

Biosafety at Ryerson

2. Biological Hazards

Training Outline

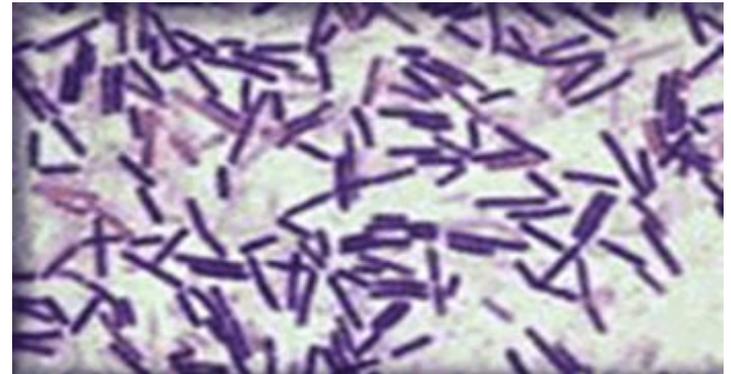
- what is a biohazard?
- risk groups
- containment levels
- risk assessment
- lab acquired infections

What is a Biohazard?

A living biological organism material produced by such an organism than can cause disease in humans or animals.

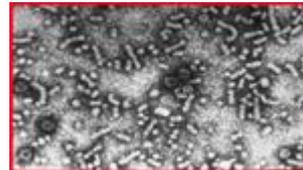
Examples:

- microorganisms such as bacteria, virus, fungus
- transformed cell lines
- infected tissue cultures
- recombinant DNA
- human or animal blood or body fluids



What is a Biohazard of Concern?

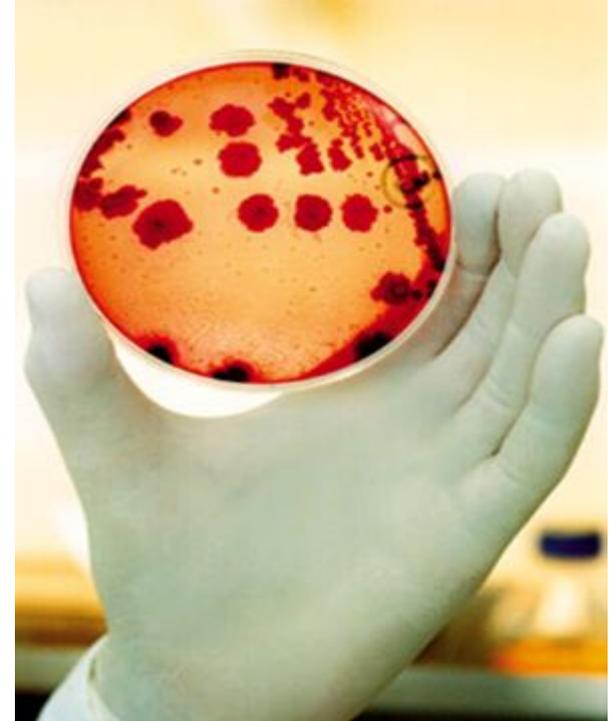
- potential for acquiring a laboratory-associated infection (LAI)
- contamination of the environment
- contamination of research
- public perception



Risk Groups

Classification of organisms according to risk groups are the traditional way that infectious organisms categorized:

- assumes growth in small volumes (<10 litres) for experimental, diagnostic or teaching purposes
- based on the relative hazards of the potential risk of causing diseases in humans and in animals
- does not take into account the procedures that are to be employed during the manipulation of a particular organism



Risk Groups

The Public Health Agency of Canada (PHAC) has defined **4 levels of risk** in classification of organisms:

Risk Group 1: Low community and low individual risk of disease

Any biological agent that is unlikely to cause disease in health workers or animals. Agents that pose little or no risk are assigned to Risk Group 1.

Examples: *Lactobacillus spp.*, *Bacillus subtilis*, *Naegleria gruberi*,
Micrococcus spp., *E. coli K12*

Risk Groups

Risk Group 2: Low community risk and moderate individual risk to disease

- can cause human disease, but under normal circumstances is unlikely to be a serious hazard to laboratory workers, the community, livestock or the environment
- lab exposures rarely cause infection leading to serious disease, effective treatment and preventive measures are available and the risk of spread is limited

Examples: *Escherichia coli* 0157:H7, *Hepatitis B virus*, *Toxoplasma spp*, *HIV (non-cultured)*, *Salmonella typhimurium*, *Measles*, *Mumps*, *Adenoviruses*, *Influenza viruses*

Risk Groups

Risk Group 3: Low community risk and high individual risk to disease

- causes serious human disease or can result in serious economic consequences but does not ordinarily spread by casual contact from one individual to another, or that causes diseases treatable by antimicrobial or antiparasitic agents

Examples: *Hantavirus*, *Yersinia pestis*, *Bacillus anthracis*, *HIV* (cultured isolates) *Bacillus anthracis*, *Mycobacterium tuberculosis*, *Creutzfeldt-Jakob*

Risk Groups

Risk Group 4: Agents with extremely high community and individual risk

- pose the greatest risk are assigned to Risk Group 4
- usually produces very serious human 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

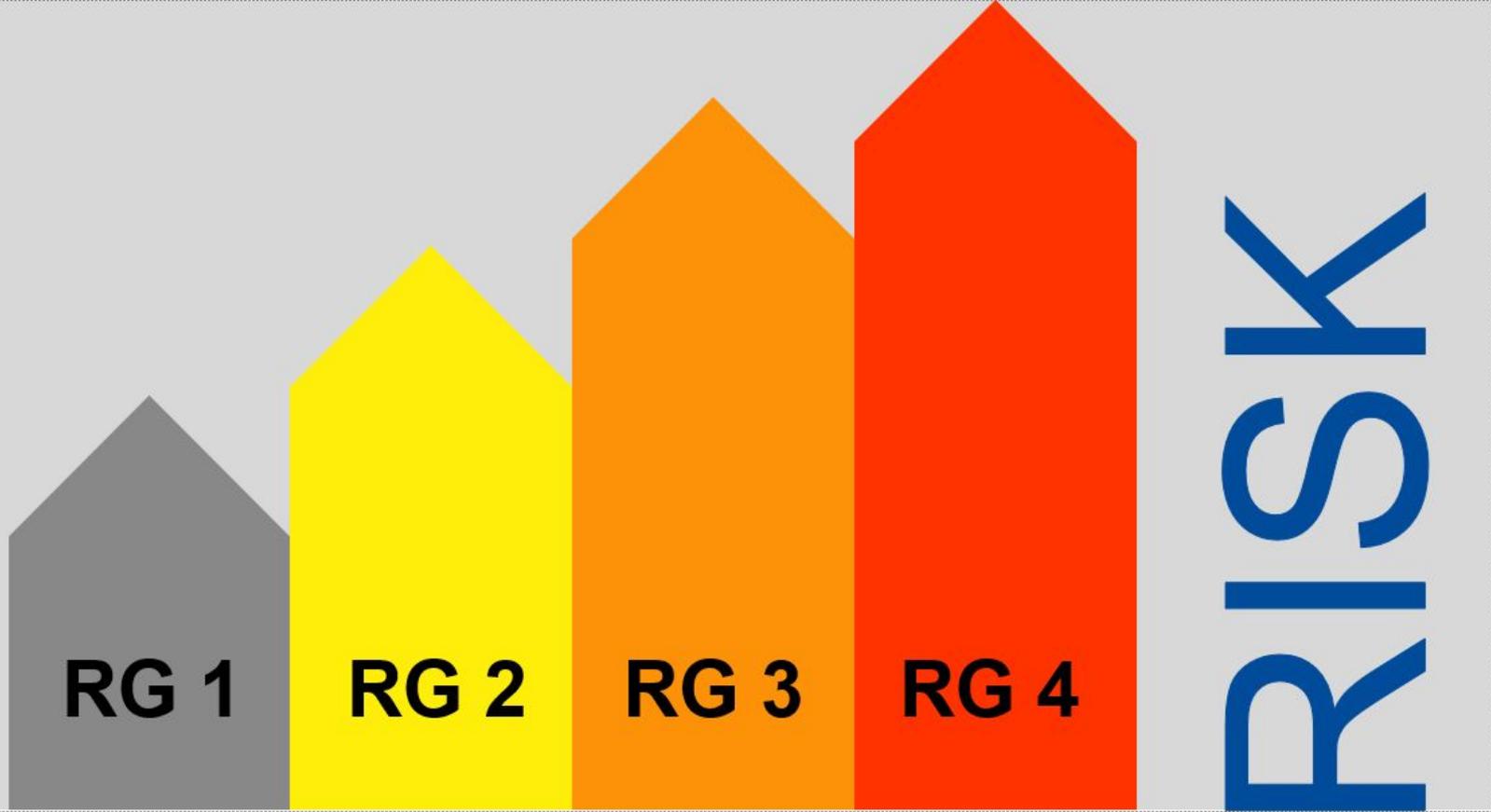
Examples: *Marburg virus*, *Ebola virus*, *Crimean-Congo hemorrhagic fever virus*

Risk Groups

As the level rises, so does:

- the risk of the organism to humans, animals, plants and/or the environment
- the procedural and facility requirements
- the level of containment required
- the degree of protection for personnel, the environment and the community





Containment Levels

Containment levels are selected to provide the user with a description of the minimum containment required for handling the organism safely in the laboratory.

PHAC outlines four containment levels.

Containment Level 1 (CL1)

- organisms requiring Containment Level 1 require no special design features beyond a basic level, well-designed functional laboratory
- work may be done on an open bench top and containment is usually achieved through the use of good work practices in a basic microbiology laboratory

Containment Level 2 (CL2)

- The primary exposure hazards associated with organisms required CL2 are through the ingestion, inoculation and mucous membrane route.
- Agents requiring CL2 facilities are not generally transmitted by airborne routes but care must be taken to avoid the generation of aerosols.
- Primary containment devices used in these types of laboratories includes such as biological safety cabinets or centrifuges in addition to proper personal protective equipment such as laboratory coats, gloves, eye protection are required.
- Minimization of contamination includes proper hand washing facilities and decontamination facilities such as autoclaves.

Containment Level 3 (CL3)

- Organisms requiring CL3 labs can cause serious or life-threatening disease and often have a low infectious dose.
- Primary and secondary barriers are required to minimize the release of infectious organism into the immediate laboratory area and the environment.
- Depending on the organism being used, both Public Health Agency of Canada and the Canadian Food Inspection Agency are required to certify the laboratory prior to start of work.

Containment Level 4 (CL4)

- Organisms are highly pathogenic, low infectious dose and have the potential for aerosol transmission and produce very serious and often fatal disease.
- Offers maximum containment and a complete sealing of the perimeter of the laboratory facility.
- CL4 laboratories are very rare in Canada.

Security Sensitive Biological Agents (SSBA)

- Agents and toxins that pose an increased biosecurity risk due to their inherent dual-use potential for bioterrorism
- Includes the following:
 - Risk Group 3 and 4 agents
 - Toxins listed in schedule 1 of the HPTA except toxins identified in HPTR sec.10(2) not prescribed in certain quantities
- An individual accessing a facility containing SSBA's must hold a security clearance issued by PHAC for that part of the facility or they are accompanied and supervised by a person who holds a security clearance.

List of Agents, Prohibited agents *and* SSBAs

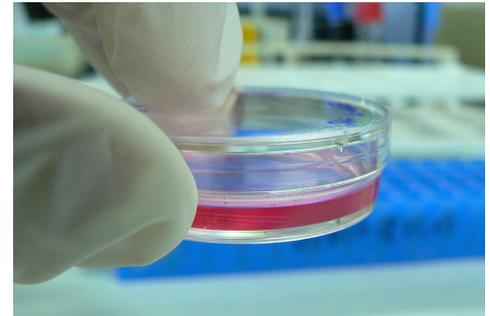
The Human Pathogens and Toxins Act includes a list of:

- *Toxins (sch.1)
- Risk Group 2 Human Pathogens: bacteria, viruses, fungi, protozoa, prions (sch.2)
- *Risk Group 3 Human Pathogens (sch.3)
- *Risk Group 4 Human Pathogens (sch.4)
- Prohibited Human Pathogens and Toxins (sch.5)

*Security Sensitive Biological Agents (SSBAs) include toxins (except toxins not prescribed in certain quantities as identified in HPTR sec.10(2), all Risk Group 3 and Risk Group 4 agents. Not all human pathogens and toxins are listed in the legislation. A human pathogen, which is not listed in any of the schedules in the Act, may still be categorized within a risk group based on individual risk assessment.

Cell Lines and Tissue Cultures

- Cell cultures derived from humans or animals suspected or known to be infected with a pathogen should be assigned to the risk group appropriate for the suspected or known pathogen and handled using the relevant containment level and work practices.
- Cell cultures may carry oncogenic or infectious particles; even well characterized lines with a history of safe use can become contaminated with infectious microorganisms
- Prudent to treat all eukaryotic cultures as moderate risk agents (Risk Group 2) and to use Containment Level 2 facilities and work practices.



Tissue Culture

- Have the potential to contain pathogenic organisms
- A detailed risk assessment should be undertaken when using a new cell line

Tissue Culture (continued...)

Source (species of origin)	Cell types or tissues	Culture type
The closer the genetic relationship of the cell culture is to humans, the higher the risk is to humans since contaminating pathogens usually have specific species barriers. <i>Be aware that some contaminating organisms might cross the usual species barrier (e.g. H5N1 influenza, BSE, SARS, etc.</i>	Consider the tumour inducing potential of cell types.	
increasing order of risk	increasing order of risk	increasing order of risk
avian and invertebrate cells	epithelial and fibroblastic cells	well-characterized cell lines
mammalian cells (other than human or primate)	gut mucosa	continuous cell lines
non-human primate cells	endothelium	primary cell lines
human cells	neural tissues	
	hematopoietic cells	

Risk Assessment

A risk assessment should take into account:

- the components of the work to be done in order to determine what procedures and activities put employees at greatest risk of having an exposure
- alternate processes considered to eliminate the risk of exposure.
- large volumes (>10 litres) and high concentrations of an organism in growth media may pose greater risk than smears of the same agent on a microscope slide.
- if infectious droplets and aerosols may be produced, containment should be elevated to the next level

Safety data sheets are available for many organisms from [PHAC's PSDS webpage](#) or their PSDS App, you can also get a link to those via [EHS' Biosafety webpage](#).

Risk Assessment

Hazard classifications of biological agents reflect the judgements made on their **inherent risks** based on:

- infectious dose
- route of infection
- pathogenicity & virulence
- host range
- vectors
- disease incidence and severity
- prevention & treatment
- whether the pathogen is indigenous to Canada
- effect on animals, plants, fish

Recombinant DNA

- In vitro incorporation of segments of genetic material from one cell into another is termed “recombinant DNA”.
- Has resulted in altered organisms that can manufacture products such as vaccines, enzymes, etc.
- Genetically engineered organisms are used for treatment of waste and spills and plants can be made resistant to disease or adverse weather conditions.
- A genetically altered organism may be directly pathogenic or toxic, and if released into the environment transfer undesirable genetic traits to wild species or mutate to pathogenic form.

Risk Assessment: *Recombinant DNA*

The risk assessment for recombinant DNA should include:

- source of the DNA to be transferred
(i.e., from an RG2 organism or an RG4 organism?)
- ability of vector to survive outside the laboratory
- interaction between transferred gene and host

When assessing the risk group and containment level for a genetic engineered protocol, **if one of the components is potentially hazardous**, a risk level appropriate to the known hazard is assigned.

Blood and Body Fluids

- Risk associated with blood and bodily fluids is the potential exposure to bloodborne pathogens which may be present in contaminated blood and bodily fluids and are capable of causing disease in exposed individuals. The pathogens of greatest concern are the **hepatitis B virus (HBV)**, the **hepatitis C virus (HCV)** and the **Human Immunodeficiency Virus (HIV)**.
- **Human Immunodeficiency Virus** is a retrovirus that causes Acquired Immunodeficiency Syndrome (AIDS). The mean incubation period is 10 years. It is difficult to become infected with HIV through a needle stick injury or other exposure to blood or other body fluids.
- The risk depends on the amount of virus to which one is exposed and the titre of HIV viral RNA. There is no vaccine for HIV, but drugs are available which reduce the risk of becoming infected with the virus. To be effective, the drugs must be started within 1 to 2 hours after the exposure.

Blood and Body Fluids

- **Hepatitis B** is caused by a potentially fatal virus that destroys liver cells and may permanently damage the liver. It can be transmitted not only by percutaneous exposures, but also via mucous membranes.
- The incubation period for hepatitis B is 45 to 160 days. Of the people infected with hepatitis B, 10% become chronic carriers and may develop cirrhosis and an increased susceptibility to liver cancer.
- Immunization is a very effective method of preventing hepatitis B. Personnel in high risk groups, must show confirmation of vaccination against hepatitis B.
- **Hepatitis C** is caused by a virus and the interval between exposure and seroconversion is approximately 8 to 10 weeks.
- At least 85% of people infected with the virus will become chronically infected. An increased risk of liver cancer does exist, especially in individuals who develop cirrhosis.

Risk Assessment: *Blood and Body Fluids*

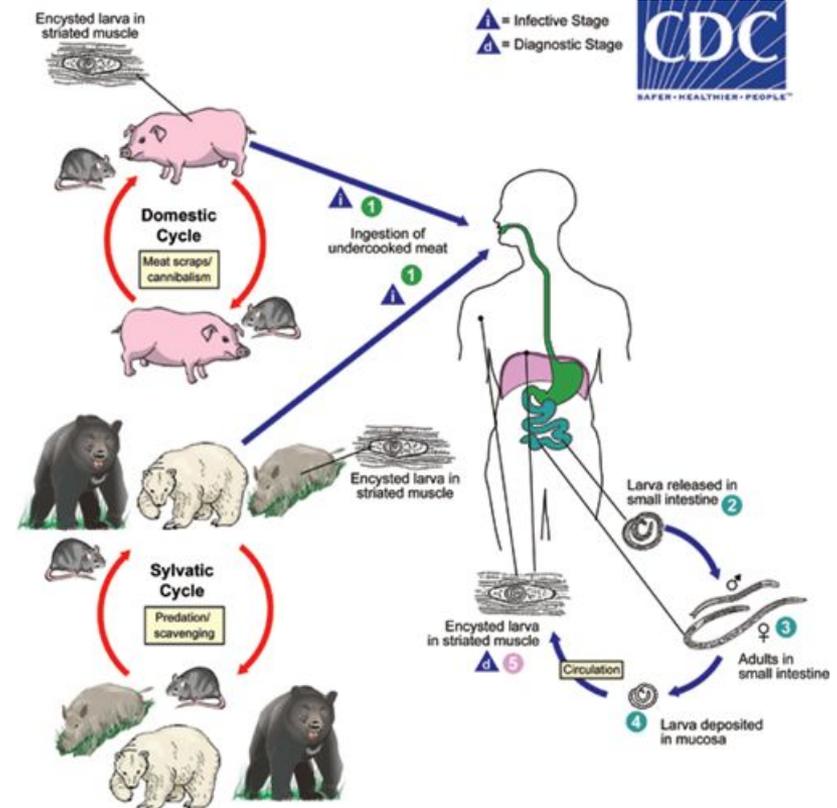
The types of body fluids capable of transmitting **HIV, HBV, and HCV** from an infected samples include:

- blood, serum, plasma and all biologic fluids visibly contaminated with blood
- laboratory specimens, samples or cultures that contain concentrated HIV, HBV, HCV
- organ and tissue transplants
- pleural, amniotic, pericardial, peritoneal, synovial and cerebrospinal fluids
- uterine/vaginal secretions or semen (unlikely able to transmit HCV)
- saliva (for HCV, HBV, and HIV if a bite is contaminated with blood and for HBV if a bite is not contaminated with blood).

Feces, nasal secretions, sputum, tears and urine are not indicated in the transmission of **HIV, HBV or HCV** unless **visibly contaminated with blood**.

RISK ASSESSMENT: *Animal Pathogens*

The level of containment will be dependent on not only the risk to human health, but also the requirements to prevent the escape of an animal pathogen into the outside environment.



Risk Assessment: *Plant Pathogens*

- The **CFIA** has [containment standards for facilities housing plant pests](#).
- The risk to laboratory personnel from plant pests is relatively low risks, since plant pests rarely infect healthy people.
- Some plant pests, pose a significant threat to agricultural production, and natural environments.
- Important that personnel working with plant pests take steps to prevent the accidental escape of potentially damaging pests into the environment.
- The level of containment required to prevent escapes will depend on specific pest biology and the impact that an escape might have on the Canadian environment.

Unconventional Pathogens

- TSE prion diseases; lethal transmissible neurodegenerative conditions
 - *Creutzfeld-Jakob disease, Variant C-J Disease, Mad Cow Disease, Scrapie, Chronic Wasting Disease.*
- Resistant to destruction by procedures that normally inactivate viruses.
- Contact EHS to assess requirements with PHAC and CFIA (containment, procedures, waste disposal, etc.)

RISK ASSESSMENT: *Unconventional Pathogens*

- Unconventional or slow viruses, e.g. prions (proteinaceous infectious particles) have been associated with transmissible degenerative disease of the central nervous system in humans (e.g., Creutzfeldt-Jacob) and in animals (such as encephalopathy).
- Resistant to destruction by chemical or physical disinfection.
- Precautions should be observed when handling neurological material from suspected infected humans or animals:
 - handle as a minimum of Risk Group 2 or higher, depending on nature of the work and amount of agent being manipulated;
 - handle tissue as if still infectious even if tissue is fixed with formalin or embedded in wax;

Lab Acquired Infections

- Health Canada reports that there have been over **5,000 reported cases of lab-acquired infections** and **190 deaths up to 1999** worldwide.
- It is also estimated that only **20%** of infections can be attributed to any known, single exposure event.
- **80%** of laboratory acquired infections (LAI's) go undetected due to long incubation periods, mild symptoms, or symptoms common to everyday illnesses (i.e. flu-like symptoms).

RISK ASSESSMENT: *Lab Acquired Infections*

Several ways in which infectious substances can enter the body and cause infection:

- ingestion
- inhalation
- contact with mucous membranes, including transfer of microorganisms to the eyes by contaminated hands or with non intact skin.

Infections are caused from exposure to infectious aerosols, spills, splashes, needle stick injuries, cuts, centrifuge accidents.

RISK ASSESSMENT: *Lab Acquired Infections*

- Exposure to aerosols is estimated to be the single largest cause of laboratory infections.
- Operational practices and techniques must be used to minimize the creation of aerosols associated with common laboratory procedures.
- Where chemical disinfection procedures are employed, effective concentrations and contact times must be used.
- Chemical disinfectants used to decontaminate materials to be removed from the laboratory must be replaced regularly.

Always wash hands

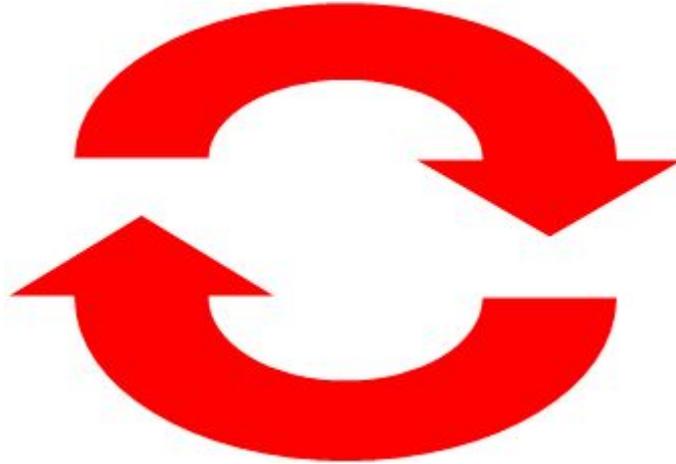


Risk Assessment: *Lab Acquired Infections*

Every incident (no matter how small) must be investigated to determine if the risk of exposure exists, and what could be done to prevent the possibility of reoccurrence.

Laboratory Associated Infections

Routes of Transmission



Laboratory Associated Infections

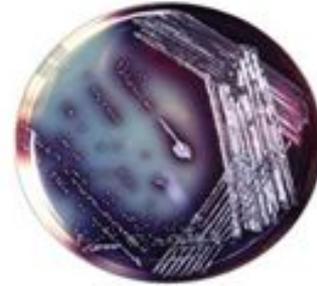
Routes of exposure

- percutaneous inoculation
- inhalation of aerosols
- contact of mucous membranes
- ingestion

Laboratory Associated Infections

Sources of infection:

- cultures and stocks
- research animals
- specimens



Susceptible host:

- immune system
- vaccination status
- age
- items contaminated with above



Laboratory Associated Infections

- **80%** unknown or unrecognized causes
- **20%** causative or defined event
 - **80%** of which are caused by human error
 - **20%** are caused by equipment failure
- Top 4 accidents resulting in infection
 1. spillages and splashes
 2. needles and syringes
 3. sharp object, broken glass
 4. bite or scratch from animals or ectoparasites

Laboratory Associated Infections

Who	What	Where	When	How
Researcher	SARS	Taiwan	December 2003	
Microbiologist	West Nile Virus	United States	August 2002	Laceration
Laboratory Worker	Meningococcal Disease	United States	2000	Aerosol?
Laboratory worker	Vaccinia virus	Europe	2002	Contact



End

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