Hydrogen peroxide	3-30% 1-60 minutes New “formulated” 6% has additives	Rapid action no residue Low toxicity Environmentally safe 30% is sporicidal	Limited sporicidal activity Corrosive to some metals, irritant Concentration dependant	Surface decontamination Instruments and equipment
Peracetic acid	0.001-0.3% 10-60 minutes gas phase 2-4% 5-120 minutes	Broad spectrum sporicidal at low temperatures Can tolerate organic load Rapid action	Pungent odour Corrosive to some metals Shelf life of dilution is less than 1 week irritant to skin and eyes	Instruments and equipment Gas phase sterilization of chambers for germ free animals
Aldehydes Gluteraldehyde	0.5-2.5% aqueous 2-30 minutes (up to 12 hours to kill all spores)	Broad spectrum Does not corrode metal Can tolerate organic load	Expensive Requires good ventilation pH, temperature dependant Pungent odour Toxic Less than 2 week shelf life	Cold sterilant and fixative Surface decontamination Instruments, equipment, glassware
Aldehydes Formalin (37% formaldehyde)	3-27% formalin in 70-90% water 10-30 minutes	Broad spectrum Inexpensive Does not corrode metal Can tolerate organic load	Pungent odour Irritant Potential carcinogen May require 24 hours to kill spores	Cold sterilant and fixative Surface decontamination Instruments and equipment
Aldehydes Formaldehyde gas (paraformaldehyde)	1-3 hours	As for formalin Effective penetration	As for formalin Toxic Flammable Poor penetration of covered surfaces	On site decontamination of biological safety cabinets, HEPA filters Enclosed area

Type	Effective concentration; contact times	Advantages	Disadvantages	Examples of uses
Ethylene oxide gas	50-1200 mg/L 1-12 hours	Broad spectrum No heat or moisture released Penetrates packaging materials	Flammable, reactive Toxic; potential carcinogen and mutagen Some sterilized items may require 24 hours for outgassing Requires specialized equipment and training	Heat or moisture sensitive supplies , equipment and instruments

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Aldehydes Formaldehyde gas (paraformaldehyde)	1-3 hours	As for formalin Effective penetration	As for formalin Toxic Flammable Poor penetration of covered surfaces	On site decontamination of biological safety cabinets, HEPA filters Enclosed area
Ethylene oxide gas	50-1200 mg/L 1-12 hours	Broad spectrum No heat or moisture released Penetrates packaging materials	Flammable, reactive Toxic; potential carcinogen and mutagen Some sterilized items may require 24 hours for outgassing Requires specialized equipment and training	Heat or moisture sensitive supplies , equipment and instruments

7.0 SPILL RESPONSE

- Spill response will vary depending on the following:
  What is spilled? How much? Where?
- Spills should be cleaned up immediately, to ensure proper decontamination
- If you must leave the scene at any time, you must leave a note informing others of the spill

- **All spills are to be reported**

- **Spills within a biological safety cabinet**

  Leave the ventilation on
  Flood the spill with 10% bleach and cover with absorbent
  Leave on for 20 minutes
  Pick up with absorbent material
  All items within the cabinet should also be disinfected (wiped down and or autoclaved)
  All waste should be autoclaved
  Ventilation should run 10-15 minutes

- **Spills outside of a biological cabinet, in a laboratory (large spills)**

  Evacuate the area for 30 minutes to allow aerosols to settle
  Assemble cleaning supplies, and PPE
  Using concentrated disinfected such as 5% hypochlorite (undiluted bleach) pour disinfectant from outside, towards inside.
  Cover with absorbent, and allow disinfectant to act for 20 min
  (If spill is of blood or other heavy organic material, wipe up most of the material, then allow disinfectant to act for 20 min)
  All adjacent areas should also be disinfected or wiped down
  All waste should be autoclaved

- **Spills occurring in a centrifuge**

  Leave lid closed and allow aerosols to settle for at least 1 hour
  Thoroughly wipe down inside of centrifuge, including the lid with paper towels soaked in disinfectant
  Disinfect the entire rotor, especially the bucket where spill occurred within the centrifuge
  Remove rotor from centrifuge and repeat disinfection.
  Rinse both rotor and inside of centrifuge with water if bleach was used.
  All waste should be autoclaved

- **Spills occurring during transport**

  Clean-up must be initiated immediately (as hallways are not negatively pressured)
  Then follow directives for spills outside of a biological safety cabinet

- **All users of biological materials should be familiar with the above procedures.**

- **Additional assistance is available from:**

  - The ERT teams by dialling extension 5411
  - EHSS by dialling extension 5892
  - Your departmental safety officer

8.0 BIOLOGICAL WASTE

- Biological waste includes the following:

  Sharps and biomedical waste (refer to the procedure)
  Tissue culture waste
  Microbiological waste, including media

- Sharps and biomedical waste are incinerated, according to the procedures

- Tissue culture and microbiological waste is decontaminated prior to disposal.

  2 choices are available: Autoclaving
  Chemical decontamination

- Autoclaving is the preferred route at the University of Ottawa

- All biological waste is to be decontaminated, including Level 1.

9. BIOHAZARD REGULATIONS

- Unlike other countries, Canada mainly oversees the use of biological agents through the Health Canada Laboratory Biosafety Guidelines.
- Granting agencies such as MRC requires that these Biosafety Guidelines are met.
- The main concern being the assurance by the University Biohazards Committee that the appropriate level of containment be available
- Certain aspects of biohazard use are more firmly regulated. These include:
- Importation of biological agents, of Risk Group 2 and above require permits. (Importation of Human Pathogens Regulations, 1994; Health of Animals Act and Regulations)
- Exportation of certain microorganisms of Risk Group 3 and above require permits. (Export Control List, Foreign Affairs and International Trade Canada)
- Transportation of infectious substances must meet packaging, labelling and documentation standards. (Transportation of Dangerous Goods Act and Regulations, Canada; IATA Dangerous Goods Regulations; ICAO Technical Instructions for the Safe Transport of Dangerous Goods by Air)
- Information, instruction and supervision must be provided. (Occupational Health and Safety Act)
- Hazard information and instruction must be provided. (WHMIS Regulation)
- Biomedical waste must be disposed of appropriately. (Regulation 347, Waste Management; The Canada Environmental Protection Act)

10.0 UNIVERSITY OF OTTAWA POLICIES AND PROCEDURES

- Biological incident reporting
- Shipping of infectious substances
- Guidelines for biomedical waste
- Biological safety cabinet certification
- Biohazardous Materials Use Certificate
- Several areas are under development
- Immunization (initiatives are already in place in some departments, and for undergraduate students)
- Post Exposure Prophylaxis policy
- Biosafety training policies
- Centralized importation permits

The above are just some of the initiatives in place, or being examined, to ensure that biological materials are handled safely at the University of Ottawa.

With proper knowledge, planning and care, biological exposure is avoidable.

Let's keep it that way!

11.0 SUGGESTED READING

WHO, 1993, Laboratory Biosafety Manual, 2nd edition, Geneva

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MMWR
Weekly
June 24, 1988 / 37(24);377-388

Perspectives in Disease Prevention and Health Promotion Update: Universal Precautions for Prevention of Transmission of Human Immunodeficiency Virus, Hepatitis B Virus, and Other Bloodborne Pathogens in Health-Care Settings

Introduction
The purpose of this report is to clarify and supplement the CDC publication entitled "Recommendations for Prevention of HIV Transmission in Health-Care Settings" (1).*

In 1983, CDC published a document entitled "Guideline for Isolation Precautions in Hospitals" (2) that contained a section entitled "Blood and Body Fluid Precautions." The recommendations in this section called for blood and body fluid precautions when a patient was known or suspected to be infected with bloodborne pathogens. In August 1987, CDC published a document entitled "Recommendations for Prevention of HIV Transmission in Health-Care Settings" (1). In contrast to the 1983 document, the 1987 document recommended that blood and body fluid precautions be consistently used for all patients regardless of their bloodborne infection status. This extension of blood and body fluid precautions to all patients is referred to as "Universal Blood and Body Fluid Precautions" or "Universal Precautions." Under universal precautions, blood and certain body fluids of all patients are considered potentially infectious for human immunodeficiency virus (HIV), hepatitis B virus (HBV), and other bloodborne pathogens.
Universal precautions are intended to prevent parenteral, mucous membrane, and nonintact skin exposures of health-care workers to bloodborne pathogens. In addition, immunization with HBV vaccine is recommended as an important adjunct to universal precautions for health-care workers who have exposures to blood (3,4).

Since the recommendations for universal precautions were published in August 1987, CDC and the Food and Drug Administration (FDA) have received requests for clarification of the following issues: 1) body fluids to which universal precautions apply, 2) use of protective barriers, 3) use of gloves for phlebotomy, 4) selection of gloves for use while observing universal precautions, and 5) need for making changes in waste management programs as a result of adopting universal precautions.

Body Fluids to Which Universal Precautions Apply

Universal precautions apply to blood and to other body fluids containing visible blood. Occupational transmission of HIV and HBV to health-care workers by blood is documented (4,5). Blood is the single most important source of HIV, HBV, and other bloodborne pathogens in the occupational setting. Infection control efforts for HIV, HBV, and other bloodborne pathogens must focus on preventing exposures to blood as well as on delivery of HBV immunization.

Universal precautions also apply to semen and vaginal secretions. Although both of these fluids have been implicated in the sexual transmission of HIV and HBV, they have not been implicated in occupational transmission from patient to health-care worker. This observation is not unexpected, since exposure to semen in the usual health-care setting is limited, and the routine practice of wearing gloves for performing vaginal examinations protects health-care workers from exposure to potentially infectious vaginal secretions.

Universal precautions also apply to tissues and to the following fluids: cerebrospinal fluid (CSF), synovial fluid, pleural fluid, peritoneal fluid, pericardial fluid, and amniotic fluid. The risk of transmission of HIV and HBV from these fluids is unknown; epidemiologic studies in the health-care and community setting are currently inadequate to assess the potential risk to health-care workers from occupational exposures to them. However, HIV has been isolated from CSF, synovial, and amniotic fluid (6-8), and HBsAg has been detected in synovial fluid, amniotic fluid, and peritoneal

fluid (9-11). One case of HIV transmission was reported after a percutaneous exposure to bloody pleural fluid obtained by needle aspiration (12). Whereas aseptic procedures used to obtain these fluids for diagnostic or therapeutic purposes protect health-care workers from skin exposures, they cannot prevent penetrating injuries due to contaminated needles or other sharp instruments.

Body Fluids to Which Universal Precautions Do Not Apply

Universal precautions do not apply to feces, nasal secretions, sputum, sweat, tears, urine, and vomitus unless they contain visible blood. The risk of transmission of HIV and HBV from these fluids and materials is extremely low or nonexistent. HIV has been isolated and HBsAg has been demonstrated in some of these fluids; however, epidemiologic studies in the health-care and community setting have not implicated these fluids or materials in the transmission of HIV and HBV infections (13,14). Some of the above fluids and excretions represent a potential source for nosocomial and community-acquired infections with other pathogens, and recommendations for preventing the transmission of nonbloodborne pathogens have been published (2).

Precautions for Other Body Fluids in Special Settings

Human breast milk has been implicated in perinatal transmission of HIV, and HBsAg has been found in the milk of mothers infected with HBV (10,13). However, occupational exposure to human breast milk has not been implicated in the transmission of HIV nor HBV infection to health-care workers. Moreover, the health-care worker will not have the same type of intensive exposure to breast milk as the nursing neonate. Whereas universal precautions do not apply to human breast milk, gloves may be worn by health-care workers in situations where exposures to breast milk might be frequent, for example, in breast milk banking.

Saliva of some persons infected with HBV has been shown to contain HBV-DNA at concentrations 1/1,000 to 1/10,000 of that found in the infected person's serum (15). HBsAg-positive saliva has been shown to be infectious when injected into experimental animals and in human bite exposures (16-18). However, HBsAg-positive saliva has not been shown to be infectious when applied to oral mucous membranes in experimental primate studies (18) or through contamination of musical instruments or cardiopulmonary resuscitation dummies used by HBV carriers (19,20). Epidemiologic studies of nonsexual household contacts of HIV-infected patients, including several small series in which HIV transmission failed to occur after bites or after percutaneous inoculation or contamination of cuts and open wounds with saliva from HIV-infected patients, suggest that the potential for salivary transmission of HIV is remote (5,13,14,21,22). One case report from Germany has suggested the possibility of transmission of HIV in a household setting from an infected child to a sibling through a human bite (23). The bite did not break the skin or result in bleeding. Since the date of seroconversion to HIV was not known for either child in this case, evidence for the role of saliva in the transmission of virus is unclear (23). Another case report suggested the possibility of transmission of HIV from husband to wife by contact with saliva during kissing (24). However, follow-up studies did not confirm HIV infection in the wife (21).

Universal precautions do not apply to saliva. General infection control practices already in existence -- including the use of gloves for digital examination of mucous membranes and endotracheal suctioning, and handwashing after exposure to saliva -- should further minimize the minute risk, if any, for salivary transmission of HIV and HBV (1,25). Gloves need not be worn when feeding patients and when wiping saliva from skin.

Special precautions, however, are recommended for dentistry (1). Occupationally acquired infection with HBV in dental workers has been documented (4), and two possible cases of occupationally acquired HIV infection involving dentists have been reported (5,26). During dental procedures, contamination of saliva with blood is predictable, trauma to health-care workers' hands is common, and blood spattering may occur. Infection control precautions for dentistry minimize the potential for nonintact skin and mucous membrane contact of dental health-care workers to blood-contaminated saliva of patients. In addition, the use of gloves for oral examinations and treatment in the dental setting may also protect the patient's oral mucous membranes from exposures to blood, which may occur from breaks in the skin of dental workers' hands.

Use of Protective Barriers

Protective barriers reduce the risk of exposure of the health-care worker's skin or mucous membranes to potentially infective materials. For universal precautions, protective barriers reduce the risk of exposure to blood, body fluids containing visible blood, and other fluids to which universal precautions apply. Examples of protective barriers include gloves, gowns, masks, and protective eyewear. Gloves should reduce the incidence of contamination of hands, but they cannot prevent penetrating injuries due to needles or other sharp instruments. Masks and protective eyewear or face shields should reduce the incidence of contamination of mucous membranes of the mouth, nose, and eyes.

Universal precautions are intended to supplement rather than replace recommendations for routine infection control, such as handwashing and using gloves to prevent gross microbial contamination of hands (27). Because specifying the types of barriers needed for every possible clinical situation is impractical, some judgment must be exercised.

The risk of nosocomial transmission of HIV, HBV, and other bloodborne pathogens can be minimized if health-care workers use the following general guidelines:**

1. Take care to prevent injuries when using needles, scalpels, and other sharp instruments or devices; when handling sharp instruments after procedures; when cleaning used instruments; and when disposing of used needles. Do not recap used needles by hand; do not remove used needles from disposable syringes by hand; and do not bend, break, or otherwise manipulate used needles by hand. Place used disposable syringes and needles, scalpel blades, and other sharp items in puncture-resistant containers for disposal. Locate the puncture-resistant containers as close to the use area as is practical.

2. Use protective barriers to prevent exposure to blood, body fluids containing visible blood, and other fluids to which universal precautions apply. The type of protective barrier(s) should be appropriate for the procedure being performed and the type of exposure anticipated.

3. Immediately and thoroughly wash hands and other skin surfaces that are contaminated with blood, body fluids containing visible blood, or other body fluids to which universal precautions apply. Glove Use for Phlebotomy

   Gloves should reduce the incidence of blood contamination of hands during phlebotomy (drawing blood samples), but they cannot prevent penetrating injuries caused by needles or other sharp instruments. The likelihood of hand contamination with blood containing HIV, HBV, or other bloodborne pathogens during phlebotomy depends on several factors: 1) the skill and technique of the health-care worker, 2) the frequency with which the health-care worker performs the procedure (other factors being equal, the cumulative risk of blood exposure is higher for a health-care worker who performs more procedures), 3) whether the procedure occurs in a routine or emergency situation (where blood contact may be more likely), and 4) the prevalence of infection with bloodborne pathogens in the patient population. The likelihood of infection after skin exposure to blood containing HIV or HBV will depend on the concentration of virus (viral concentration is much higher for hepatitis B than for HIV), the duration of contact, the presence of skin lesions on the hands of the health-care worker, and -- for HBV -- the immune status of the health-care worker. Although not accurately quantified, the risk of HIV infection following intact skin contact with infective blood is certainly much less than the 0.5% risk following percutaneous needlestick exposures (5). In universal precautions, all blood is assumed to be potentially infective for bloodborne pathogens, but in certain settings (e.g., volunteer blood-donation centers) the prevalence of infection with some bloodborne pathogens (e.g., HIV, HBV) is known to be very low. Some institutions have relaxed recommendations for using gloves for phlebotomy procedures by skilled phlebotomists in settings where the prevalence of bloodborne pathogens is known to be very low.

   Institutions that judge that routine gloving for all phlebotomies is not necessary should periodically reevaluate their policy. Gloves should always be available to health-care workers who wish to use them for phlebotomy. In addition, the following general guidelines apply:

4. Use gloves for performing phlebotomy when the health-care worker has cuts, scratches, or other breaks in his/her skin.

5. Use gloves in situations where the health-care worker judges that hand contamination with blood may occur, for example, when performing phlebotomy on an uncooperative patient.

6. Use gloves for performing finger and/or heel sticks on infants and children.

7. Use gloves when persons are receiving training in phlebotomy. Selection of Gloves

   The Center for Devices and Radiological Health, FDA, has responsibility for regulating the medical glove industry. Medical gloves include those marketed as sterile surgical or nonsterile examination gloves made of vinyl or latex. General purpose utility ("rubber") gloves are also used in the health-care setting, but they are not regulated by FDA since they are not promoted for medical use. There are no reported differences in barrier effectiveness between intact latex and intact vinyl used to manufacture gloves. Thus, the type of gloves selected should be appropriate for the task being performed.

   The following general guidelines are recommended:

8. Use sterile gloves for procedures involving contact with normally sterile areas of the body.

9. Use examination gloves for procedures involving contact with mucous membranes, unless otherwise indicated, and for other patient care or diagnostic procedures that do not require the use of sterile gloves.

10. Change gloves between patient contacts.

11. Do not wash or disinfect surgical or examination gloves for reuse. Washing with surfactants may cause "wicking," i.e., the enhanced penetration of liquids through undetected holes in the glove. Disinfecting agents may cause deterioration.

12. Use general-purpose utility gloves (e.g., rubber household gloves) for housekeeping chores involving potential blood contact and for instrument cleaning and decontamination procedures. Utility gloves may be decontaminated and reused but should be discarded if they are peeling, cracked, or discolored, or if they have punctures, tears, or other evidence of deterioration. Waste Management

    Universal precautions are not intended to change waste management programs previously recommended by CDC for health-care settings (1). Policies for defining, collecting, storing, decontaminating, and disposing of infective waste are generally determined by institutions in accordance with state and local regulations. Information regarding waste management regulations in health-care settings may be obtained from state or local health departments or agencies responsible for waste management. Reported by: Center for Devices and Radiological Health, Food and Drug Administration. Hospital Infections Program, AIDS Program, and Hepatitis Br, Div of Viral Diseases, Center for Infectious Diseases, National Institute for Occupational Safety and Health, CDC. Editorial Note: Implementation of universal precautions does not eliminate the need for other category- or disease-specific isolation precautions, such as enteric precautions for infectious diarrhea or isolation for pulmonary tuberculosis (1,2). In addition to universal precautions, detailed precautions have been developed for the following procedures and/or settings in which prolonged or intensive exposures to blood occur: invasive procedures, dentistry, autopsies or morticians' services, dialysis, and the clinical laboratory. These detailed precautions are found in the August 21, 1987, "Recommendations for Prevention of HIV Transmission in Health-Care Settings" (1). In addition, specific precautions have been developed for research laboratories (28). References

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40. Centers for Disease Control. 1988 Agent summary statement for human immunodeficiency virus and report on laboratory-acquired infection with human immunodeficiency virus. MMWR 1988;37(suppl no. S4:1S-22S). *The August 1987 publication should be consulted for general information and specific recommendations not addressed in this update. **The August 1987 publication should be consulted for general information and specific recommendations not addressed in this update. Copies of this report and of the MMWR supplement entitled Recommendations for Prevention of HIV Transmission in Health-Care Settings published in August 1987 are available through the National AIDS Information Clearinghouse, P.O. Box 6003, Rockville, MD 20850.