Biohazard and Biosafety
Topics covered

Quick Introduction

Some definitions

Pathogens and biosafety levels

Good microbiological techniques

Helpful equipment
Introduction
Why do we care about biosafety?

Some unfortunate examples of disease outbreaks in research labs:

1950-1976:
• A survey of 5000 labs showed 3921 cases of disease outbreaks
• Most commonly reported were: Hepatitis, tuberculosis, typhoid, brucellosis, rabbit fever

More recently (since 2003):
• SARS infects researchers in a lab in Singapore
• A US and a Russian scientist are infected by Ebola. One survives, one dies.
• Polio virus escapes from two idian labs
• Scientists from Boston University contract rabbit fever (a serious bacterial disease)
• Anthrax exposure in a Huston lab due to aerosols leaked inside an unshielded centrifuge
Some Definitions

**Biohazard**: An agent of biological origin that has the capacity to produce deleterious effects on humans, i.e. microorganisms, toxins and allergens derived from those organisms; and allergens and toxins derived from higher plants and animals.

**Biosafety**: The containment principles, technologies and practices that are implemented to prevent the unintentional exposure to pathogens and toxins, or their accidental release

**Biosecurity**: Control of accidental and deliberate release of biohazardous material
Pathogens
Types of pathogens

**Bacteria**
- Size: 0.3 to 2 μm
- Single-celled organisms
- Various morphologies
- Examples: *Salmonella* spp., *E. coli*, *Vibrio* spp. (Cholera), *Mycobacterium* (Tuberculosis)
Types of pathogens (cont.)

**Virus**
- Size: 18-200 nm
- Basic structure: capsid (protein) + nucleic acid
- Obligate parasites
- Enveloped vs. non-enveloped
- Examples: Hepatitis, polio, HIV
Types of pathogens (cont.)

**Protozoa**

- Size: 5-10 μm
- Single-celled eukaryotes
- Numerous morphologies
- Examples: *Cryptosporidium* spp., *Plasmodium* spp. (Malaria), *Giardia* spp.

*Giardia*

Malaria patient
Types of pathogens (cont.)

**Helminths**
- Size: 20-100 μm
- Multi-cellular eukaryotes
- For transmission mainly concerned with eggs

*Ascaris lumbricoides* egg
- Causes human roundworm
- 70 μm length
Types of pathogens (cont.)

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Patient receiving treatment for *Ascaris*
Pathogen risk assessment

To analyze the biological risk, we must take into account:

- Pathogenicity/infectivity
- Virulence/lethality
- Infective dose
- Therapy/Prophylaxes
- Epidemic potential
- Resistance
- Survival in the environment
- Geographic spread (endemic)
- Mode of transmission
Transmission routes

Pathogens can be waterborne, bloodborne, foodborne, airborne, ...

**Routes of entry:**

Skin typically forms a good barrier, but infectious agents can enter the body through:

- Contact with open wounds, cuts, acne, sunburn or blisters
- Mucous membranes of mouth, nose and eyes
- Ingestion (eating in the lab)
- Insect/animal bites (5-10 per year at EPFL)
- Inhalation of dust, aerosols
- Personal hygiene practices
- Injections (unprotected needles)
Transmission routes you might not think off

Think about your hands before you touch any of the following:
Classification of pathogens (and GMOs)

Biosafety level 1:

Definition:
GMOs or pathogen agents that are generally speaking harmless for humans.

Examples:
E.coli (non-pathogenic strains), yeast

Requirements:
Labs must have doors and sinks for hand washing
Work surfaces must be easy to clean
Classification of pathogens (and GMOs)

Biosafety level 2:

Definition:
GMOs or pathogen agents that may cause a person (or animal) to be affected by a disease but whose propagation through the community is pretty improbable. Immunization or antibiotic treatment are available.

Examples:
Measles, Salmonella, Hepatitis B

Requirements:
Biohazard signs
Doors can be locked, limited access
Directional airflow (no air circulating into other labs)
Some procedures conducted in biological safety cabinets
Classification of pathogens (and GMOs)

Biosafety level 3:

Definition:
GMOs or pathogen agents that may induce a serious disease to humans or animals but for which an efficient treatment is available.

Examples:
Tuberculosis, Hepatitis C, HIV

Requirements:
Separate building or isolated zone
Double entry door
Respiratory protection when necessary
All procedures must be conducted in BSC
Classification of pathogens (and GMOs)

Biosafety level 4:

Definition:
GMOs or pathogen agents that induce serious diseases in humans or animals. They are often propagated via air routes and no efficient vaccines nor therapy exist to cure their effects.

Examples:
Ebola, Smallpox

Requirements:
Dedicated exhaust and vacuum system
Door, windows, etc. are sealed
Emergency breathing air, generator and exit
Positive pressure personnel suit
Etc.

Level 4 labs are rare! None at EPFL
Good Microbiological Techniques (GMT)
Basic principles for biosafety:

It’s not just about protecting YOU!

It’s also about:

• Protection of the public
• Protection of the experiment
• Protection of the environment

Very important to remember:
Your experiment can be part of the public even if you don’t think of it, e.g., by way of contact with the custodial staff!
Information in the lab

GMTs and other information

• Access
• Personal protection
• Procedures
• Working area
• Equipment
• Etc.

Lab access

The international biohazard warning symbol and sign must be displayed on the doors of the rooms where microorganisms of Biosafety level 2 or higher are handled.

Only authorized persons should be allowed to enter the laboratory working areas.

Laboratory doors should be kept closed.

Children should not be authorized or allowed to enter laboratory working areas.
Personal protection

Wear protective clothing, eye protection and gloves.

Do NOT wear laboratory clothing outside the laboratory, e.g. in offices, libraries and toilets.

Do NOT wear open toe shoes.

Wash hands after handling infectious materials and before leaving the laboratory.

No eating, drinking, handling of contact lenses or cosmetics.
Procedures

No mouth pipetting!

All procedures should be performed in a way that minimizes the formation of aerosols and droplets

All spills, accidents and exposures to infectious materials must be reported to the laboratory supervisor

Contaminated liquids must be decontaminated (chemically or physically) before discharge to the sink

Written documents that are expected to be removed from the laboratory need to be protected from contamination
Working area

The laboratory should be kept neat, clean and free of materials that are not pertinent to the work.

Work surfaces must be decontaminated after any spill and at the end of the day.

All contaminated materials must be decontaminated before disposal or cleaning.

Steam autoclaving is the preferred method for decontaminating loose material. Chemical decontaminants (e.g., bleach) can be used to decontaminate lab benches.
Biological waste

In the lab, waste must be contained in a biohazard box with an autoclavable biohazard bag (at EPFL: usually yellow)

Inactivate liquids either chemically (e.g., with bleach) or autoclave.

Sterilize solids by autoclaving, then transfer into a different bag (at EPFL: red-white) to indicate that the waste has been deactivated.

Special treatment for radioactive waste and waste of a biosafety level of 3 and higher (see EPFL biohazard coordinator)
Needles, blades and glass

AVOID INJECTION OF BIOLOGICAL MATERIALS!

Handle all needles with extreme caution

If possible, do NOT recap needles

Avoid glassware, use plastic instead. In labs with a biosafety level 2 and higher, glass is forbidden.

Dispose of broken glass and needles in a sharps containers. Full and sealed sharps containers can be added to solid waste.
Centrifuges and other tricky instruments

Centrifuges should be placed at a level that one can see into the bowl

Always close centrifuge tubes

Make sure centrifuge rotor is equilibrated and locked

Pressure can build up in sonicators, shakers, etc.

After use of these instruments, open containers in the biosafety cabinet.

Avoid splashing.
Necessary and helpful equipment
Autoclave

An autoclave is a steam pressure vessel

Steam and pressure increases the boiling point of water, sterilizes without boiling

Sterilization usually complete after 20 min at 121 °C

Can also use in dry heat mode to sterilize glassware

Things that CANNOT be autoclaved: animals, solvents, acid, corrosive materials, radioactive materials, certain plastics, explosives, batteries, bulbs, etc.

Attention: things are HOT when you remove them from the autoclave
Biosafety cabinet

NOT a fume hood!

Protects the worker and/or the product from contamination

Exhaust air is filtered (HEPA filter)

Has UV lamp for disinfection (protect your eyes!)

Must be in a location that is free of turbulence and air flow

Should be in an area of low personnel traffic

Different classes:
Class 1: protection of worker, no product protection
Class 2: protection of worker and product
Class 3: Process isolation
For Biosafety at EPFL

http://biosafety.epfl.ch/

Biosafety coordinator: Stephane Karlen

• Basic training and prevention
• Risk evaluation
• Waste handling
• Emergency care
• Etc.

Each lab working with biohazards must have a biosafety officer who:

• supervises the activities linked to pathogens or GMOs
• verifies that the good microbiological techniques of laboratory are respected
• prepares the standard operating procedures (SOP) and ensures that they are implemented
Questions?

"THE VIRUS IS THAT BAD, HUH?"