http://www.youtube.com/watch?v=mPD2ilLGDf4

BIOSAFETY

An introduction

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by Jörn Klein, Ph.D. in Virology

Why focusing on biosafety ?

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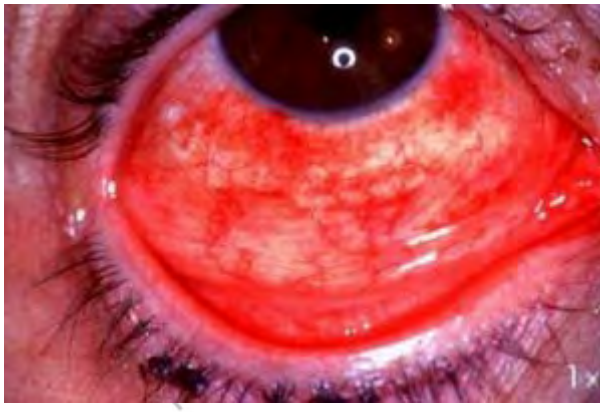

Many people are not aware about potential biohazard.
Molecular biologists and physicians are generally not trained in biosafety and it is only by chance that he/she knows e.g.
that adenoviral vectors do not have to be replication competent to cause corneal and conjunctival damage
or
that Epstein Baar virus transformed cell lines are still infectious during the first passages and can cause cancer.

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TO BE OR NOT TO BE

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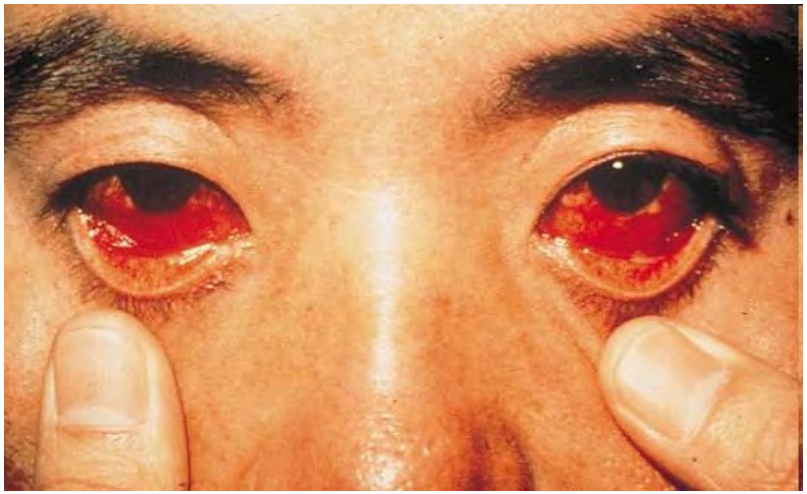
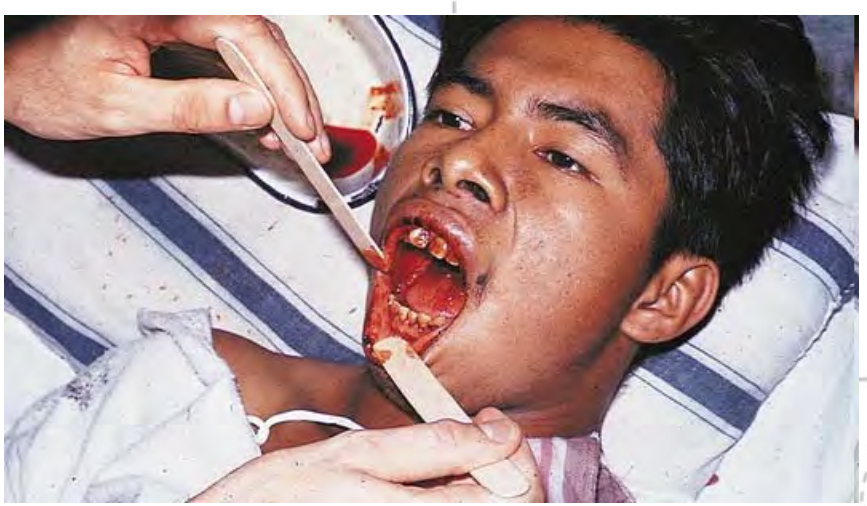
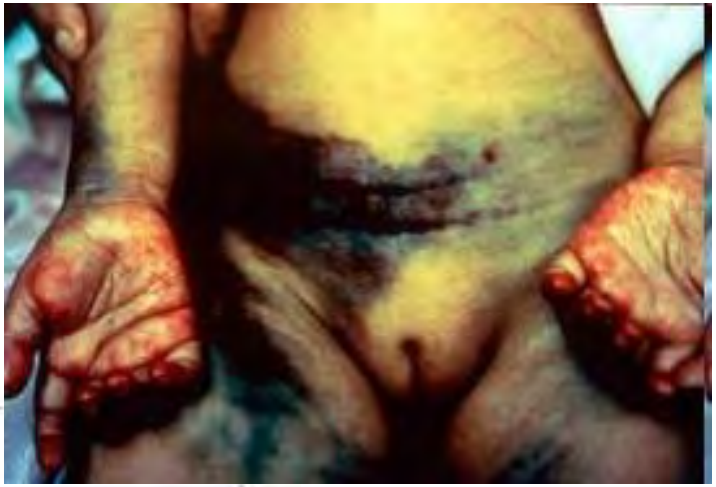
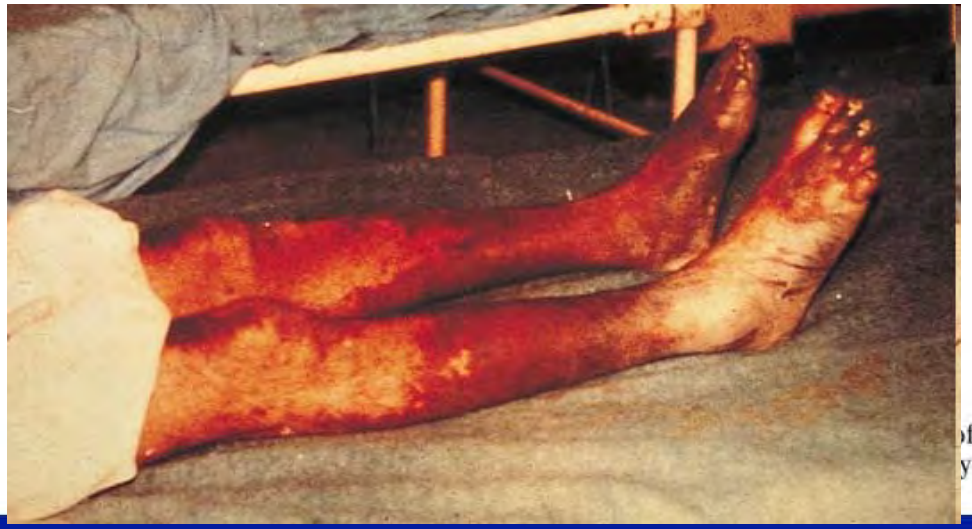




Hantaan-virus





World Health Organization

The World Health Organization's Laboratory Biosafety Manual states that

"no biosafety cabinet or other facility or procedure alone guarantees safety unless the users operate safe techniques based on informed understanding."

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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.

What is a Lab?

Labs are a workplace where people do unusual things with hazardous materials

Incidence of laboratory-acquired infections

Surveys for lab-associated infections
More than 5,000 labs the last 10 years in the western world
Cumulative total of 3,921 cases cited

Most commonly reported:

- **Hepatitis**
- **Tuberculosis**
- **Typhoid**
- **E.coli (HUS)**
- **Brucellosis**
- **Tularemia**

More

Laboratory infection

A woman in her 20s fell ill with bloody diarrhoea on 1 July 2011. She had been in contact with the outbreak strain during the incubation period while working in a microbiology laboratory. The outbreak strain was isolated from her stool sample. She had not travelled to northern Germany and not eaten sprouts during the incubation period. She lives in an area without known outbreak clusters and had no known link to Company A or the two private parties.

Reasons for laboratory accidents

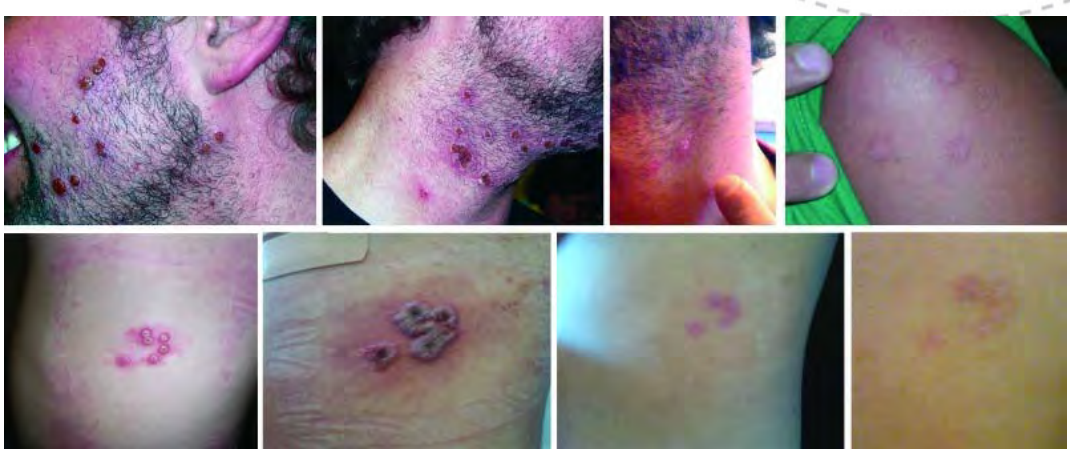
- Lack of instruction in safe procedures for handling infected material
- The removal of trained and experienced workers
- The emergence of new and resurgence of old diseases which have caught some people unawares
- Lack of protection of workers
- Inadequate system of inspection of premises

It's not only you !!!

Secondary and Tertiary Transmission of Vaccinia Virus from US Military Service Member

Gregory E. Young, Christina M. Hidalgo, Ann Sullivan-Frohm, Cynthia Schulte, Stephen Davis, Cassandra Kelly-Cirino, Christina Egan, Kimberly Wilkins, Ginny L. Emerson, Kimberly Noyes, and Debra Blog

During February and March 2010, the New York State Department of Health investigated secondary and tertiary vaccinia contact transmission from a military vaccinee to 4 close contacts. Identification of these cases underscores the need for strict adherence to postvaccination infection control guidance to avoid transmission of the live virus.



SURVEILLANCE AND OUTBREAK REPORTS

Secondary transmissions during the outbreak of Shiga toxin-producing *Escherichia coli* O104 in Hesse, Germany, 2011

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Why Biosafety Practices?

Protection:

- **workers**
- **co-workers**
- **lab support personnel**
- **environment (Hey, that means your family and friends)**
- **“products”**

Exercise

What is Biosafety?

- Principles and practices employed to protect laboratory personnel and the environment from exposure or infection while working with living organisms, biological materials, or agents.
  - Included are any materials that may be potentially infectious.
  - Includes recombinant DNA research

to reduce the risk

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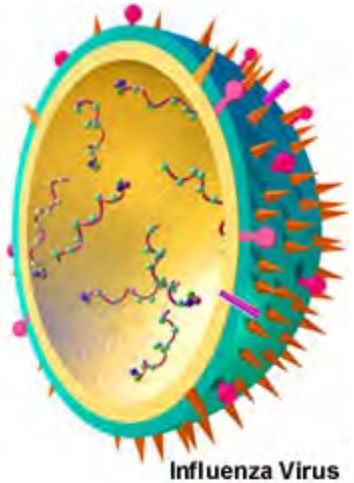
Agents and Risks



- The “agent” is the what creates risk
- Risks to the worker or environment are often unknown
- Determining “acceptable risk”?

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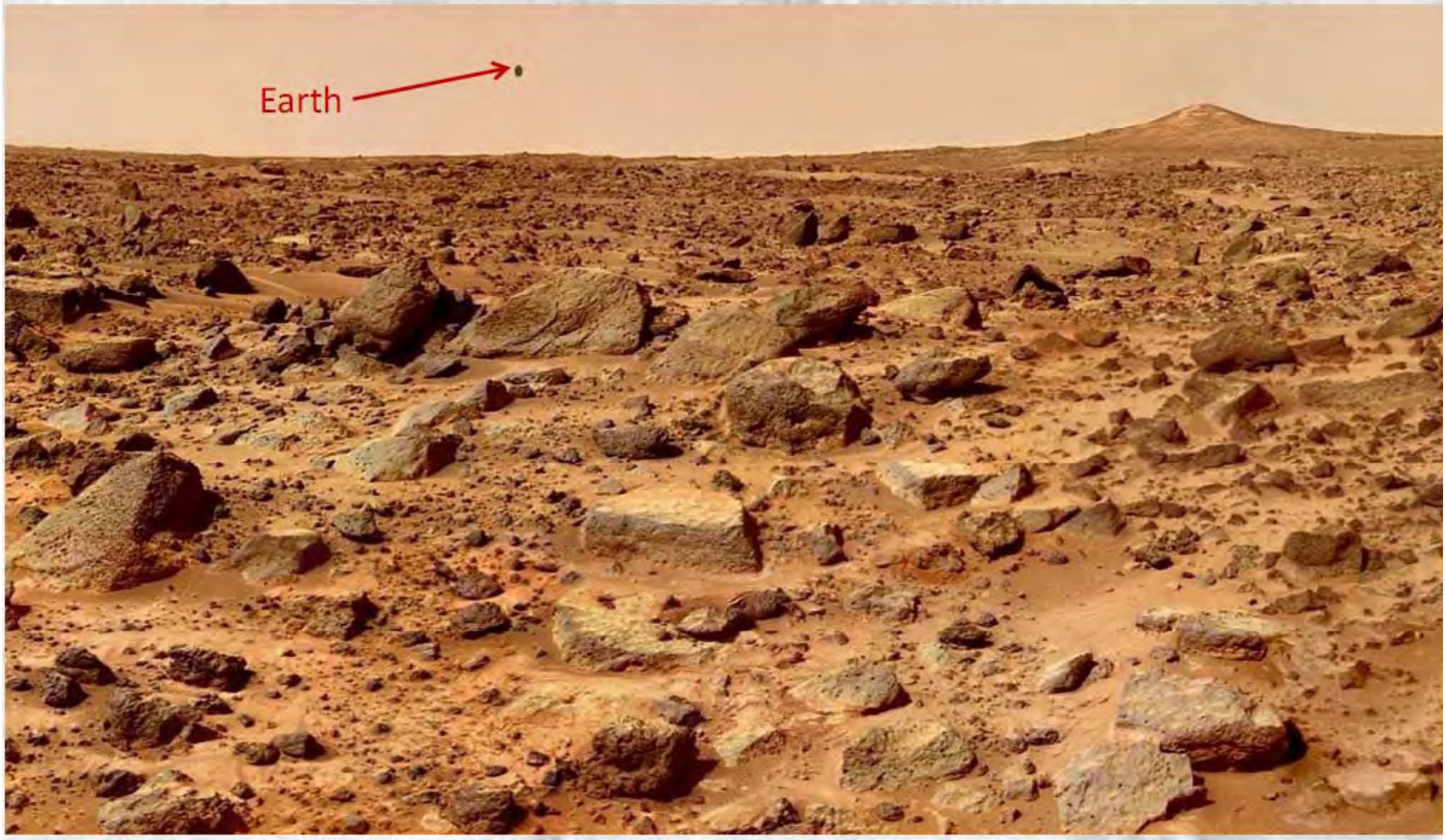
100 % Safety and/or Sustainability CAN be achieved*



*Don’t build a facility or perform research with biological agents. On Mars.

Assessing Risk

- There is always risk!
  - The risk must be identified
  - The risk is evaluated
  - The risk must be measured
  - Plan to minimize the risk

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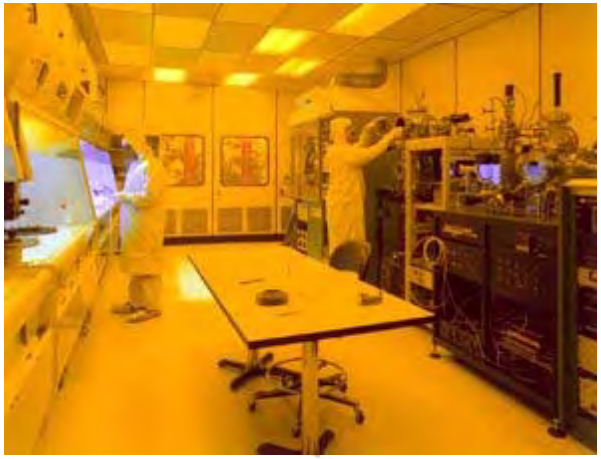
Identifying Risk



- Understand the biology of the agent
- Susceptibility and transmission within the host
- Hazards associated with equipment and procedures
- <u>Goal:</u>
  - *Provide the highest practical protection and the lowest practical exposure*

Evaluating Risk Acceptability

- Worst case scenario -What might happen?
- Likelihood of an event
- Seriousness of the incident
- Actions needed to resolve the problems

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What is Acceptable Risk?

- Since there is no such thing as “no risk”
- “Safe” means risk has been judged acceptable
- Judging risk is a subjective- humans make decisions

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Pathogen Safety Data Sheets and Risk Assessment

Pathogen Safety Data Sheets (PSDSs) are technical documents that describe the hazardous properties of a human pathogen and recommendations for work involving these agents in a laboratory setting.

http://www.phac-aspc.gc.ca/lab-bio/res/psds-ftss/index-eng.php

Biosafety levels

Biosafety Level

A Biosafety Level is the level of the biocontainment precautions required to isolate dangerous biological agents in an enclosed facility. The levels of containment range from the lowest biosafety level 1 to the highest at level 4.

Council Directive 90/679/EEC of 26 November 1990 on the protection of workers from risks related to exposure to biological agents at work, OJ No. L 374, p. 1

Biosafety Level 1

This level is suitable for work involving well-characterized agents not known to consistently cause disease in healthy adult humans, and of minimal potential hazard to laboratory personnel and the environment

canine hepatitis, Escherichia coli, varicella

Biosafety Level 2

This level is similar to Biosafety Level 1 and is suitable for work involving agents of moderate potential hazard to personnel and the environment.

Includes various bacteria and viruses that cause only mild disease to humans, or are difficult to contract via aerosol in a lab setting, such as C. diff, hepatitis A, B, and C, influenza A, Lyme disease, Salmonella, mumps, Bacillus subtilis, measles, scrapie.

Biosafety Level 3

This level is applicable to clinical, diagnostic, teaching, research, or production facilities in which work is done with indigenous or exotic agents which may cause serious or potentially lethal disease as a result of exposure by the inhalation route.

Anthrax, West Nile virus, Venezuelan equine encephalitis, Eastern equine encephalitis, SARS, tuberculosis, typhus, Rift Valley fever, Hantavirus, Dengue virus, yellow fever.

Biosafety Level 4

This level is required for work with dangerous and exotic agents that pose a high individual risk of aerosol-transmitted laboratory infections, agents which cause severe to fatal disease in humans.

Variola, Marburg virus, Ebola virus, Lassa fever, Crimean-Congo hemorrhagic fever, and other various hemorrhagic

Biosafety Level 1

Suitable for work involving well characterized agents *not known to cause disease in healthy adult humans and of minimal potential hazard* to laboratory personnel and the environment.

General Lab Requirements

- **Knowledgeable supervisor**
- **Knowledgeable personnel**
- ***Aware of potential hazards***
- ***Proficient in practices & techniques***
- **Lab specific biosafety manual**

General Lab Requirements

- Biosafety Levels (BSLs)
- Laboratory Practice and Technique
- Safety Equipment (Primary Barriers)
- Facility Design and Construction
- (Secondary Barriers)

General Lab Requirements

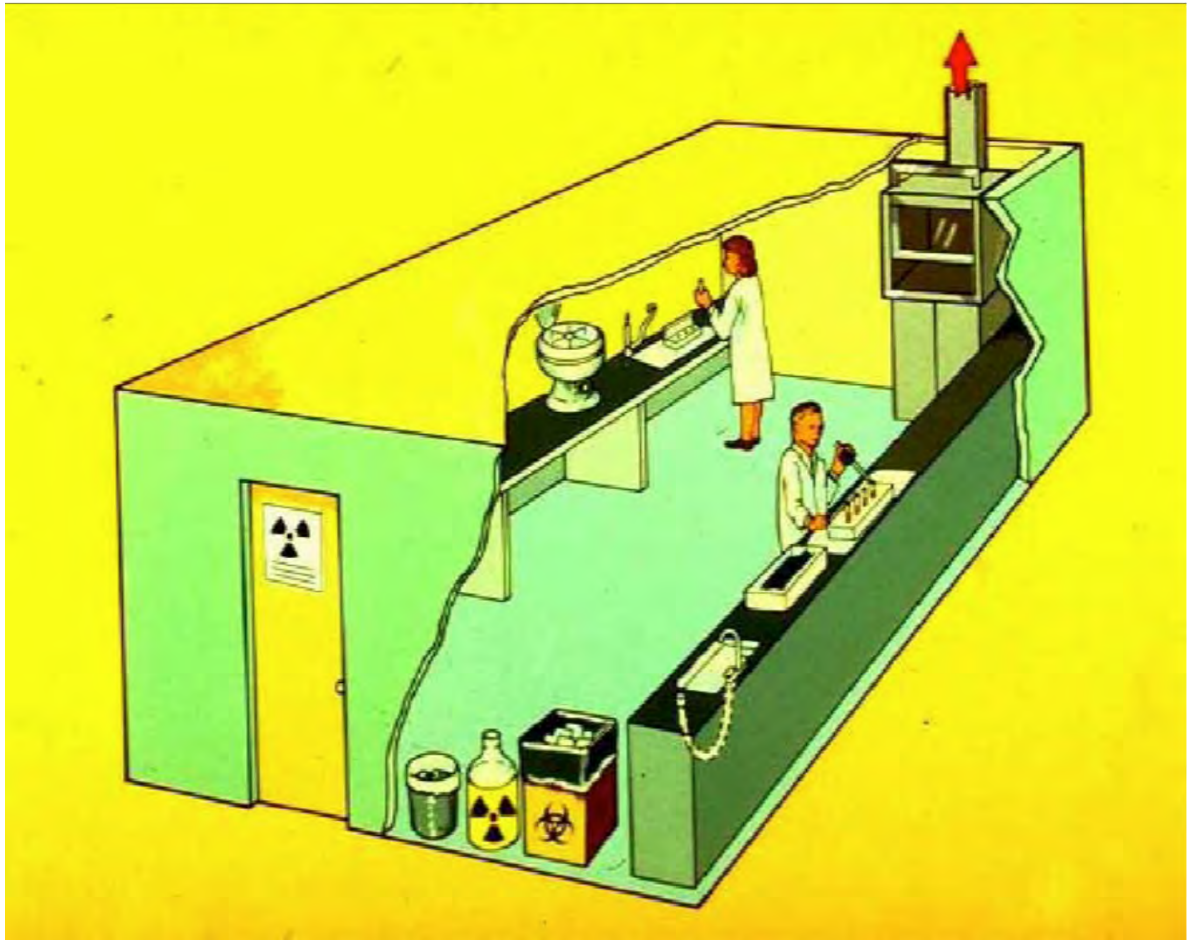
- Biosafety cabinets (BSCs) - BSL 2/3
- Personal protective clothing
- Pipetting Devices
- Safety centrifuge cups and rotors

Biosafety Level 1

Examples:

Bacillus subtilis
Naegleria gruberi
Infectious canine hepatitis virus

Biosafety Level 1



Biosafety Level 1

Facility Design (Secondary Barrier) Requirements:

- ***Laboratories have doors***
- ***Sink for hand washing***
- ***Work surfaces easily cleaned***
- ***Bench tops are impervious to water***

Biosafety Level 1

Easily cleaned and decontaminated

Biosafety Level 1

Requirements:

- ***Location - not separated***
- ***Structure - normal construction***
- ***Ventilation - none***

Biosafety Level 1

Standard Microbiological Practices

- **Restrict or limit access when working**
- **Prohibit eating, drinking and smoking**
- **Prohibit mouth pipetting**

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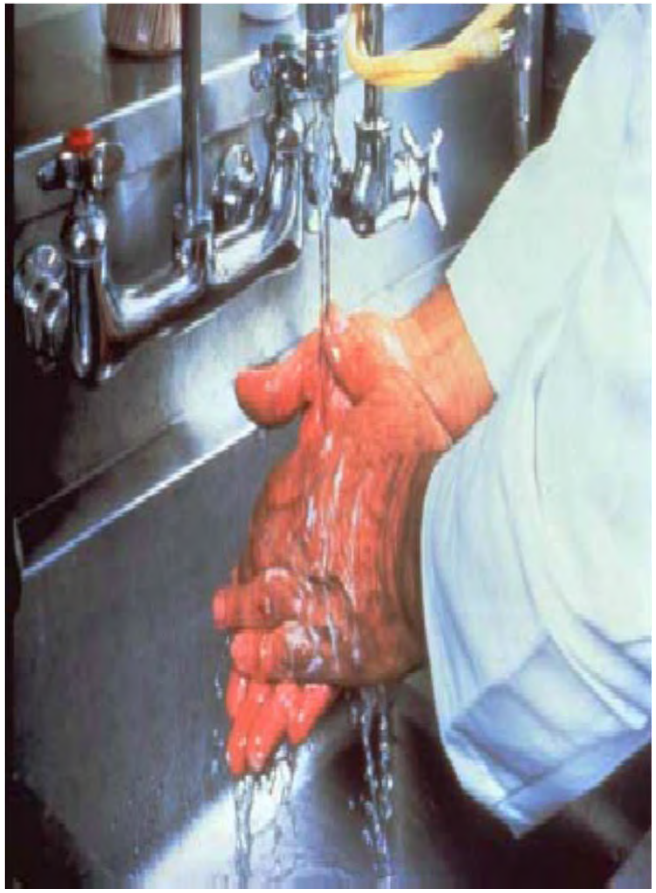
Biosafety Level 1

Use mechanical pipetting devices

Biosafety Level 1

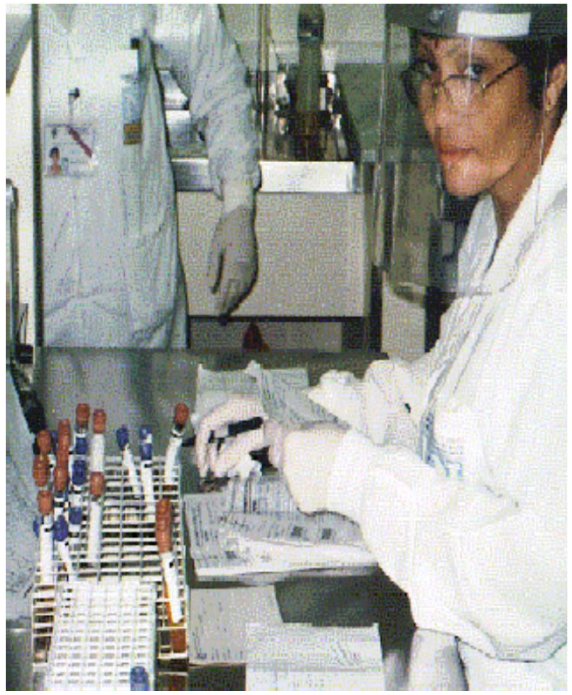


Wash hands

Biosafety Level 1

Protective clothing
Lab coat
Gloves

Biosafety Level 1



Personal protective equipment
Face protection
Eye protection

Biosafety Level 1

Special Practices

NONE

Biosafety Level 1

Training Requirements

Supervisor

Scientist with general training

Lab Personnel

Specific training in lab procedures

Biosafety Level 2

Biosafety Level 2

Suitable for work involving *agents of moderate potential hazard* to personnel and the environment.

Dr Adu-Bobie – an britsh expert on meningococcal vaccines (BSL-2) - had been in New Zealand only 20 days and at the university lab about seven working days when she contracted meningococcal septicaemia in March 2005. She spent several months in hospital and had both legs, her left arm and the digits of her right hand amputated.

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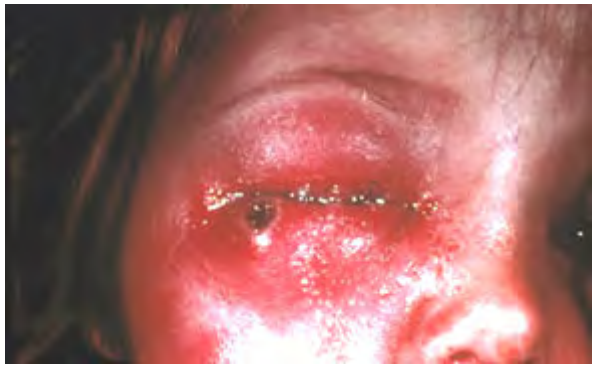
Biosafety Level 2

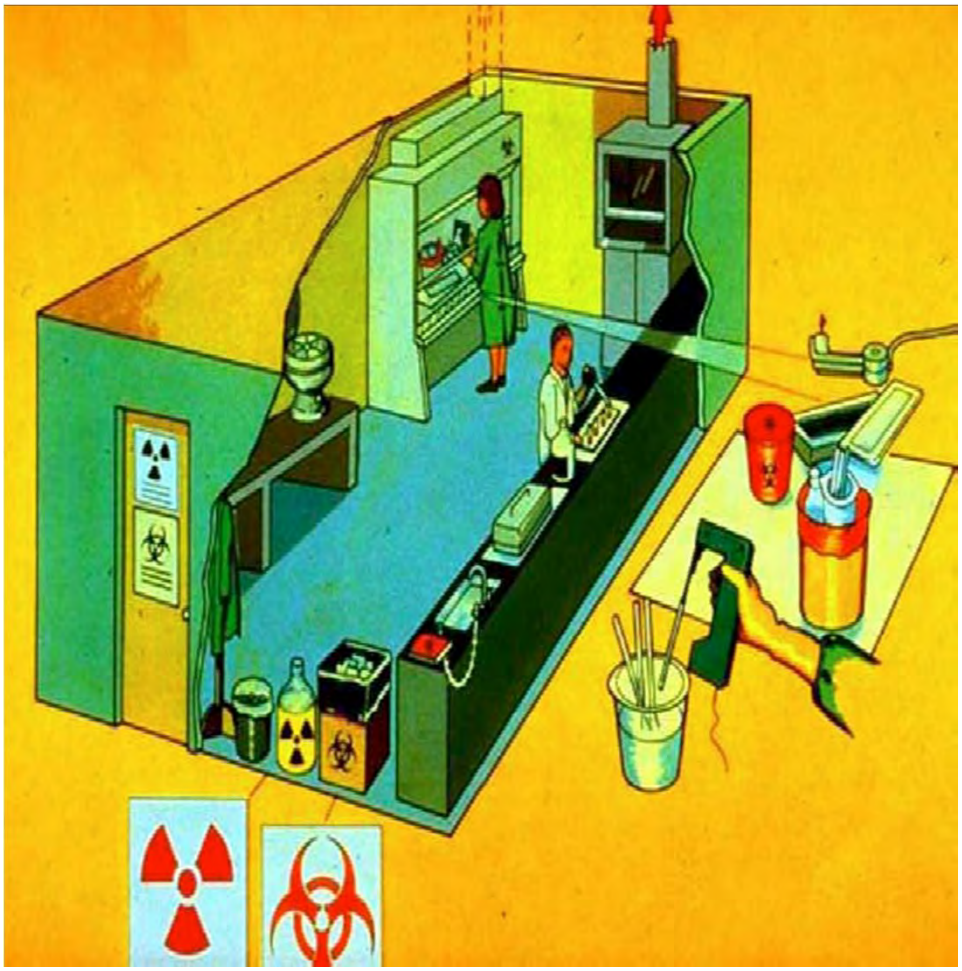
Examples:

Measles virus
Salmonellae
Toxoplasma spp.
Hepatitis B virus
Vaccina virus



Accidental auto-inoculation of eyelid with vaccinia virus with concurrent cellulitis.

Biosafety Level 2



Biosafety Level 2

Facility Design (Secondary Barriers)

Requirements:

- *Laboratories have lockable doors*
- *Sink for hand washing*
- *Work surfaces easily cleaned*
- *Bench tops are impervious to water*
- *Sturdy furniture*
- *Biological safety cabinets*
- *Adequate illumination*
- *Air flows into lab without re-circulation to non-lab areas*
- *Windows fitted with flyscreens*

Biosafety Level 2

Restricted access when work in progress

Biosafety Level 2

Laboratory Facilities (Secondary Barriers)

BSL-1 Facilities PLUS:
Autoclave available

Biosafety Level 2

Facility Construction (Secondary Barrier)

Requirements:

- ***Location - separated from public areas***
- ***Structure - normal construction***
- ***Ventilation - directional***

Biosafety Level 2

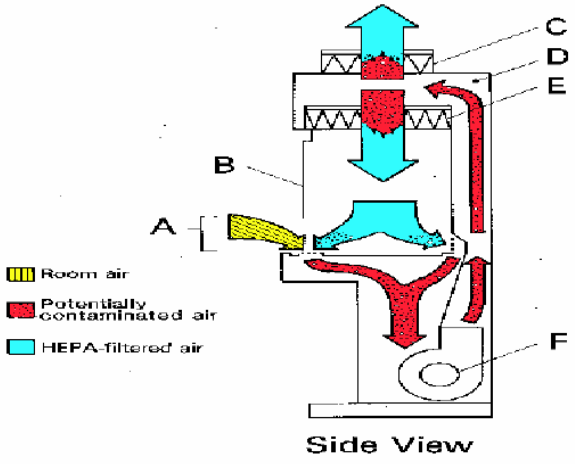
Standard Microbiological Practices,
as in BSL 1

Biosafety Level 2

Safety Equipment (Primary Barriers)

Use biosafety cabinets (class II) for work with infectious agents involvin

- **Aerosols and splashes**
- **Large volumes**
- **High concentrations**



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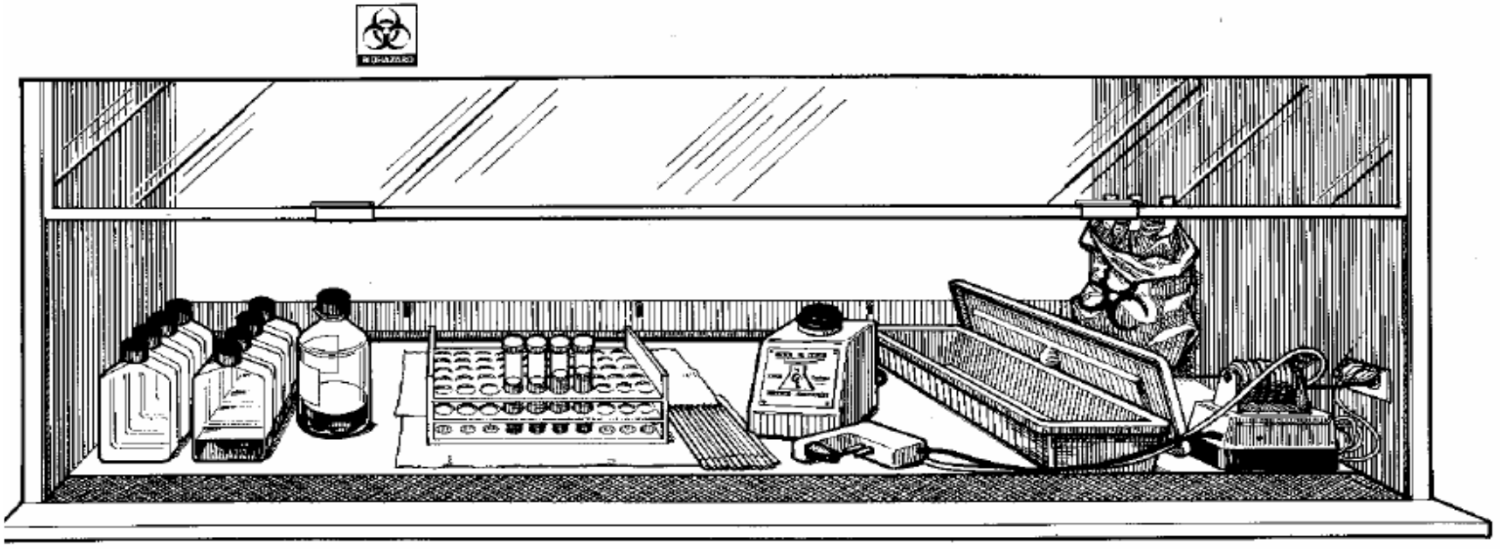
Biosafety Level 2

Safety Equipment (Primary Barriers)

Class II Biosafety Cabinet
Equipment layout



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Working under
a Biosafety
cabinet,
it's easy to say!

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Biosafety Level 2

Needles & Sharps Precautions

- ***Use sharps containers***
- ***DON'T break, bend, re-sheath or reuse syringes or needles***

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Biosafety Level 2

Needles & Sharps Precautions (cont.)

DON'T place needles or sharps in office waste containers

Biosafety Level 2

Needles & Sharps Precautions (cont.)

DON'T touch broken glass with hands

Biosafety Level 2

Needles & Sharps Precautions (cont.)

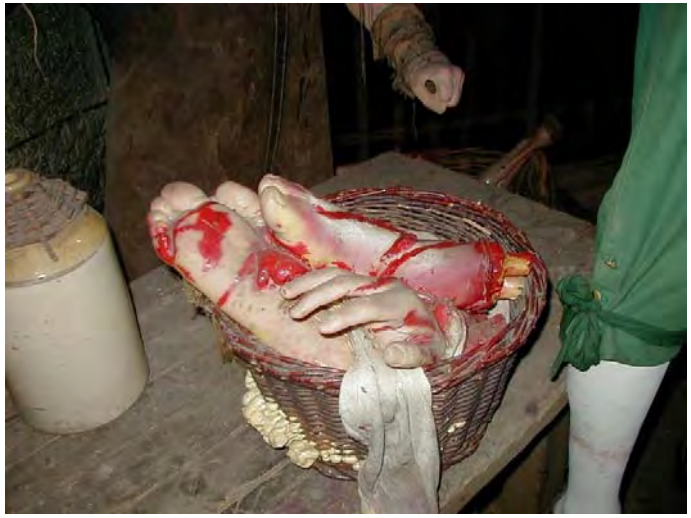
Use plasticware

Carcasses and Body Parts

- Human tissues
  - Unfixed tissues are medical waste
  - Make waste unrecognizable!

- Animal tissues, carcasses
  - When generated in infectious disease or recombinant DNA research, these are medical waste

- These items must be stored in biolabeled, leakproof containers for incineration.



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Biosafety Level 2

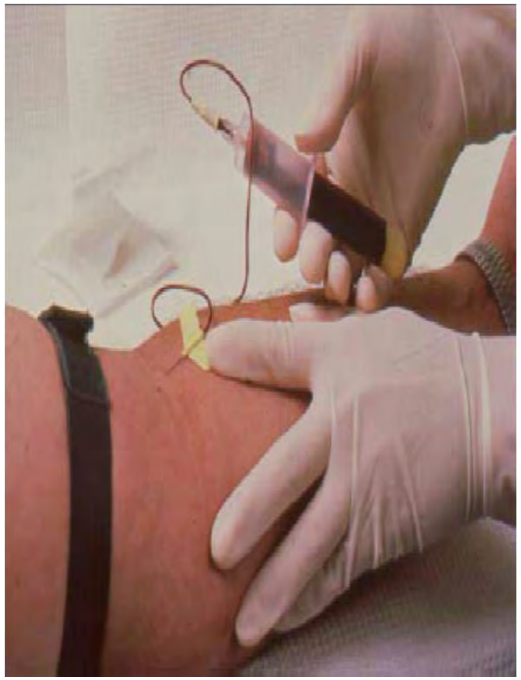
Special Practices

- **Policies and procedures for entry**
- **Biohazard warning signs**
- **Biosafety manual specific to lab**
- **Training with annual updates**

Biosafety Level 2

Special Practices

- **Immunizations**
- **Baseline serum samples**



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Biosafety Level 2

Special Practices

- Decontaminate work surfaces
- Report spills and accidents
- No animals and no children in laboratories

Biosafety Level 2

Supervision

Supervisor is a competent scientist with increased responsibilities

Limits access if immunocompromised

Restricts access to immunized

Lab Personnel

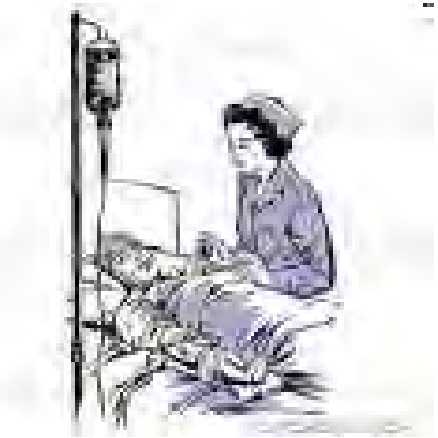
Aware of potential hazards

Proficient in practices/techniques

Biosafety Level 3

Biosafety Level 3

Suitable for work with infectious agents which *may cause serious or potentially lethal disease* as a result of exposure by the inhalation route.



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Biosafety Level 3

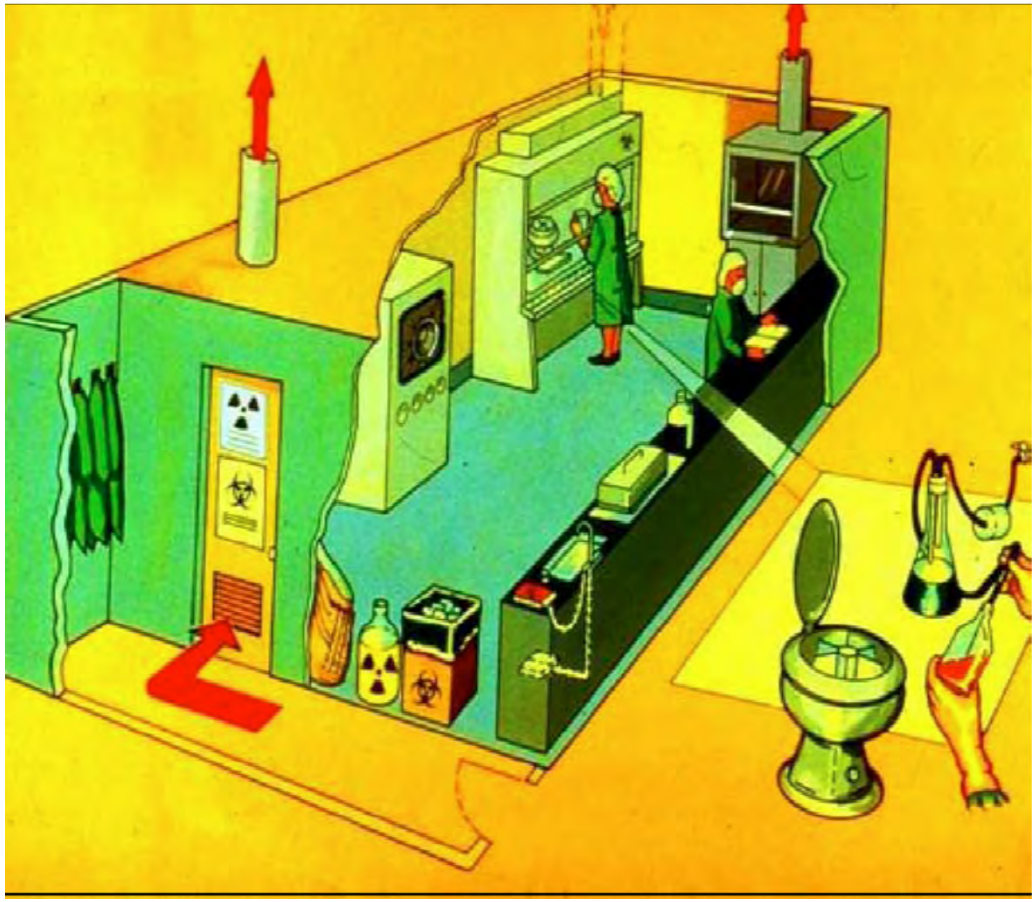
Exposure potential to pathogens
spread by aerosol
Infections are serious, possibly lethal

Examples:

M. tuberculosis

St. Louis encephalitis virus

Biosafety Level 3



Biosafety Level 3

Laboratory Facilities (Secondary Barriers)

BSL-1 and 2 Facilities PLUS:

- ***Separate building or isolated zone***
- ***Double door entry***
- ***Directional inward airflow***
- ***Single-pass air; 10-12 air changes/hour***

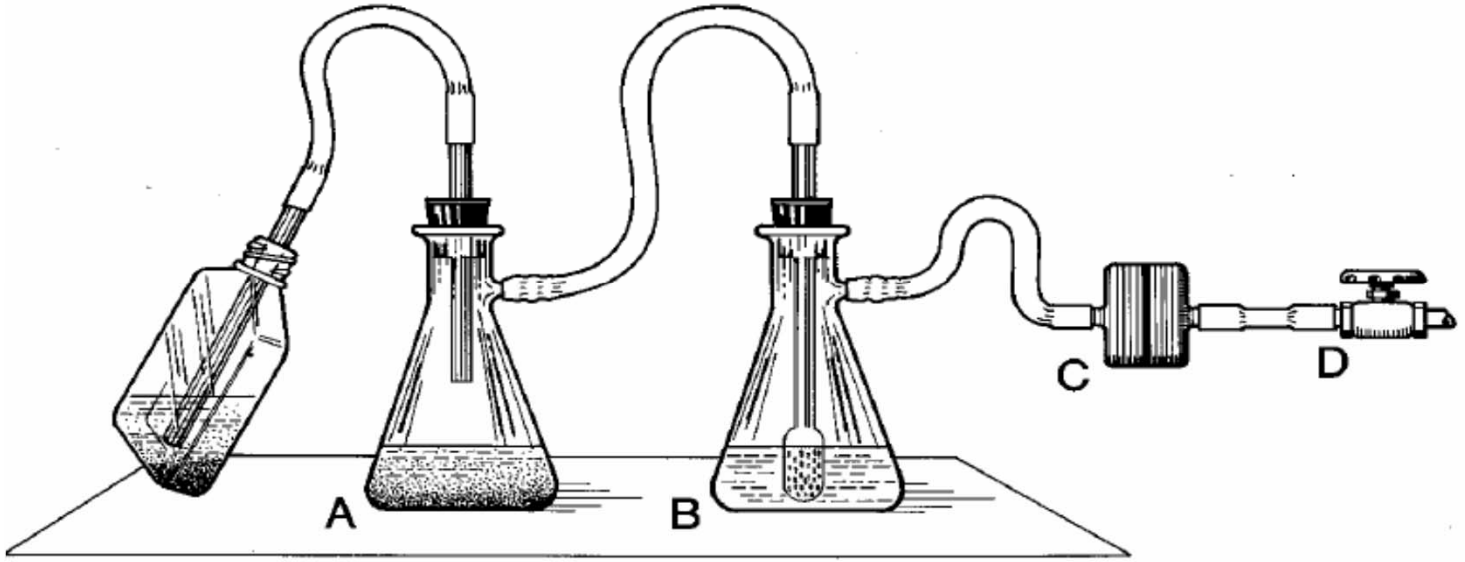
Biosafety Level 3

Laboratory Facilities (Secondary Barriers)

- ***Room penetrations sealed***
- ***Walls, floors and ceilings are water resistant for easy cleaning***

Biosafety Level 3

Vacuum lines protected with liquid disinfectant traps or HEPA filters



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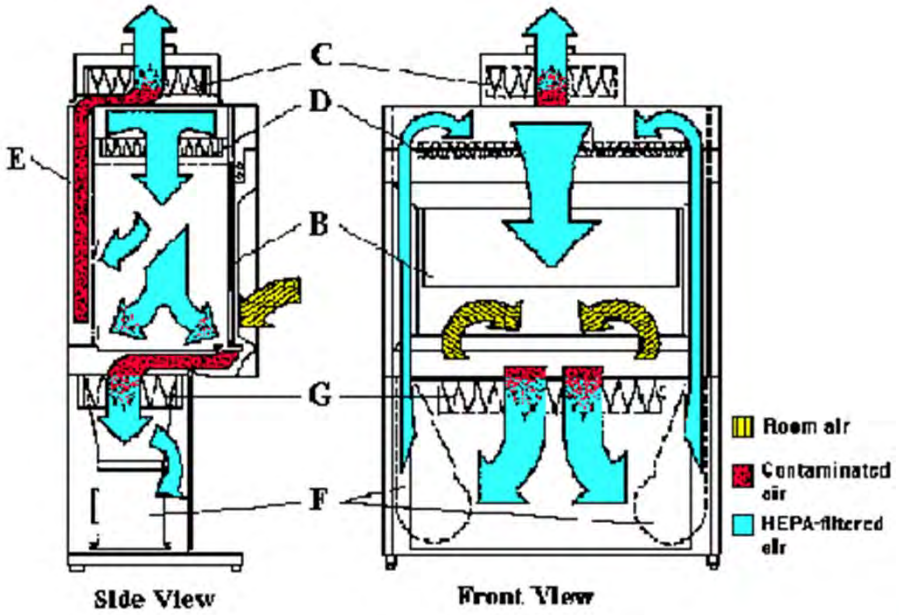
Biosafety Level 3

Safety Equipment (Primary Barriers)

BSL-1 and 2 Safety Equipment PLUS:

BSC class II or III to manipulate infectious material



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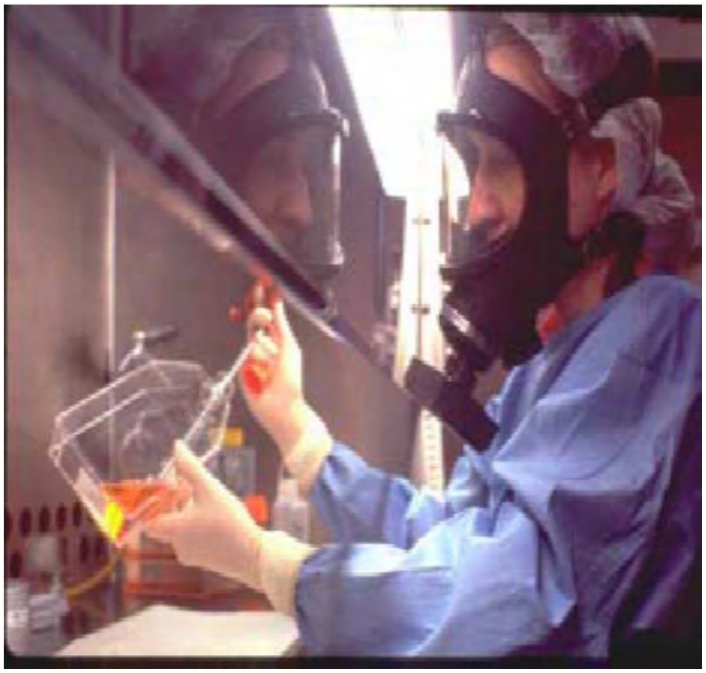
Biosafety Level 3

BSL-1 and 2 Safety Equipment PLUS:

Respiratory protection may be indicated



Biosafety Level 3

Standard Microbiological Practices as in BSL1 and 2

Biosafety Level 3

BSL-2 Special Practices PLUS:

- ***Work in certified BSC***
- ***Decontaminate spills promptly***
- ***Wear gloves and wrabaround gown, whenever entering the BSL 3 lab.***
- ***Wear dobbel gloves, face-shield and surgical mask, whenever handling the agent***

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Biosafety Level 3

Special Practices

Supervision

Supervisor is a competent scientist experienced working with agents

Establishes criteria for entry
Restricts access
Develops policies/procedures
Trains lab personnel

Biosafety Level 3

Special Practices

Lab Personnel

- ***Strictly follow guidelines***
- ***Demonstrate proficiency***
- ***Receive appropriate training***
- ***Report incidents***

Biosafety Level 4

Suitable for work with dangerous and exotic agents that *pose a individual risk of aerosoltransmitted laboratory infections and life-threatening disease.*

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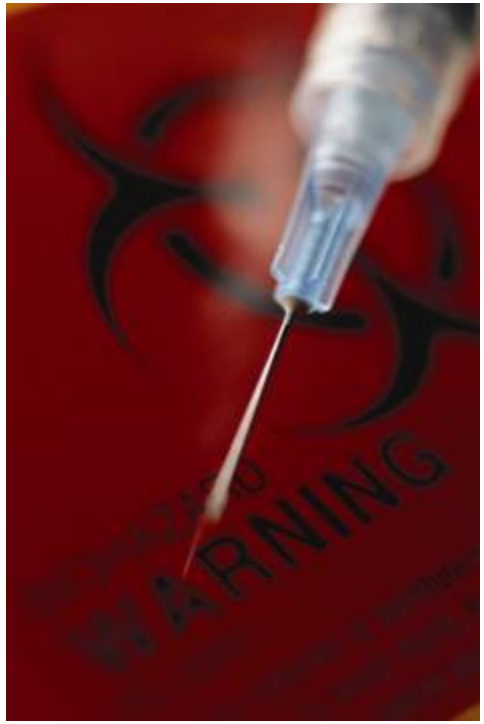
Table 2. ***Relation of risk groups to biosafety levels, practices and equipment***

RISK GROUP	BIOSAFETY LEVEL	LABORATORY TYPE	LABORATORY PRACTICES	SAFETY EQUIPMENT
1	Basic – Biosafety Level 1	Basic teaching, research	GMT	None; open bench work
2	Basic – Biosafety Level 2	Primary health services; diagnostic services, research	GMT plus protective clothing, biohazard sign	Open bench plus BSC for potential aerosols
3	Containment – Biosafety Level 3	Special diagnostic services, research	As Level 2 plus special clothing, controlled access, directional airflow	BSC and/or other primary devices for all activities
4	Maximum containment – Biosafety Level 4	Dangerous pathogen units	As Level 3 plus airlock entry, shower exit, special waste disposal	Class III BSC, or positive pressure suits in conjunction with Class II BSCs, double-ended autoclave (through the wall), filtered air

BSC, biological safety cabinet; GMT, good microbiological techniques (see Part IV of this manual)

- International -
Laboratory Biorisk Management Standard
CWA 15793:2008



biorisk@icid.com • www.biorisk.eu

Overview

- In 2007, the document was adopted, and published as CWA 15793:2008 by CEN in 2008
  - 76 participants from 24 countries developed a management system approach to biosafety and biosecurity in the laboratory
  - CEN in Brussels facilitated the process with funding by the European Commission

CWA 15793:2008

is a

Management System Standard

Consistent with other international standards such as ISO 9001 / 14001 and OSHAS18001

Contains definitions, requirements and notes for guidance

BioSafety Cabinet

http://www.youtube.com/watch?v=5icV_mctrkE

Biosafety cabinet

Purpose

- **Product protection**
- **Personal protection**
- **Environmental protection**

Biosafety cabinet

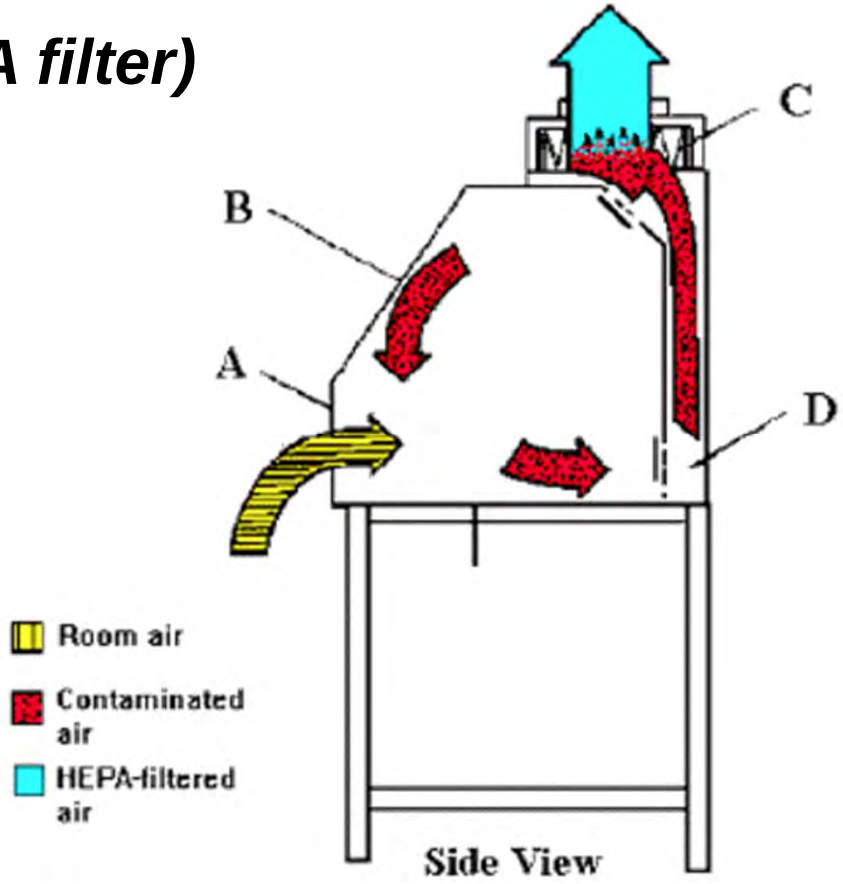
Class I
(w/wo HEPA filter)

Class II
sterile" work area

Class III
totally enclosed, ventilated, air-tight suitable for work with BSL3/4 agents

Biosafety cabinet

Class I
(w/wo HEPA filter)



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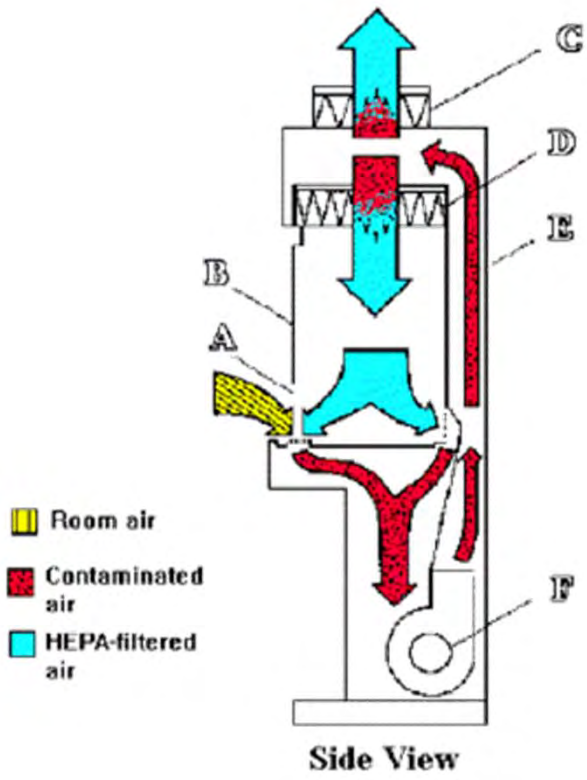
Biosafety cabinet

BioSafety Cabinet Class IIA

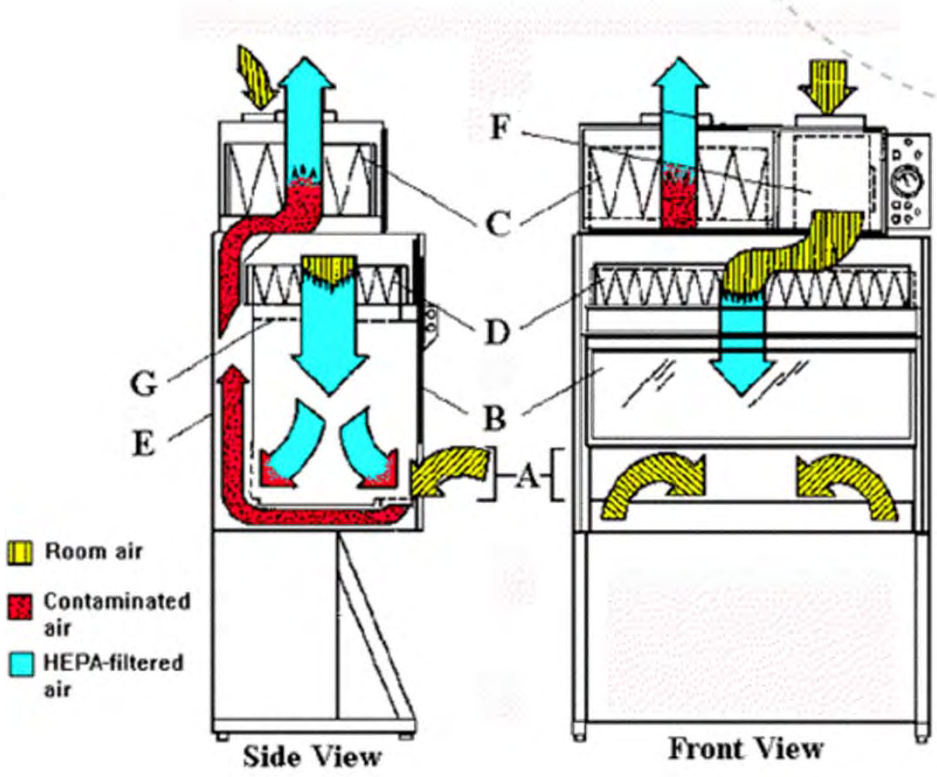


30% exhausted to room

BioSafety Cabinet Class IIB2

Connection to building exhaust system required.

100% exhausted to outside



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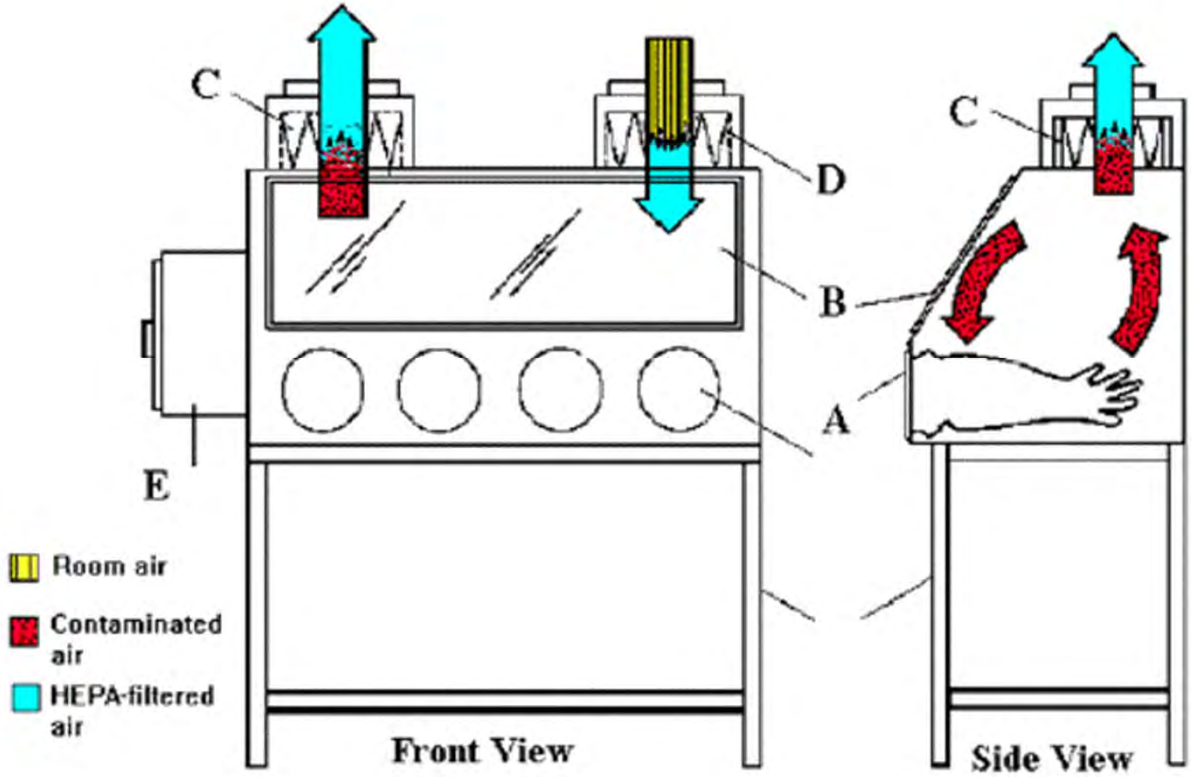
Biosafety cabinet

BioSafety Cabinet Class III

Connection to building exhaust system required.



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Biosafety cabinet

HEPA Filter

"High efficiency particulate air" filter

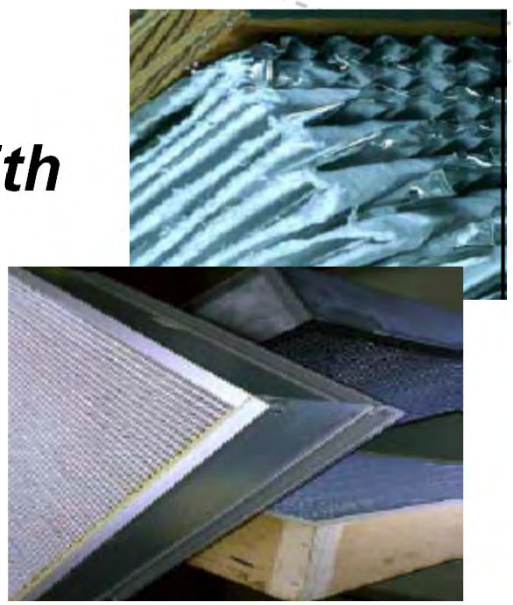
Traps particulates only;
chemicals, fumes, vapors pass through

Traps particulates 0.3 µm

Biosafety cabinet

HEPA Filter

- *Metal or wood framed*
- *Continuous sheet of flat filter medium with aluminum separators*
- *Adhesive bond between filter pack and frame*



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Biosafety cabinet

Operating Procedure

1. Load BSC with all needed supplies.
2. Turn BSC on and allow to run for 10-15 minutes.
3. Check inward airflow with a piece of tissue.
4. Enter straight into cabinet and perform work in a slow, methodical manner.
5. At end of work, decontaminate all items to be taken out of cabinet.
6. Decontaminate interior of BSC.
7. Allow cabinet to run for 10-15 minutes.
8. Shut off.

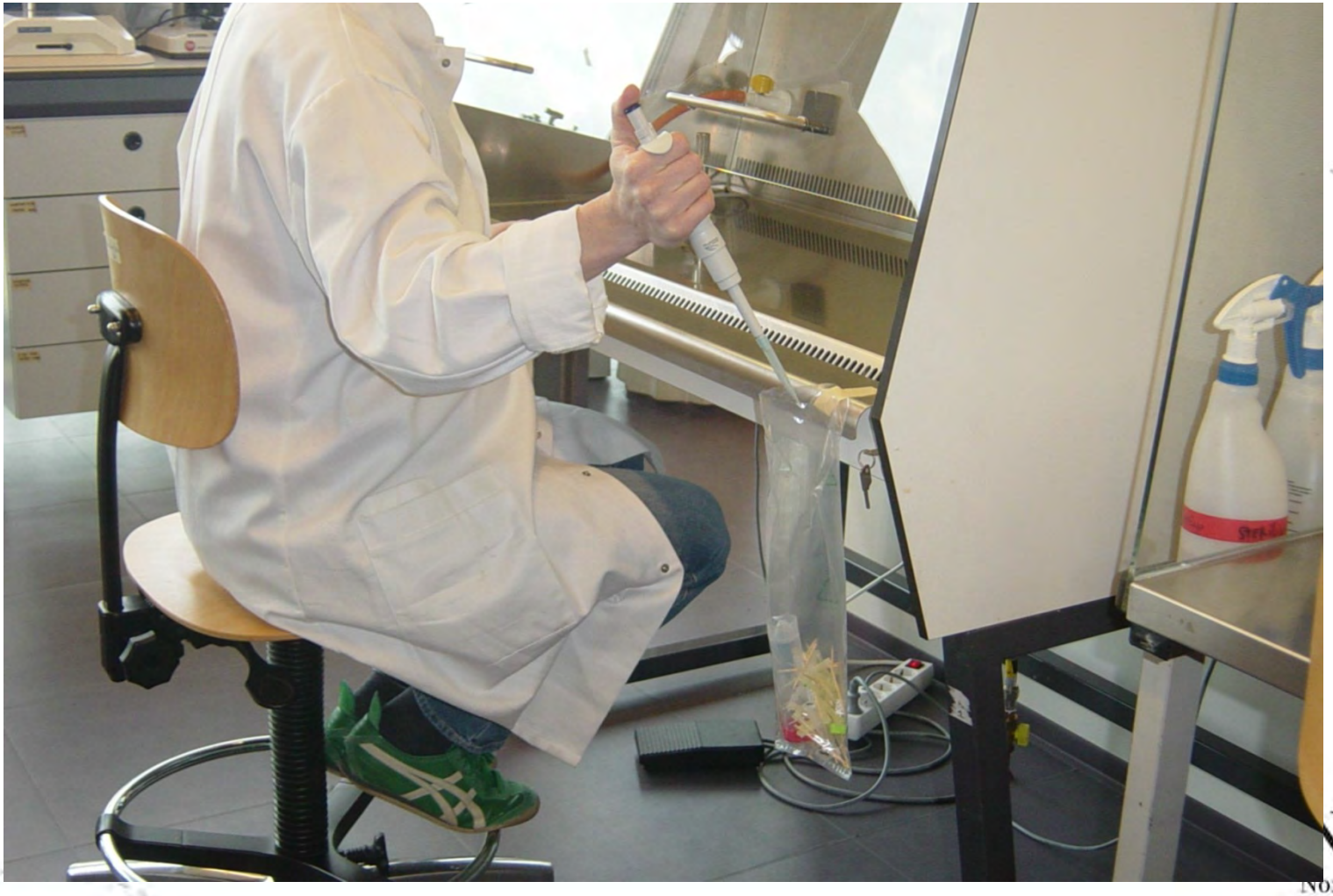
Biosafety cabinet

Safe Operation

Always enter straight into cabinet - no sweeping motions

Watch for disruptions of laminar air flow

Decontaminate materials **before** removal from cabinet



Place materials well within the cabinet – not on front grill

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Biosafety cabinet

CAUTIONS

Chemicals may damage HEPA filter

Exposure risk - chemical/infectious agents
Volatile chemicals NOT retained by HEPA filter

Exposes personnel if not exhausted

BSC fans NOT spark proof
Chemical use may result in fire/ explosion

Centrifuges

Centrifuges

Hazards

- Mechanical failure of machine
- Lab equipment failure (tubes etc.)
- Aerosol generation
- Operator error

Centrifuges

Operating Procedure

1. Check tubes for cracks/chips.
2. Use matched sets of tubes, buckets etc.
3. Tightly seal all tubes and safety cups.
4. Ensure that rotor is locked to spindle and bucket seated.
5. Close lid during operation.
6. Allow to come to complete stop before opening.

Centrifuges

Safe Operation

Disinfect after all spills or breakage's

Do not use rotors that have been dropped

Decontamination

Decontamination

Sterilization

The use of a physical or chemical procedure to destroy all microbial life, including large numbers of highly resistant bacterial spores.

Decontamination

Disinfection

The use of a physical or chemical procedure to virtually eliminate all recognized pathogenic microorganisms but not all microbial forms (bacterial endospores) on inanimate objects.

Decontamination

Antisepsis

A germicide that is used on skin or living tissue for the purpose of inhibiting or destroying microorganisms.

Decontamination

Agent Selection

- **Degree of microbial killing required**
- **Nature of item/surface to be treated**
- **Ease of use**
- **Safety**
- **Cost**

Decontamination

Agent Efficacy

Type of organism
Number of organisms
Amount of organic material present
Type & configuration of material to be treated
Type & concentration of germicide
Time and temperature or exposure
Humidity

Decontamination

Methods

- Heat
- Chemical
- Radiation

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Decontamination

HEAT*

Types

Moist – steam

Dry

Incineration

***The most effective method of sterilization**

Decontamination

Steam sterilization practices

Ensure proper functioning of autoclave

Vessels should not be capped or plugged

Large loads require longer contact time

Excessive amounts of liquid should not be added to load

Decontamination

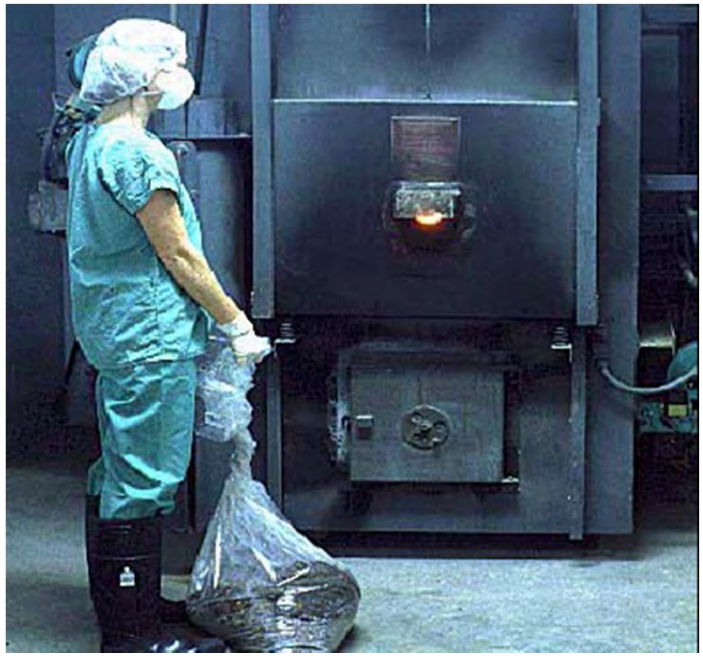
Dry heat sterilization

Denaturation of proteins: 1600 – 1700 C/2-4 hours
Effective on impervious non-organic materials like glass

Decontamination

Incineration

Method of choice
for animal carcasses



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Decontamination

Chemical

Types
Liquids, I.e. chlorox, hydrogen peroxide

Gases, I.e. ethylene oxide

Decontamination

Chemicals

Agent selection – complexity

Over 14,000 registered products
Over 300 active ingredients
14 ingredients present in 92% of products

Decontamination

Chemical

Agent selection – activity

HLD – high level disinfection
ILD – intermediate level disinfection
LLD – low level disinfection

Decontamination

Chemical

High level disinfection –
sporocides
Kills all microorganisms except high numbers of bacterial spores
Require 5-10 min. exposure

Examples: aldehydes, hydrogen peroxide, paracetic acid

Decontamination

Chemical

Intermediate level disinfection -
tuberculocides
Kills M. tuberculosis var. bovis and all vegetative bacteria, fungi, and most viruses

Examples: phenolics, iodophores, chlorine compounds, alcohols

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Decontamination

Chemical

Low level disinfection – hospital germicides used for housekeeping

Kills most vegetative bacteria and some fungi, but not M. tuberculosis var. bovis
Require minimum 20 min. exposure

Examples: quartenary ammonium compounds

Decontamination

Disinfectians do not replace standard microbial practices

Biosafety and recombinant DNA technology

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Recombinant DNA technology or genetic engineering was first used to **clone DNA segments** in bacterial hosts in order to **overexpress specific gene products** for further studies.

Recombinant DNA molecules have also been used to create GMOs such as transgenic and “knock-out” animals and transgenic plants.

Molecular biologists and physicians are generally not trained in biosafety

Biosafety considerations for expression vectors:

1. The expression of DNA sequences derived from pathogenic organisms may increase the virulence of the GMO

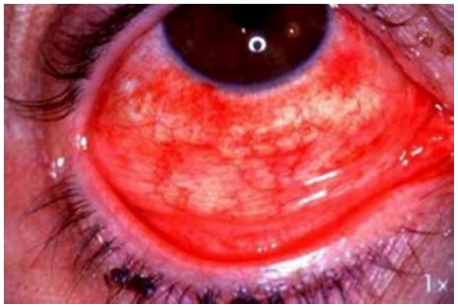
2. Inserted DNA sequences are not well characterized, e.g. during preparation of genomic DNA libraries from pathogenic microorganisms

3. Gene products have potential pharmacological activity

4. Gene products code for toxins.

Viral vectors for gene transfer

Viral vectors, e.g. adenovirus vectors, are used for the transfer of genes to other cells.



Such vectors lack certain virus replication genes and are propagated in cell lines that complement the defect.

Stocks of such vectors may be contaminated with replication-competent viruses, generated by spontaneous recombination events in the propagating cell lines, or may derive from insufficient purification.

Transgenic and “knock-out” animals

Animals carrying foreign genetic material should be handled in containment levels appropriate to the characteristics of the products of the foreign genes.

Animals with targeted deletions of specific genes (“knock-out” animals) do not generally present particular biological hazards.

Hazards arising directly from the inserted gene (donor organism)

Assessment is necessary in situations where the product of the inserted gene has known biologically or pharmacologically active properties that may give rise to harm, for example:

1. Toxins
2. Cytokines
3. Hormones
4. Gene expression regulators
5. Virulence factors or enhancers
6. Oncogenic gene sequences
7. Antibiotic resistance
8. Allergens.

Hazards arising from the alteration of existing pathogenic traits

Many modifications do not involve genes whose products are inherently harmful, but adverse effects may arise as the result of alteration of existing non-pathogenic or pathogenic traits. Modification of normal genes may alter pathogenicity. In an attempt to identify these potential hazards, the following points may be considered (the list is not exhaustive).

1. Is there an increase in infectivity or pathogenicity?

2. Could any disabling mutation within the recipient be overcome as a result of the insertion of the foreign gene?

3. Does the foreign gene encode a pathogenicity determinant from another organism?

Hazards arising from the alteration of existing pathogenic traits

4. If the foreign DNA does include a pathogenicity determinant, is it foreseeable that this gene could contribute to the pathogenicity of the GMO?

5. Is treatment available?

6. Will the susceptibility of the GMO to antibiotics or other forms of therapy be affected as a consequence of the genetic modification?

7. Is eradication of the GMO achievable?

Emergency response

Emergency response
by needle stick, splash or any direct contact

1. Stay calm

2. Clean exposed surface with soap/water, eyewash (eyes), or saline (mouth)

3. Report to medical clinic immediatly

4. Notify supervisor

Post-exposure prophylaxis drugs should be administred within 2 hours of exposure to HIV !!!

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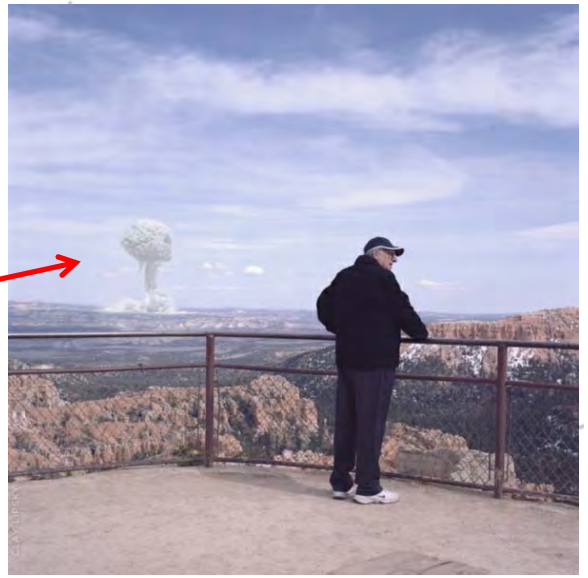
Emergency response
by potentially infectious aerosol release, e.g. spills outside BSC



1. **Inform your co-workers**
2. **Evacute immediately**
3. **Signs should be posted indicating that entry is forbidden**
4. **Notify supervisor**

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Reasons for potential exposures

- Working in disorganized laboratory bench setting
- Working too fast
- Failure to receive proper training
- Assumption that agent is not infectious to humans
- Assumption that agent(s) are no longer viable
- Using defective equipment

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http://www.youtube.com/watch?v=p-AkTaK0WX8

New course in molecular virology from next spring-semester (MOL3020)

Are you interested in:

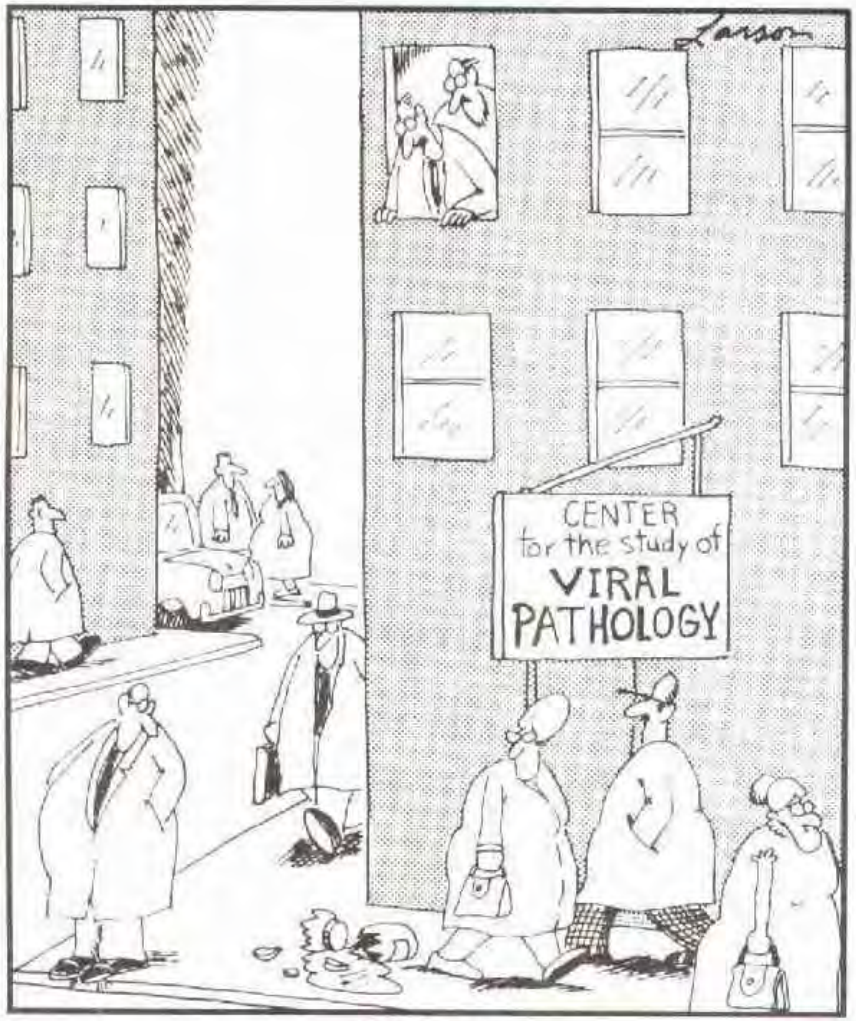
how a virus is build up and how it makes you sick ?

learning more about viral evolution and viral ecology ?

how to develop virus vaccines ?

how you can use viruses as tools in nanotechnology ?

biosafety ?



"Uh-oh."

Takk

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