Biological Safety

Dr Samuel Yu, DEnv, CIH

HSEO

HKUST

Important Reminder

- This and other HSE safety courses only provide basic safety principles
- Your supervisor must give you specific safety instructions and hands-on training
- Your rights as a worker:
  - to know about hazards in your work and ways to control them
  - to have a safe and healthy work environment
  - to have proper personal protective equipment

What We Will Talk About...

- What is biohazard? And the major types.
- What is bioaerosol, and why do we care?
- How does infection occur? And the facts about occupational infection.
- Controls of biohazards
  - biological safety cabinet and HEPA filter
  - control of specific biohazards
- Disinfection & biological wastes.
- HKUST(GZ) Biological Safety Program.

What is Biohazard?

Definition of Biohazards

Biological agents or substances which present a hazard to human health or well being.

These include certain bacteria, fungi, viruses, rickettsiae, chlamydiae, parasites, recombinant DNA products, allergens, cell cultures, toxins and clinical specimens.

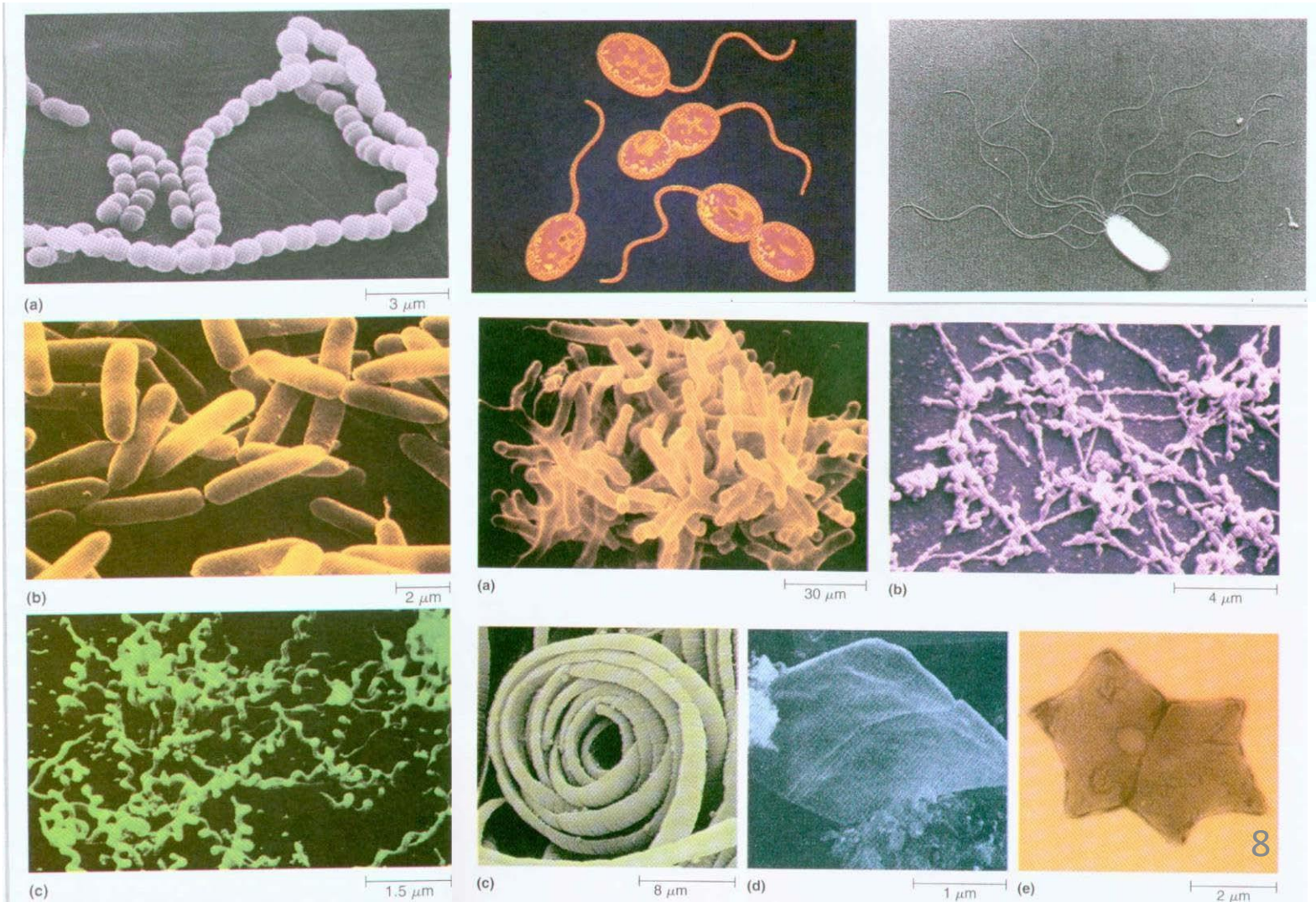
Major Types of Biohazards

- Infectious agents
  - Microorganisms and arthropod vectors
  - Clinical specimens (blood-borne pathogens)
  - Cell cultures and viral vectors
  - Experimental materials (e.g. sewage)
  - Indoor air
- Laboratory animals
- Biological toxins
- Recombinant DNA molecules

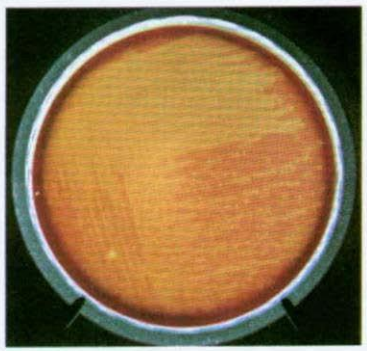
Infectious Agents-- Pathogens and Vectors

- Bacteria
- Fungi
- Virus
- Protozoans
- Arthropod vectors
- Prion

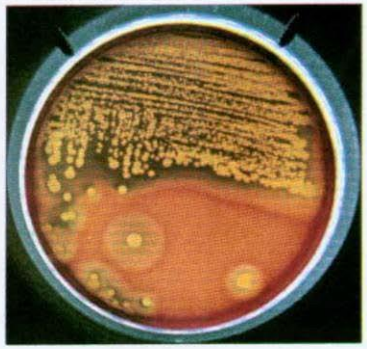
Bacteria appear in many different sizes and shapes...



...and they look different in colonies



(a)



(b)

FIGURE 5-16 Hemolysis on blood agar. Blood agar is a differential and enriched medium. Hemolytic reactions are useful in identifying bacteria. On these plates, alpha hemolysis produced by *Enterococcus faecalis* (*a*) and beta hemolysis produced by *Streptococcus pyogenes* (*b*) are easily distinguished from each other.

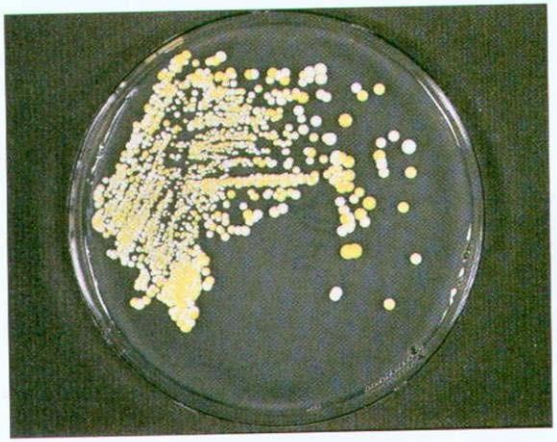
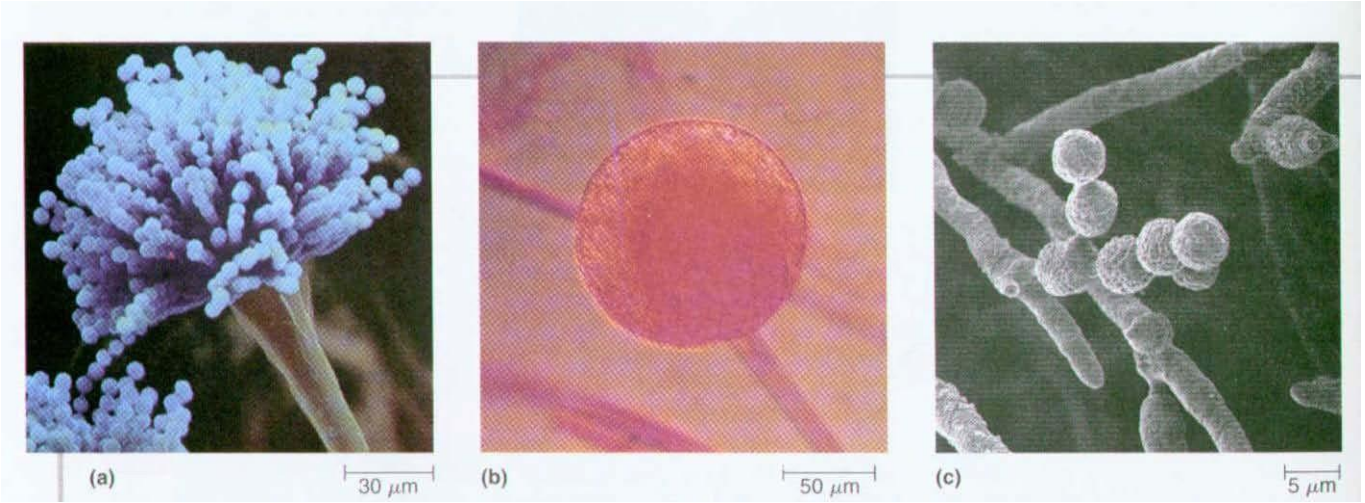
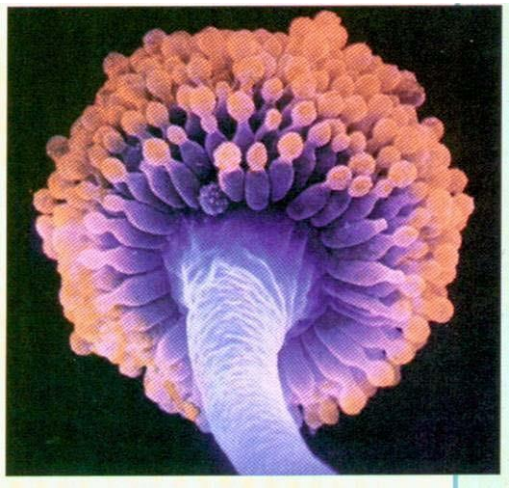


***Figure* 5.17** Results of a three-way streak of four strains of *Micrococcus luteus*. A dark yellow strain and a white mutant. A light yellow strain and its pink mutant. (Courtesy of Wesley Kloos)

Fungi look like miniature plants





Viruses are submicron sized intracelluar parasites

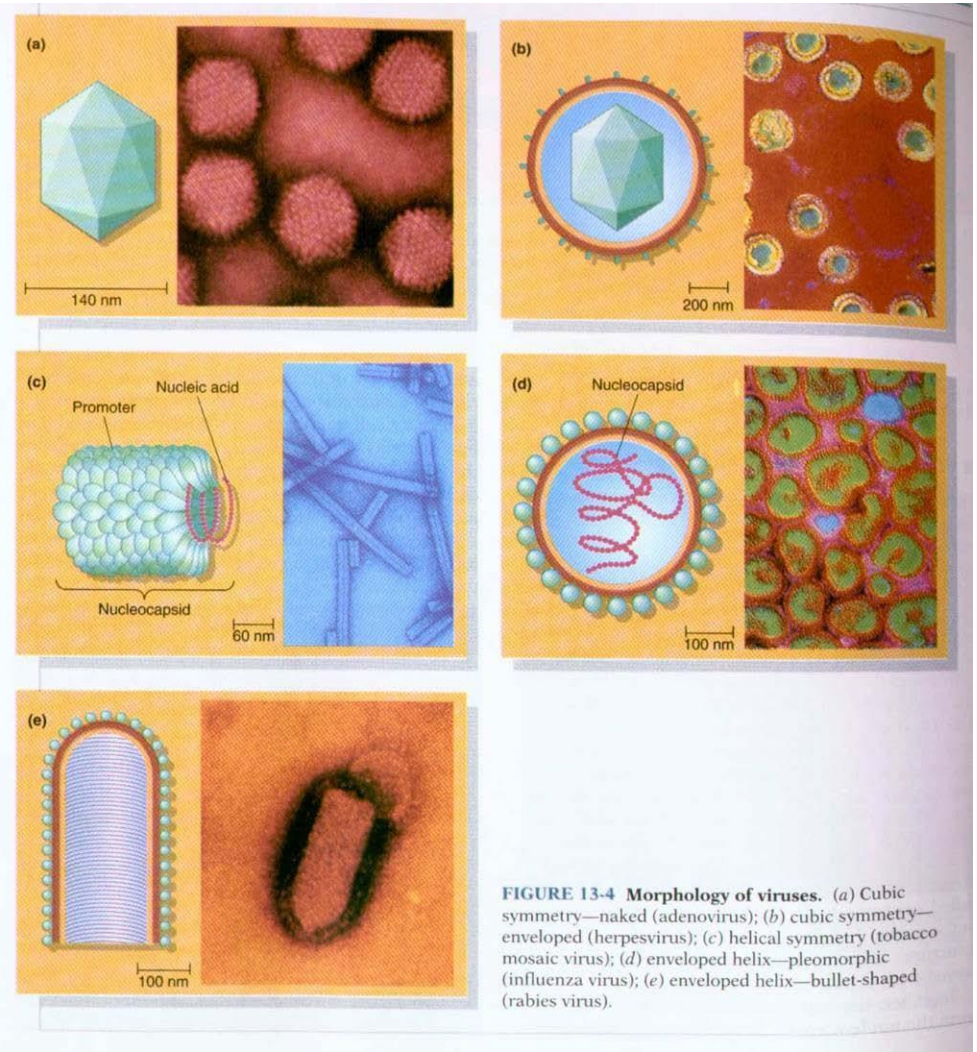


FIGURE 13-4 Morphology of viruses. (*a*) Cubic symmetry—naked (adenovirus); (*b*) cubic symmetry—enveloped (herpesvirus); (*c*) helical symmetry (tobacco mosaic virus); (*d*) enveloped helix—pleomorphic (influenza virus); (*e*) enveloped helix—bullet-shaped (rabies virus).

There are many kinds of protozoan pathogens...

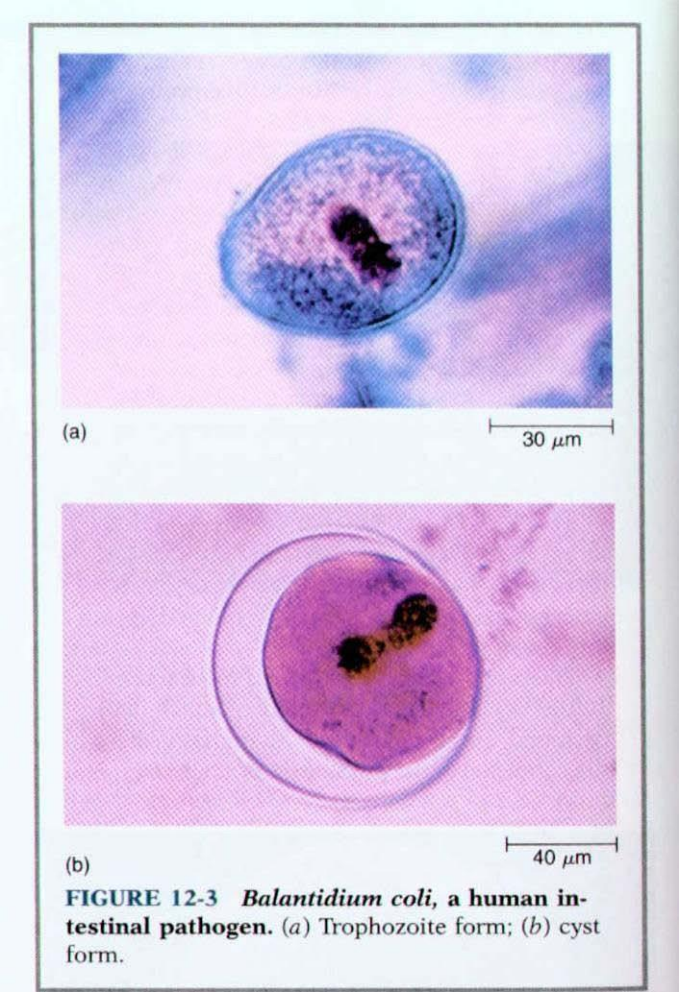
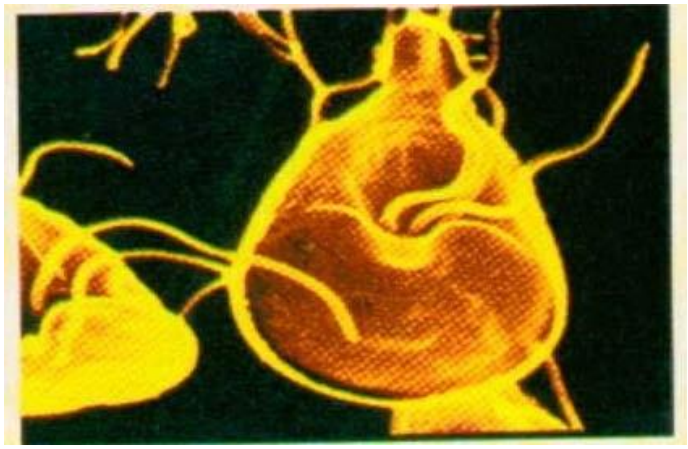
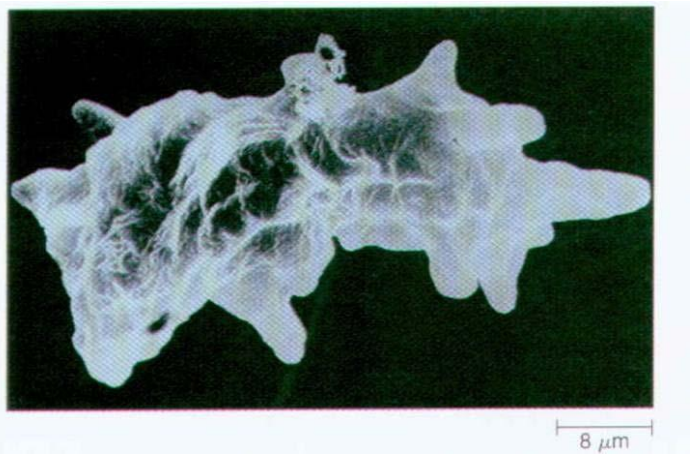
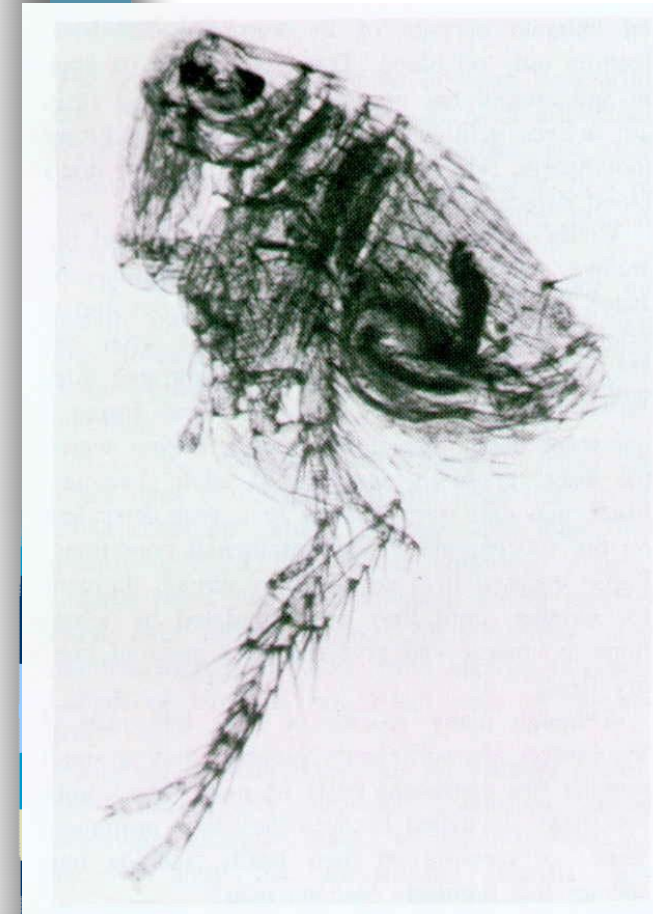


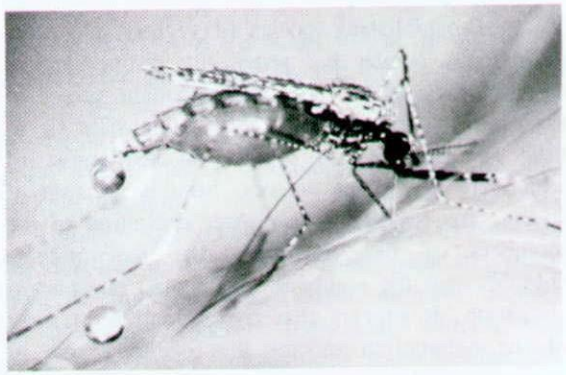
FIGURE 12-3 ***Balantidium coli,*** **a human intestinal pathogen.** (*a*) Trophozoite form; (*b*) cyst form.

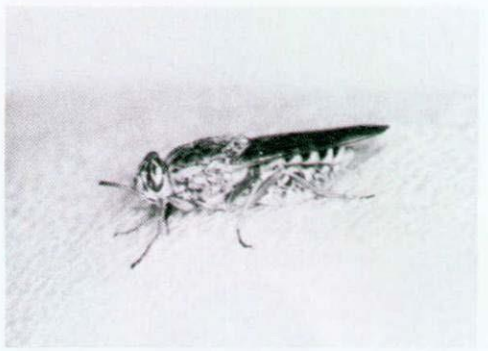


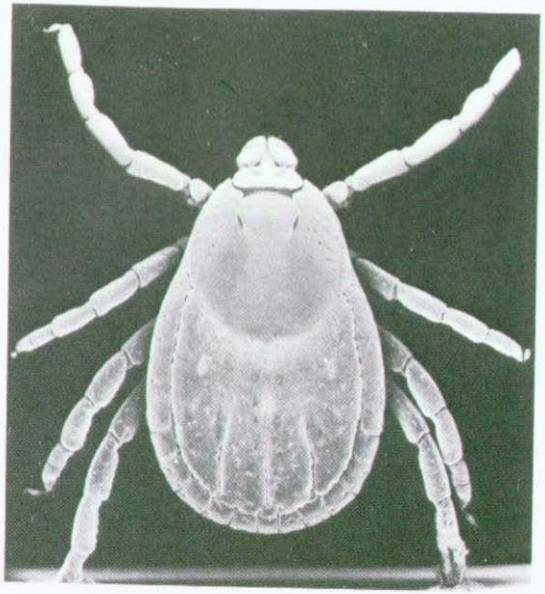


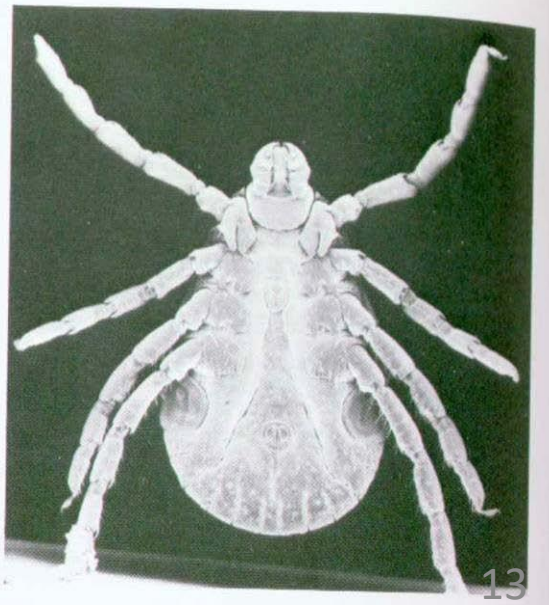
...and many types of arthropods which are vectors of pathogens











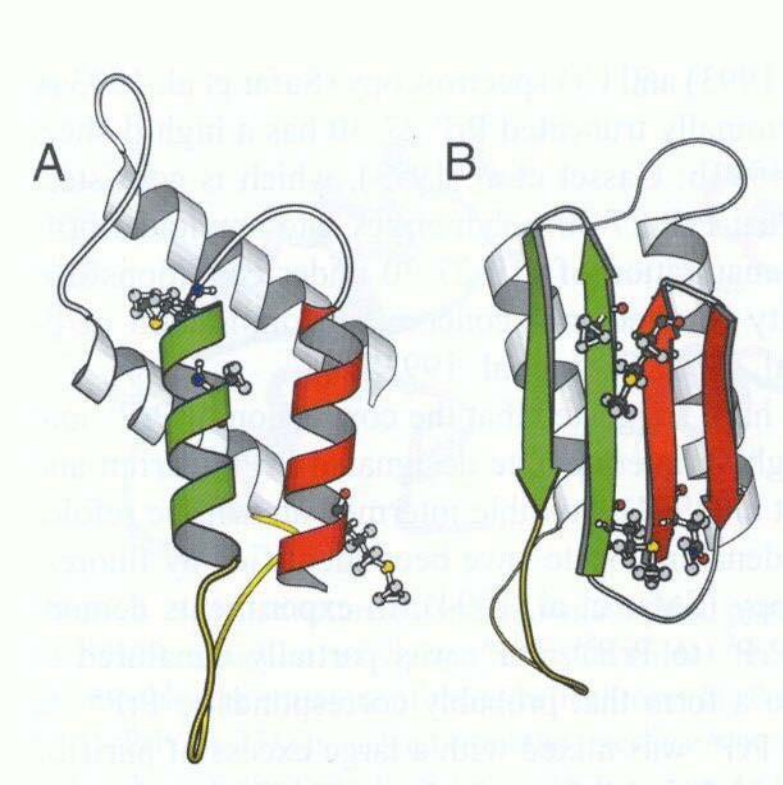
Prion--A Unique Infectious Agent

- Cause a group of animal and human fatal neurological diseases, e.g. Scrapie (sheep); Bovine Spongiform Encephalopathy (“Mad Cow Disease”); Creutzfeldt-Jakob Disease, Kuru (humans)
- Result in devastating degeneration of the CNS without inflammatory or other immune responses
- Could be genetic, infectious or sporadic

What is Prion?

- Prions are protein molecules
- Prion disease is caused by a modified isoform of a pathogenic prion
- Some non-pathogenic prions are found to be essential to learning and memory
- Normal prion is converted into the disease form through a conformational change of the secondary structure (two α-helix regions changed to β-sheet)

Prion Protein Conversion



- A is the normal cellular prion protein with 4 α-helix regions
- B is the disease form of the protein with 2 of the α-helix converted to β-sheet (colored red and green)

Source: Prusiner, S.B.(Ed) 1999. Prion Biology and Diseases. Cold Spring Harbor Laboratory Press.

How Does Prion work?

- The converted prion protein has profoundly different physicochemical properties from the normal form, and cause devastating neurodegeneration
- The converted prion protein acts as a template to refold other normal prion proteins into the disease form and thus propagate the disease (a “conformational cascade”)

Prion Infection Routes

- Consumption of organs (especially brain) and meat of infected animals
- Subsequently data indicated bloodborne transmission
- Bloodborne transmission risk appears to be related to stage of disease of donor, later the stage, higher the risk

Infectious Agents--Clinical Specimens

- Blood borne pathogens are the major concern
  - Hepatitis B Virus (HBV)
  - Human Immunodeficiency Virus (HIV)
  - Viruses causing other hepatitis, e.g. HCV
- Clinical specimens of blood-containing organs and bodily fluids other than blood can also carry significant amount of pathogens

Infectious Agents--Cell Cultures and Viral Vectors

- Infectious agents harbored in cells are major concerns
- Viral vectors used for introducing genes into cells is a potential source of infection
- Naked DNA, another means of transfecting cells, may also be infectious
- Introduced genes may activate dormant infectious agents or oncogenes in cells

Infectious Agents--Experimental Materials

- Many experimental materials, such as environmental samples, may contain biological agents
- Freshwater, seawater, wastewater, sewage samples
- Soil, sediment, sludge samples
- Food samples

Infectious Agents--Indoor Air

- Bacteria--Legionnaire's Disease
  - *Legionella pneumophila*
- Fungus
  - *Stachybotrys chartarum*
  - *Aspergillus sp.*
  - *Penicillium sp.*
- Fungal effects: allergic, pathogenic, toxic
- Sick Building Syndrome and Building Related Infections

Laboratory Animals

- Physical injuries inflicted by the animals, such as bites and scratches
- Zoonoses, i.e. diseases that infect both animals and humans
  - indigenous (naturally carried by the species) e.g. rabies in mammals, hantavirus in rodents
  - experimentally introduced
- Allergens of animal origins
- Other introduced hazards, such as radioisotopes

Biological Toxins

- Bacterial toxins (botulinum toxin, tetanus toxin)
- Fungal toxins (aflatoxin)
- Algal toxins (saxitoxin)
- Animal toxins (snake venom)

Recombinant DNA Molecules

- Genes encoding biological toxins
- Genes related to virulence of pathogens
- Oncogenes and other genetic materials that may create adverse health effects
- Promoters and other constructs that lead to over-expression and abnormal secretion, etc
- Vectors that infect animals and humans
- Transgenic organisms in the environment

Classification of Biohazards

- Many agencies classify microorganisms according to their degree of hazard
  - US Centers for Disease Control and Prevention (CDC)
  - US National Institute of Health (NIH)
  - Health Canada
  - European Council (EC)
- Usually four classes/risk groups ranking from low to high hazard

Classification of Biohazards

- All lists use similar criteria but there are minor differences in classification
- Primarily based on human pathogenicity
- The lists are dynamic in nature
- Classification is “flexible” with appropriate special risk assessment and controls

Classification of Biohazards In China

分类	病原微生物描述
第一类 （高致病性病原微生物）	能够引起人类或者动物非常严重疾病的微生物，以及我国尚未发现或者已经宣布消灭的微生物
第二类 （高致病性病原微生物）	能够引起人类或者动物严重疾病，比较容易直接或者间接在人与人、动物与人、动物与动物间传播的微生物
第三类	能够引起人类或者动物疾病，但一般情况下对人、动物或者环境不构成严重危害，传播风险有限，实验室感染后很少引起严重疾病，并且具备有效治疗和预防措施的微生物
第四类	在通常情况下不会引起人类或者动物疾病的微生物

《病原微生物实验室生物安全管理条例》（2018年修订版）

What about SARS Coronavirus?

- Non-SARS human coronavirus is Class 2
- Clinical specimens potentially containing SARS coronavirus can be handled in a Level 2 facility
- Culturing of SARS coronavirus requires a Level 3 facility

Avian Flu Virus?

- H5N1 Avian Flu Virus needs to be handled under Biosafety Level 3
- Normal seasonal flu viruses can be handled under Biosafety Level 2

Swine Flu (2009 H1N1 Influenza A) Virus?

- Initially US CDC recommended Biosafety Level 3
- Later (Aug 2009) US CDC lowered the requirement to Biosafety Level 2

What is Bioaerosol?
And why do we care?


New York
The battle against disease has been helped by understanding the relationships between micro-organisms, humans and other species, and how they are affected by environmental changes. 'Epidemic! The World of Infectious Disease' provides an imaginative, graphic, lively and interactive history of various infections. Ongoing at the American Museum of Natural History. Tel 769 5800.
GAZE INTERNATIONAL/ANNE MANIGLIER

Definition of Bioaerosols

- Definition: Biological materials suspended in air, which may be hazardous to human health.
- Generated by various processes, animals and humans
- Range in size - 0.5 to 100 μm

Nature of Bioaerosols Found in Indoor Environment

- Dirt and debris
- Respiratory pathogens
- Skin flakes
- Dust mite and insect parts and excreta
- Animal shedding and excreta
- Fungal spores & hyphae

Settling Rates of Airborne Particles

Particle	Microns (μm)	Settling Velocity (m/s)
droplets	100 - 400	0.3 - 2.5
dust	10 - 100	3×10^{-3} - 0.3
droplet nuclei	1 - 10	3.6×10^{-5} - 3×10^{-3}
droplet nuclei	0.1 - 1	8.1×10^{-7} - 3.6×10^{-5}

Distances Traveled by Particles from a Height of 1.5 Meter

Size (μm)	Distance (m) before Impact
100	0.6 - 1.5
50	7.6
30	15.2
20	27.4
10	61.0 - 91.4
01	Indefinite

Particle Deposition in Human Airway

- >10 μm - removed in nasal passage
- 5 - 10 μm - deposit in upper respiratory tract
- <5 μm - deposit in deeper region in lung

Potential Hazards of Bioaerosols

- Respiratory distress
- Microbial infection
- Allergic reaction
- Respiratory sensitization
- Toxicological reaction

Why Do We Care?

- Respiratory route estimated to account for 65-75% of all occupational infection cases
- When things are airborne they spread easily and are difficult to contain
- We can stop eating and drinking, but not breathing

Many Laboratory Operations Generate Aerosols

- Blending
- Centrifugation
- Pipetting
- Opening screw cap containers
- Withdrawing material from vacuum bottles
- Streaking with wire loop
- Lyophilization procedures
- Flow cytometer operations
- Improper use of biosafety cabinets

How does infection occur? And what do we know about occupational infections?

Bodily Response to Exposure

- Exposure may not lead to infection
  - Route of exposure
  - Dose of exposure
  - Pathogenicity of organism
  - Host susceptibility
- Infection response
  - asymptomatic
  - subclinical
  - clinical
- Antibody formation

Mode of Transmission (1)

- Airborne
  - by droplets or nuclei, 1-4 μm
  - long-term suspension in air
- Contact
  - by large droplets
  - close contact with patient
  - direct or indirect contact

Mode of Transmission (2)

- Vehicle
  - fomite (contaminated items)
  - food
  - water
- Vector
  - external vectors (e.g. flies)
  - internal vectors (e.g. mosquitoes, fleas)

What about Transmission of SARS and Flu Viruses?

- Contact transmission for sure
- Indications of limited airborne transmission
- Remember the distinction between “airborne” and “contact” is ARTIFICIAL

Infective Dose Varies With Microbes

Shigella sp.	<100
Salmonella sp.	100,000
Vibrio cholera	100,000,000
Giardia lamblia	<100
Coxiella burnetii	10
Mycobacterium tuberculosis	1

We have defense mechanisms against infection (1)

FIRST line of defense

- Digestive tract:
  - Stomach acid
  - Normal flora
  - Peristalsis
- Respiratory tract:
  - Ciliated epithelial cells
  - Mucous membranes
  - Macrophages

We have defense mechanisms against infection (2)

- Skin:
  - Normal flora
  - Impermeable to microbes

SECOND line of defense

- Immune System
  - Cell-mediated--lymphocytes, T-cells, etc.
  - Humoral--antibodies

Occupational Infections Do Happen!

- >4000 cases reported in 30 yrs (Pike, 1979), many more unreported cases
- Fatality rate ~4%
- 4X more cases in research vs clinical labs
- Involved different agents: bacteria, viruses, rickettsiae, fungi, chlamydiae and parasites
- Only 20% of the cases followed a recognized accident

Common Accidents Resulting in Occupational Infections

- Cut, bite or scratch by lab animals
- Splatter or spill of microbial culture
- Syringe or needle stick
- Mouth pipetting
- Others

Probable Causes of Other Occupational Infections

- Working with infective agents
- Handling infected animal or arthropod
- Exposure to aerosol
- Handling clinical specimen
- Conducting human autopsy
- Handling discarded glassware

Incidents Involving Biological Agents Other Than Microbes

- Cell Culture: Needle stick transmission of colonic adenocarcinoma, nodule formation despite of HLA disparities (Gugel & Sanders, 1988)
- Recombinant DNA: Cancer from alleged oncogene exposure at the Pasteur Institute: 5 molecular biologist contracted cancer. All worked with tumor viruses / oncogenes in two adjacent labs (Bartel, 1987)

Controls of Biohazards

General Principals to Prevent Occupational Infections

- Ingestion route
  - frequent hand washing
  - proper use of gloves
  - no eating or drinking in lab
- Inhalation route
  - avoid aerosol generation
  - contain aerosol generating processes
- Skin penetration
  - proper handling and disposal of sharps

General Approaches to Control Occupational Hazards

- Engineering Controls
  - "built-in"equipment or facility safety measures
  - most effective and preferred
- Administrative Controls
  - rely on worker compliance
- Personal Protective Equipment
  - the last resort
  - sometimes used for additional protection

Biological Safety Levels

- Based on US CDC definitions
- Corresponds to Classes of microbes (BSL 1-4 for Class 4-1 organisms)
- Animal BSL (ABSL 1-4) for animal work
- Each level builds on the previous level
- Main elements:
  - laboratory practice and techniques
  - safety equipment (primary barriers)
  - facility design (secondary barriers)

Engineering Controls for Biohazards

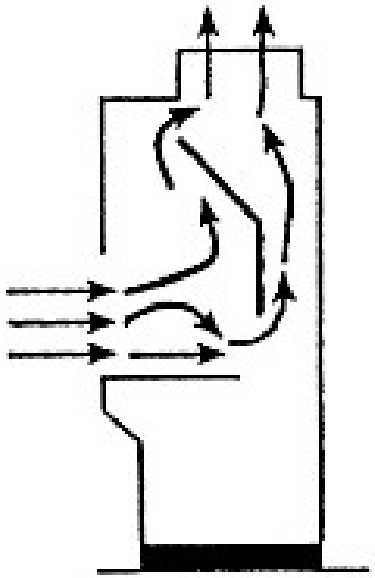
- Primary Barrier mainly achieved by Biological Safety Cabinets
- Secondary Barrier by facility features
  - isolated/sealed rooms
  - special ventilation, e.g. pressure regime, air locks, HEPA filtered air exhaust
  - special drainage, e.g. holding tanks
  - waste disinfection facilities
  - other features, e.g. UV lamp

Primary Barrier: Biological Safety Cabinets (BSC) and HEPA Filters

Chemical Fume Hood

- Designed to capture chemical vapors /fumes and sweep them away from the worker.
- Offers personnel protection from vapors/ fumes.
- Does not protect the product.
- Environment may be protected by air cleaning devices in ventilation system.
- Blower is on roof and pulls air through fume hood.

Chemical Fume Hood

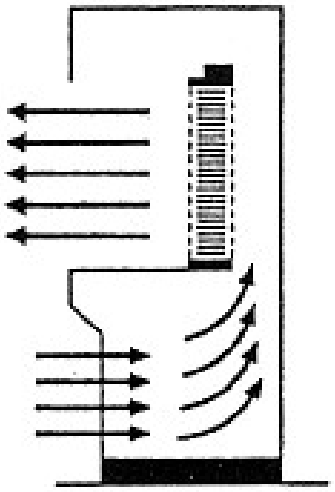


Chemical
Fume Hood

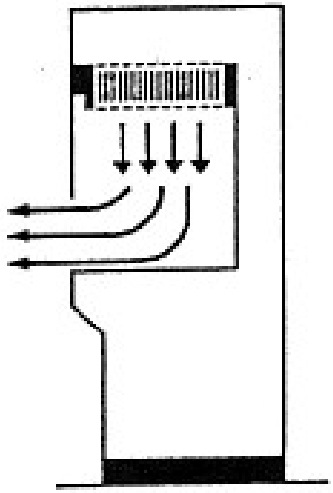
Laminar Flow Clean Bench

- Offers product protection only.
- Horizontal Flow: HEPA filtered air sweeps horizontally across workspace.
- Vertical Flow: HEPA filtered air descends vertically, then sweeps horizontally across workspace.

Laminar Flow Clean Benches



Horizontal Laminar Flow
Clean Bench

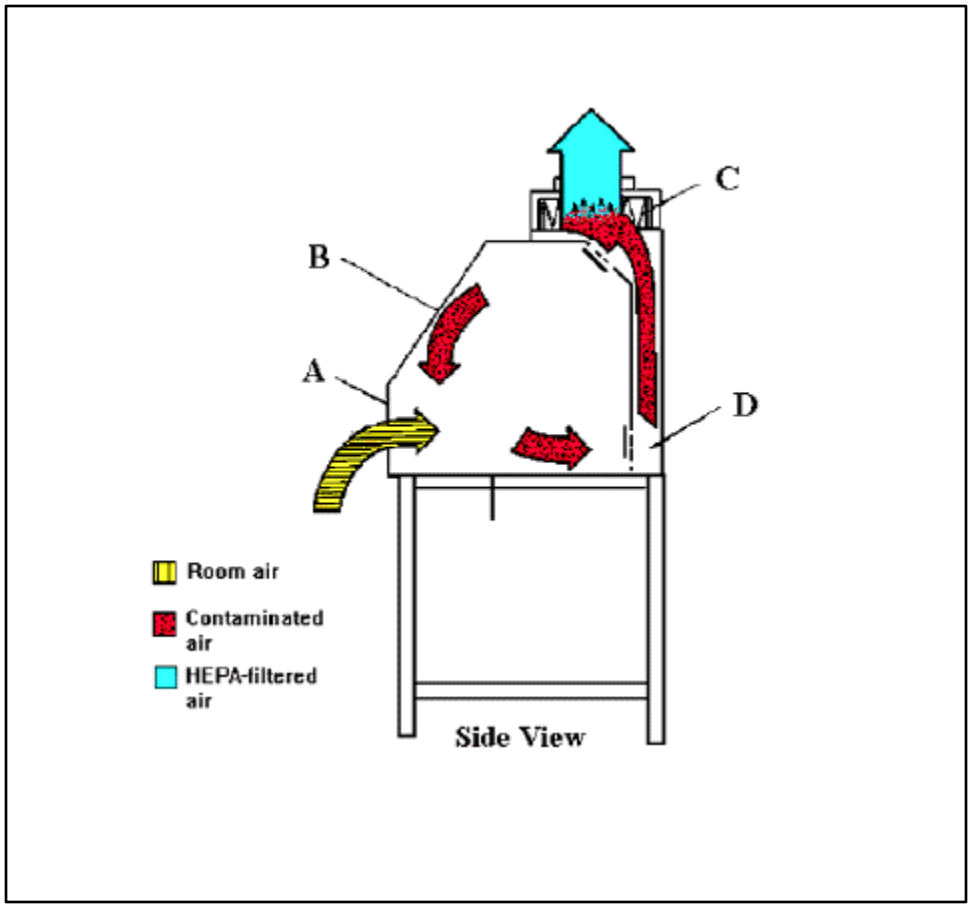


Vertical Laminar Flow
Clean Bench

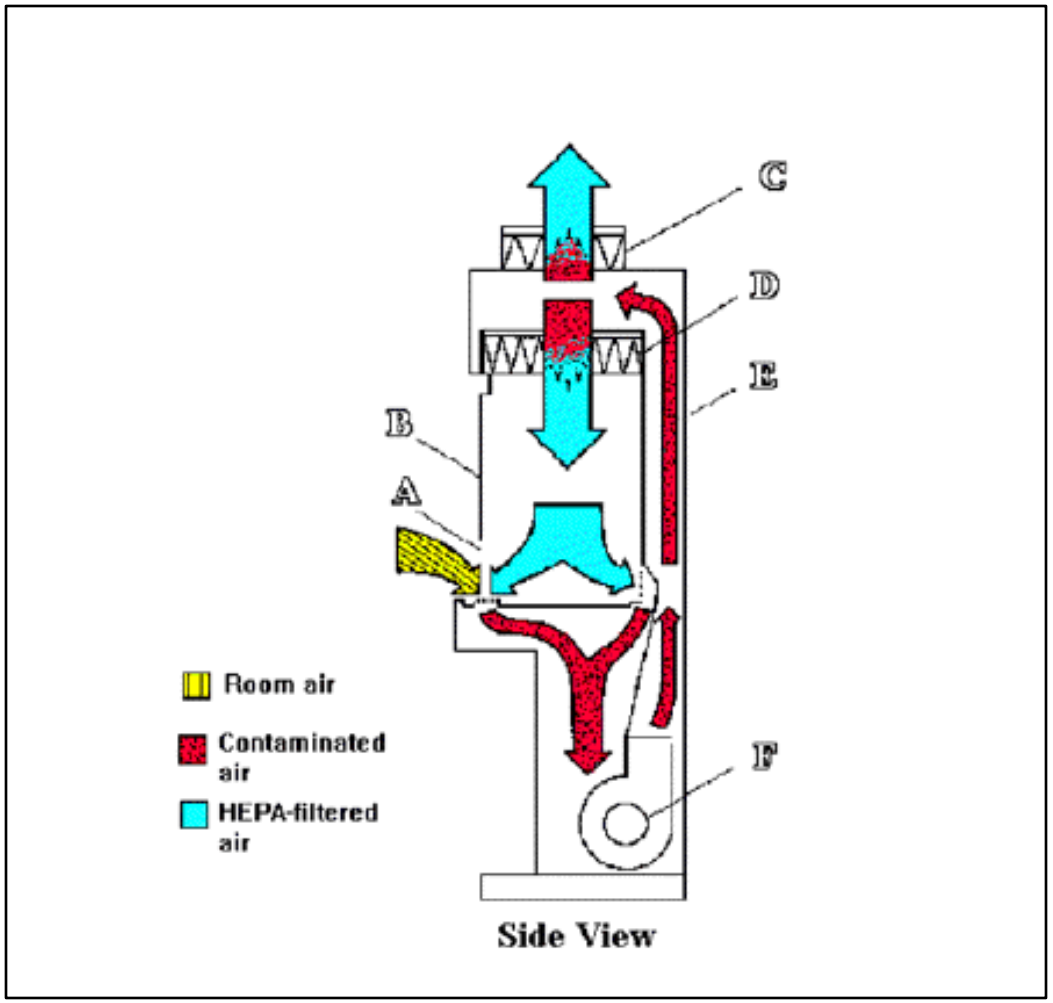
Biological Safety Cabinet

- A Biological Safety Cabinet is basically a leak tight box containing HEPA Filters and a motor/blower system to provide controlled air movement through the box and filters.

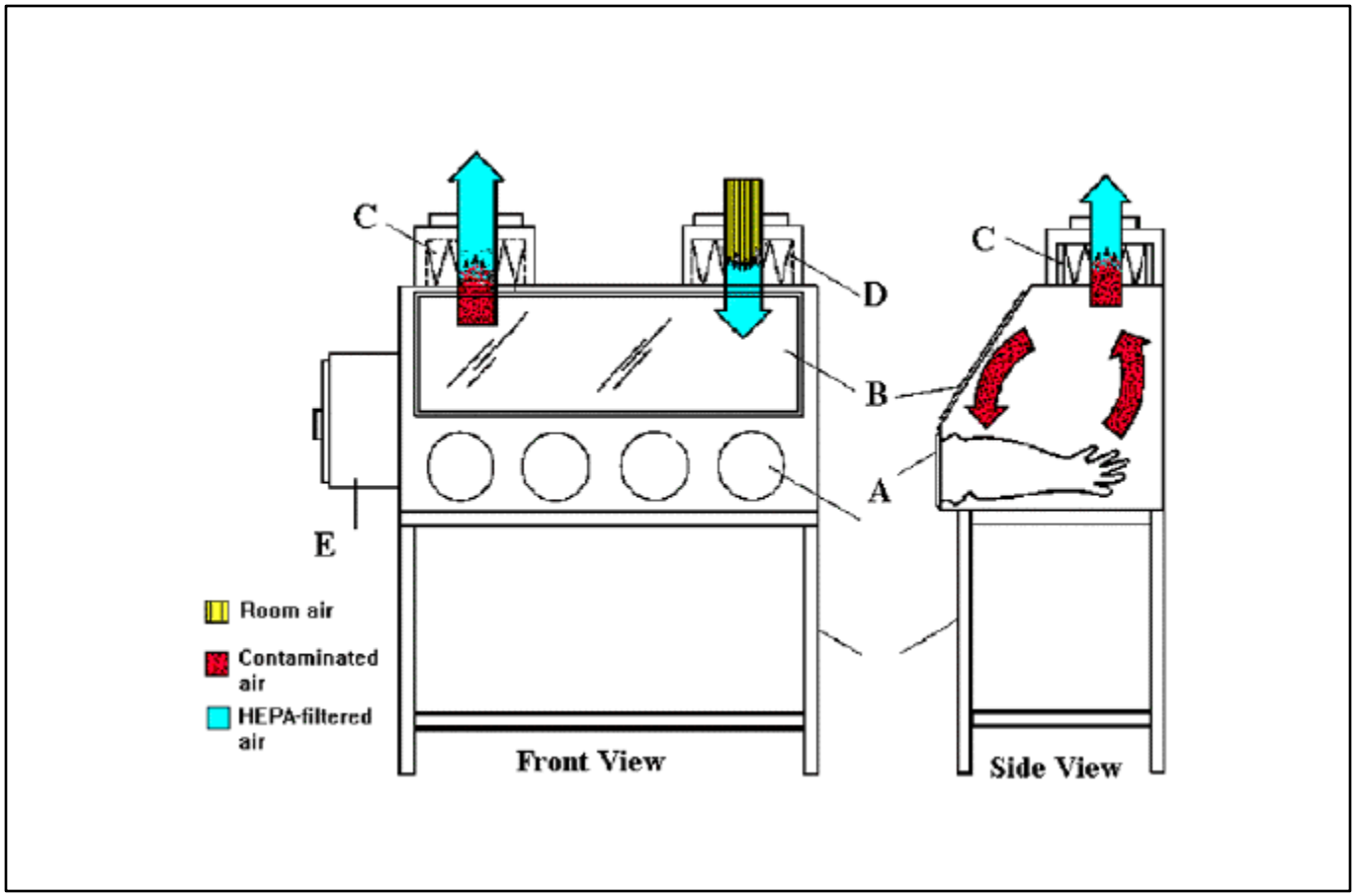
Class I BSC Air Flow Diagram



Class II BSC Air Flow Diagram



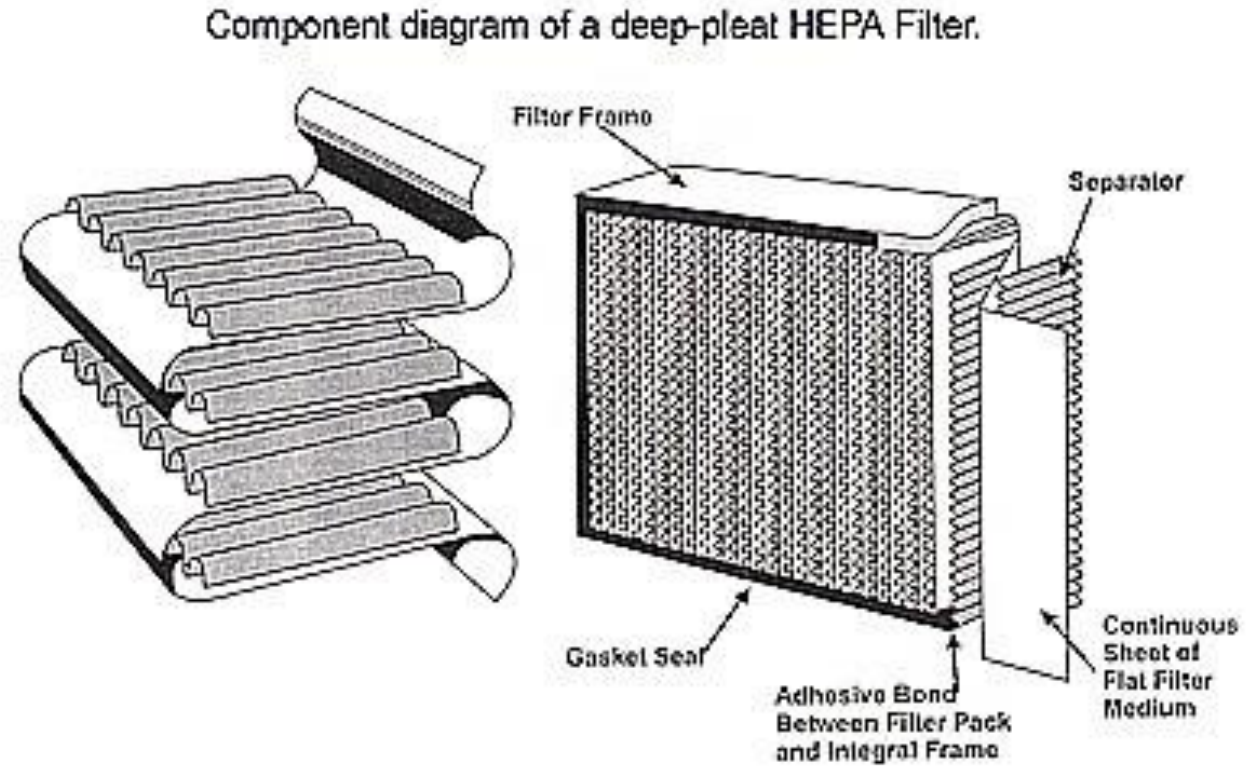
Class III BSC Air Flow Diagram



Definition of HEPA Filter

- A throw-away extended/pleated medium dry-type filter with:
  - rigid casing enclosing the full depth of the pleats
  - minimum particulate removal of 99.97% for thermally generated DOP smoke particles or equivalent with a diameter of 0.3 micron

HEPA Filter

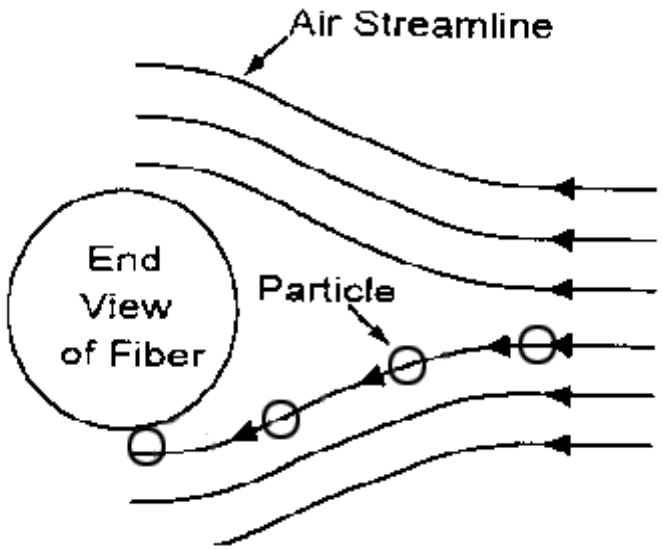

Component diagram of a deep-pleat HEPA Filter.
Filter Frame
Separator
Gasket Seal
Adhesive Bond Between Filter Pack and Integral Frame
Continuous Sheet of Flat Filter Medium

Particulate Capture Mechanisms

- Filtering devices (e.g. sieves and filter papers) for large particles rely on pore sizes
- Different mechanisms remove submicron particles down to the range of large gas molecules:
  - impact and interception (efficient for larger particles)
  - diffusion (efficient for smaller particles)
- 0.1-0.3 is at the boundary where neither mechanism is efficient

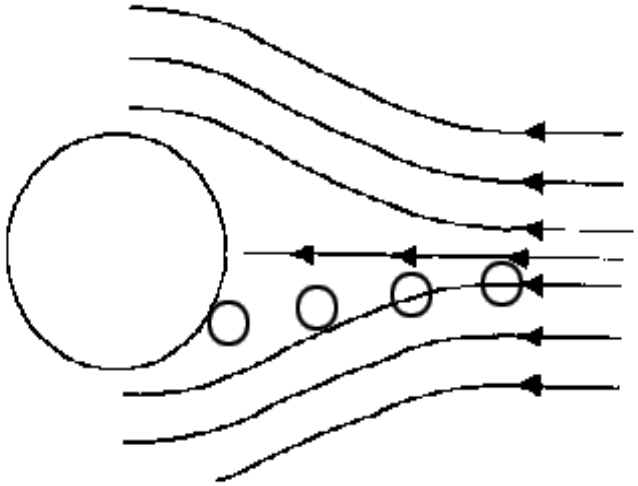
Direct Interception

- Air stream brings particle within one particle radius of a filter fiber
- Particle touches fiber surface and captured



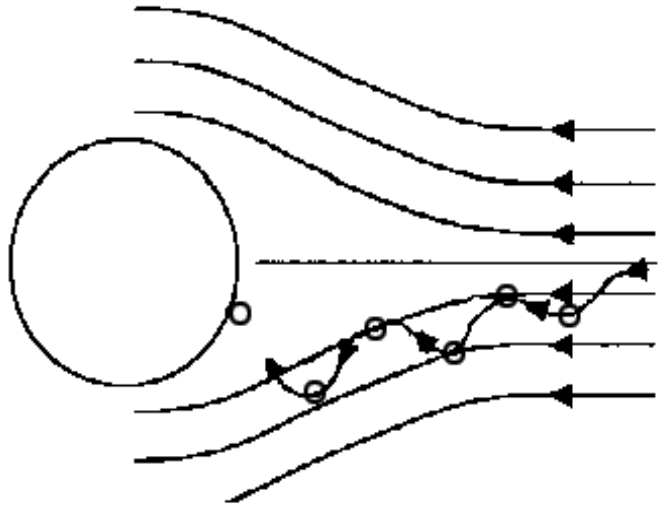
Inertial Impaction

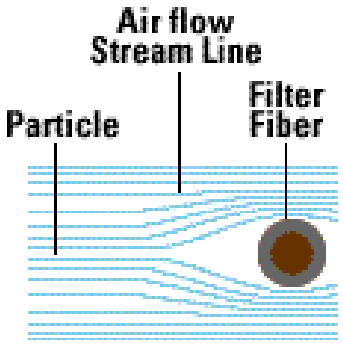
- Particle too large to adjust to changes in air stream direction
- Particle continues by inertia and hit filter fiber
- Predominant in high gas velocities and dense packing of filter media



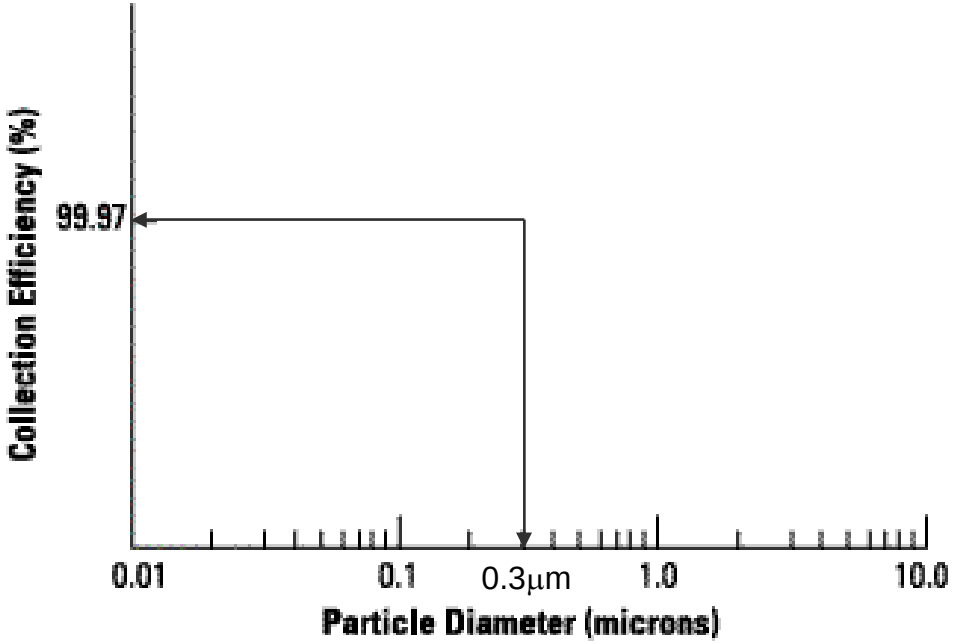
Diffusion

- Result of random Brownian motion of gas molecules
- Small particles (<0.1 µm) hit fiber after bumped by gas molecules
- Predominant with low gas velocities and smaller particles



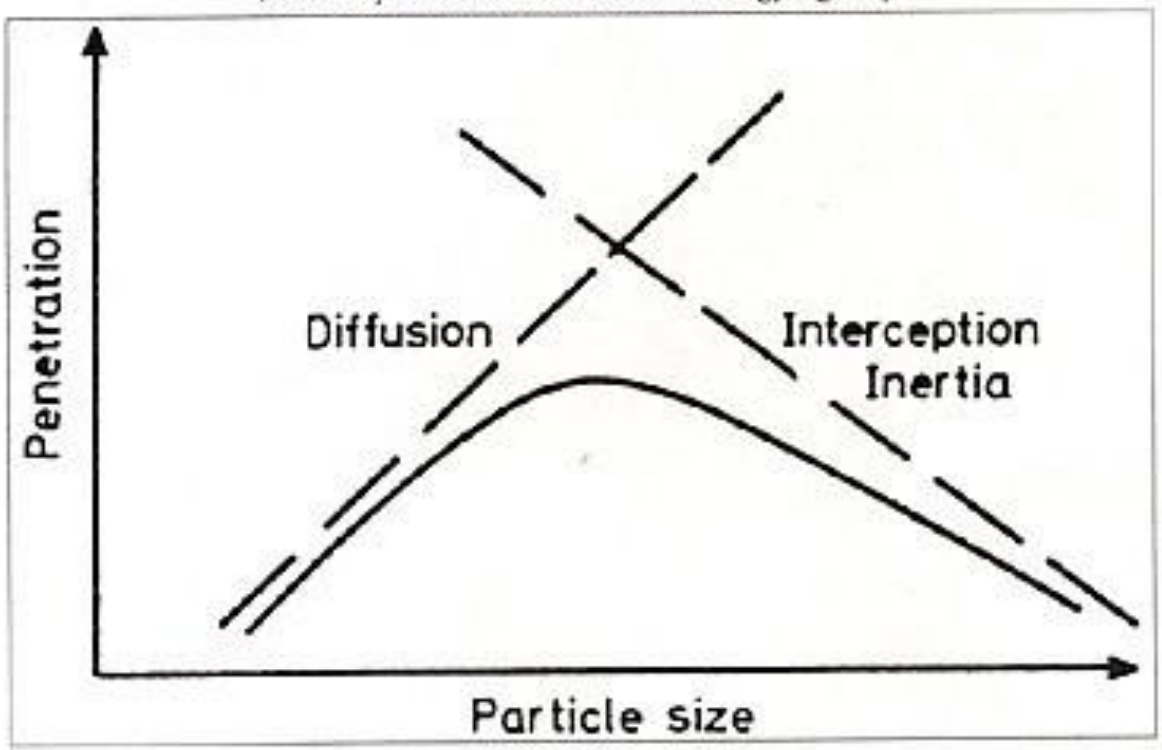


Inertial Impaction
Particle inertia causes it to leave flow stream lines and impact on fiber

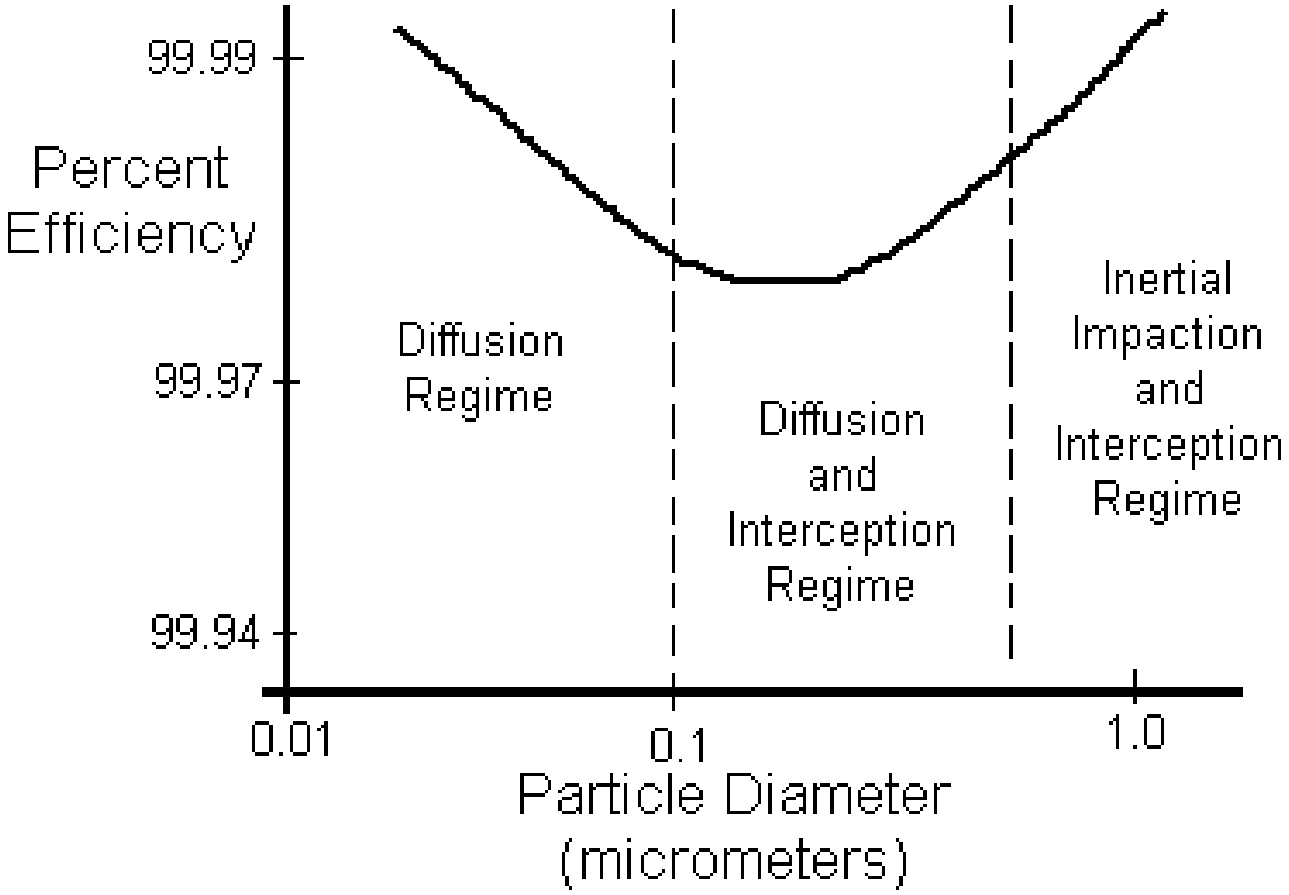


Theoretical HEPA Filter Collection Efficiency

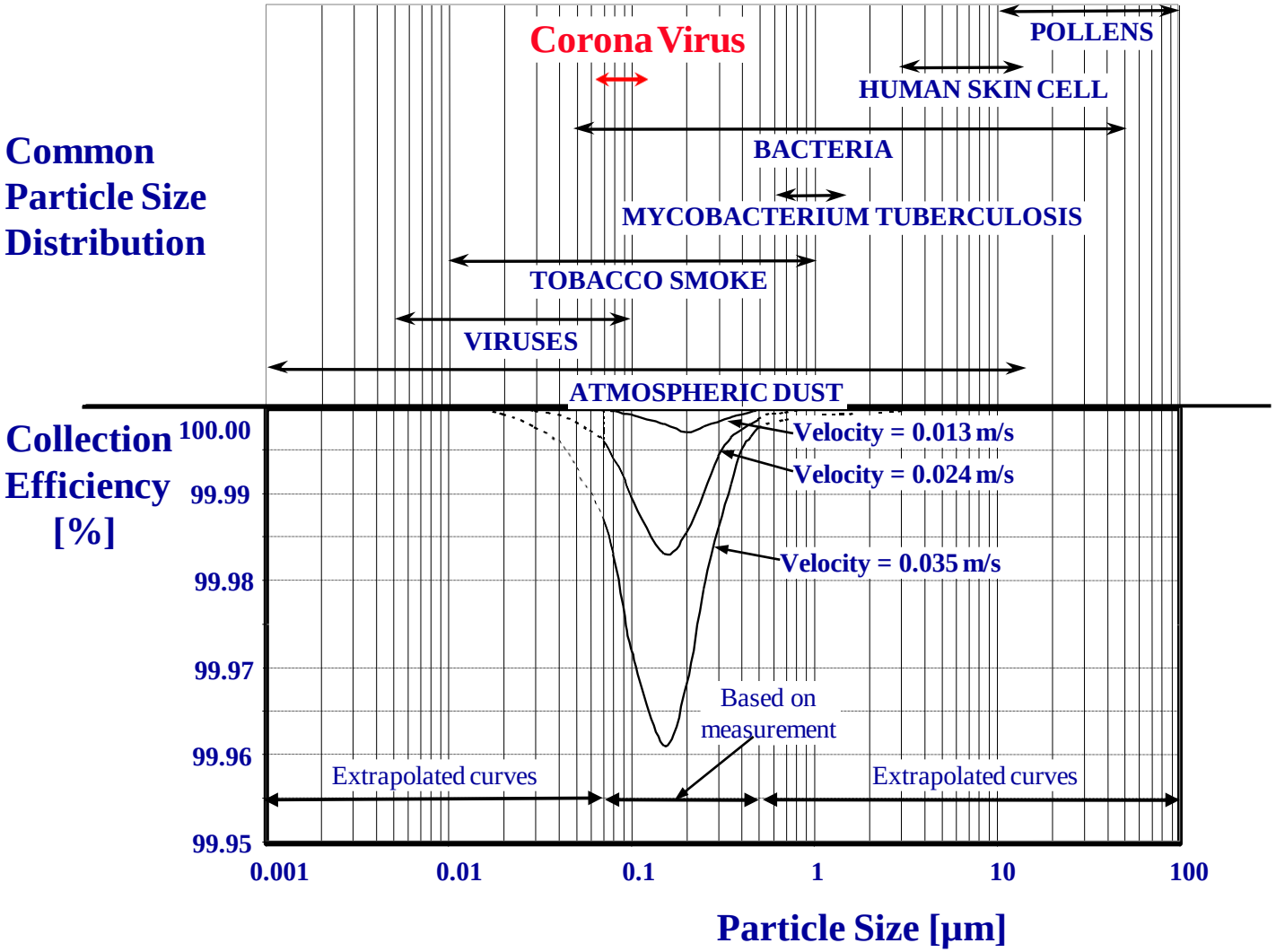
The effects of inertia, diffusion, and interception on the penetration-particle-size curve. (Courtesy International Atomic Energy Agency)



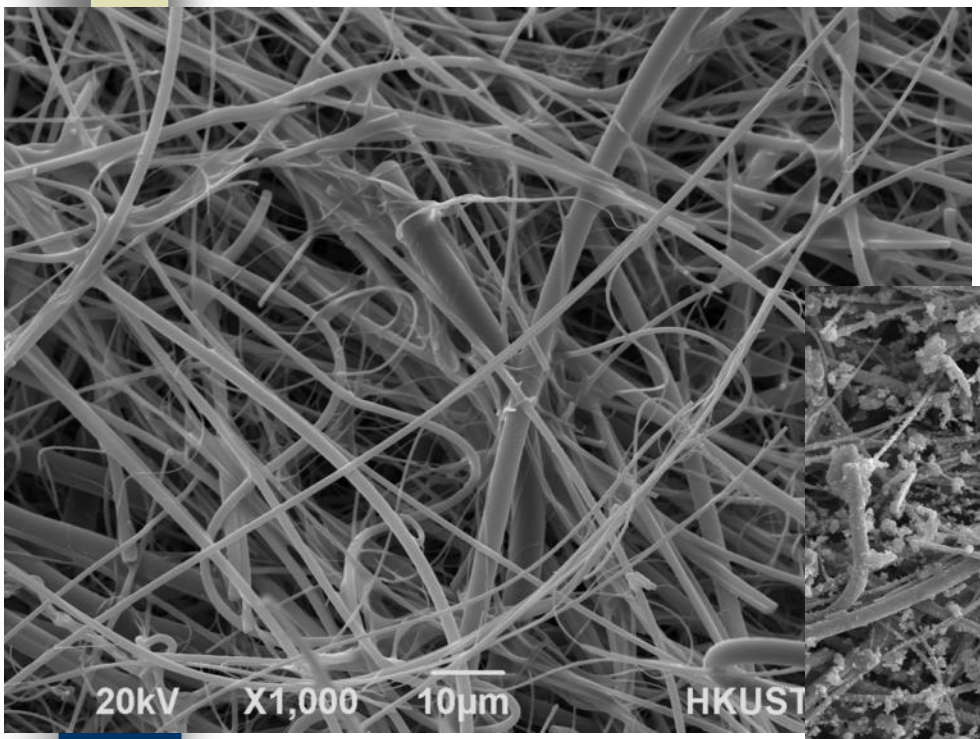
Particle Size and Filtration Efficiency



HEPA Filtration Efficiency

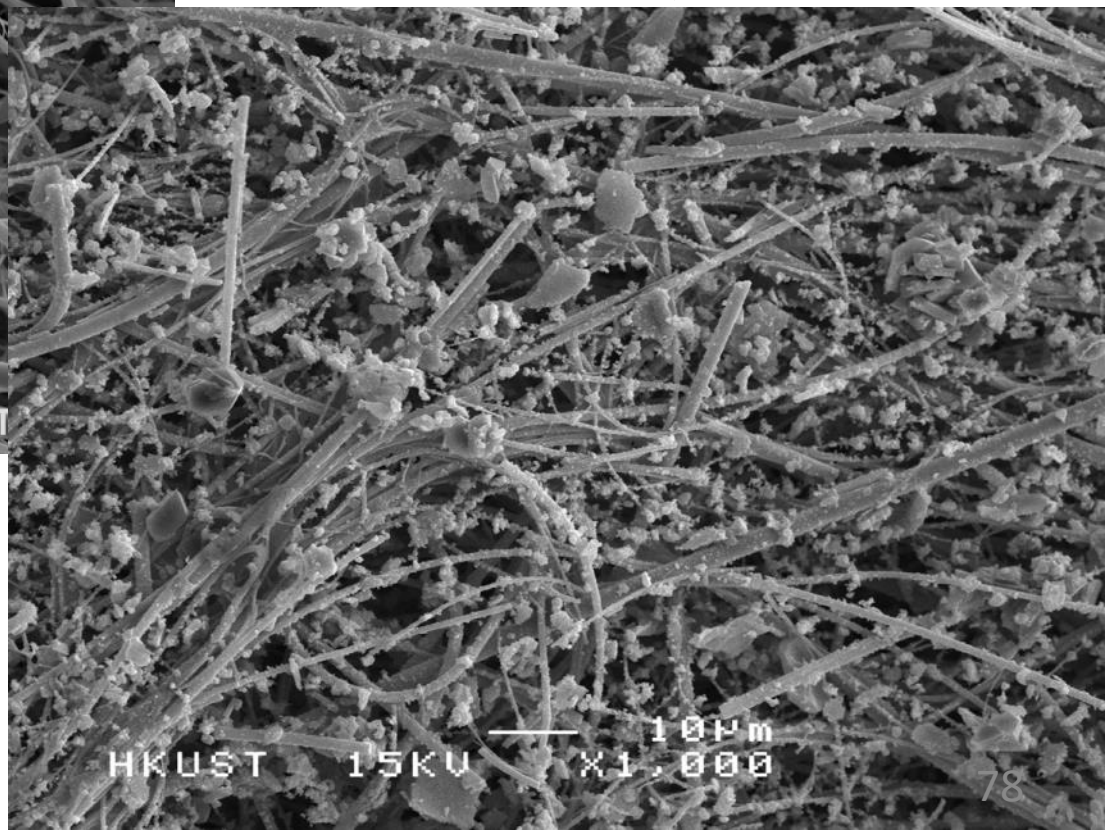


SEM of Fibrous Filter 1000 x

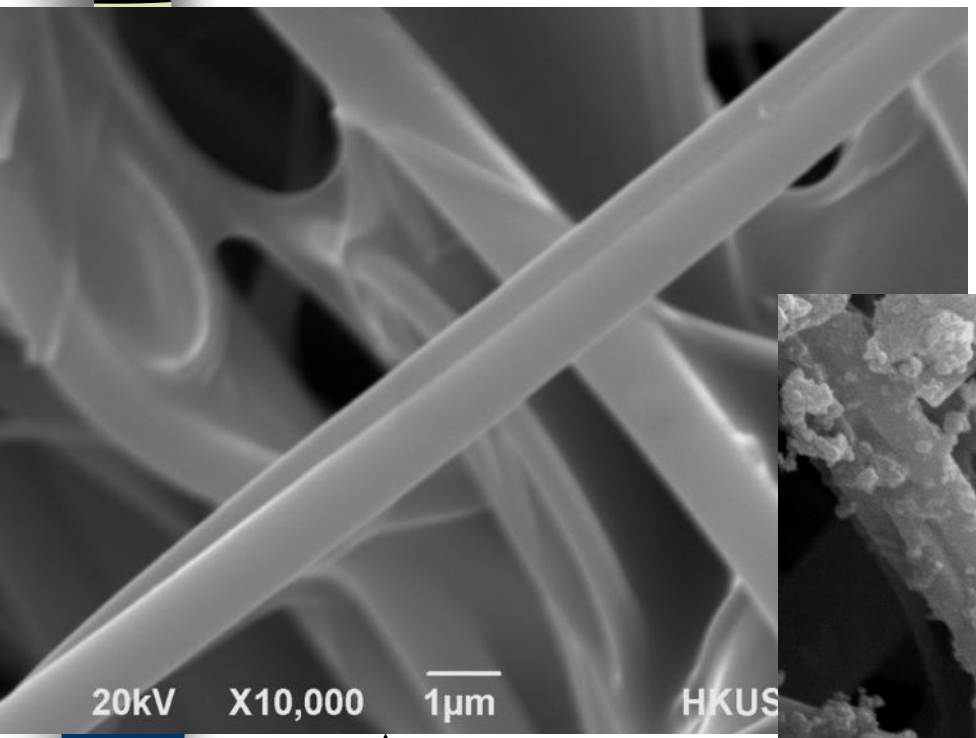


New Filter

Used Filter

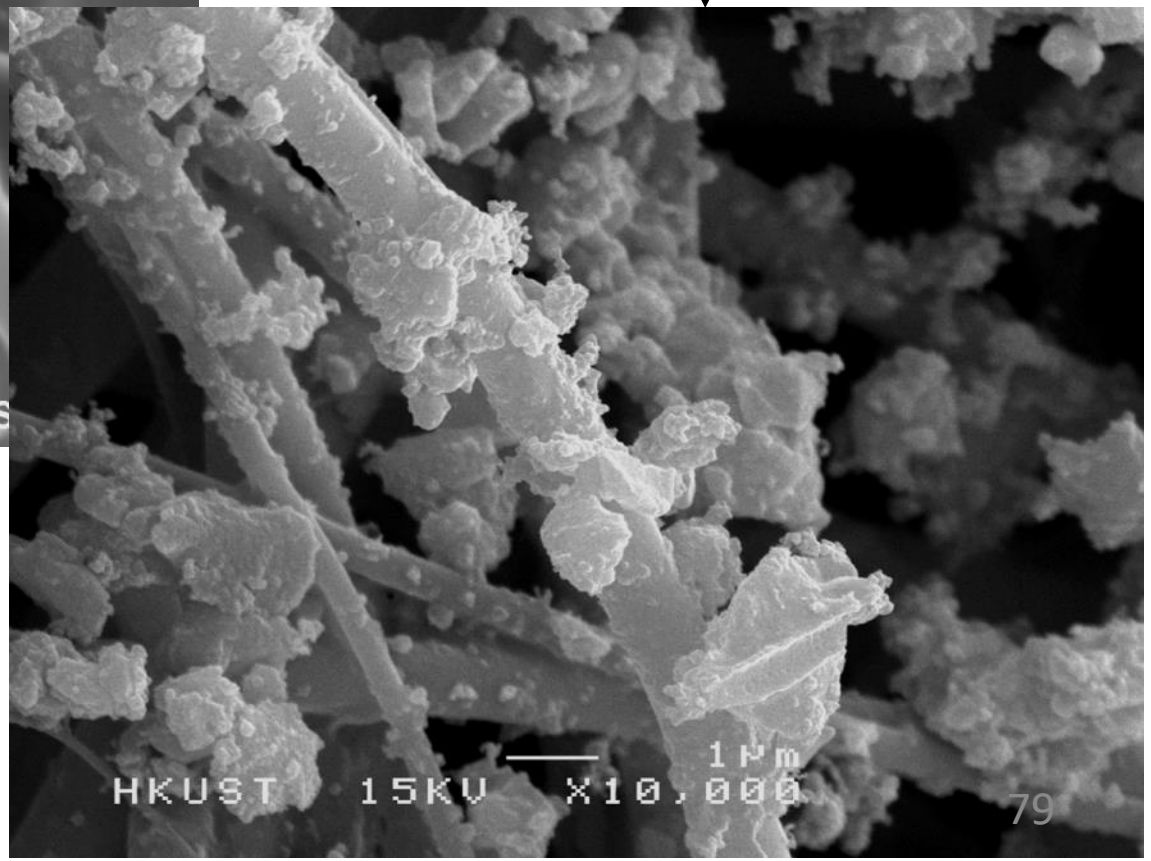


SEM of Fibrous Filter 10000 x



New Filter

Used Filter



Using Volatile Chemicals in BSC

- HEPA Filters DO NOT filter out gases and vapors, they only filter particulate
- Different types of BSC are designed for biohazard work which involves volatile chemicals and/or radioisotopes
- Class I

  Class II (Type A1, A2, B1, B2)

  Class III

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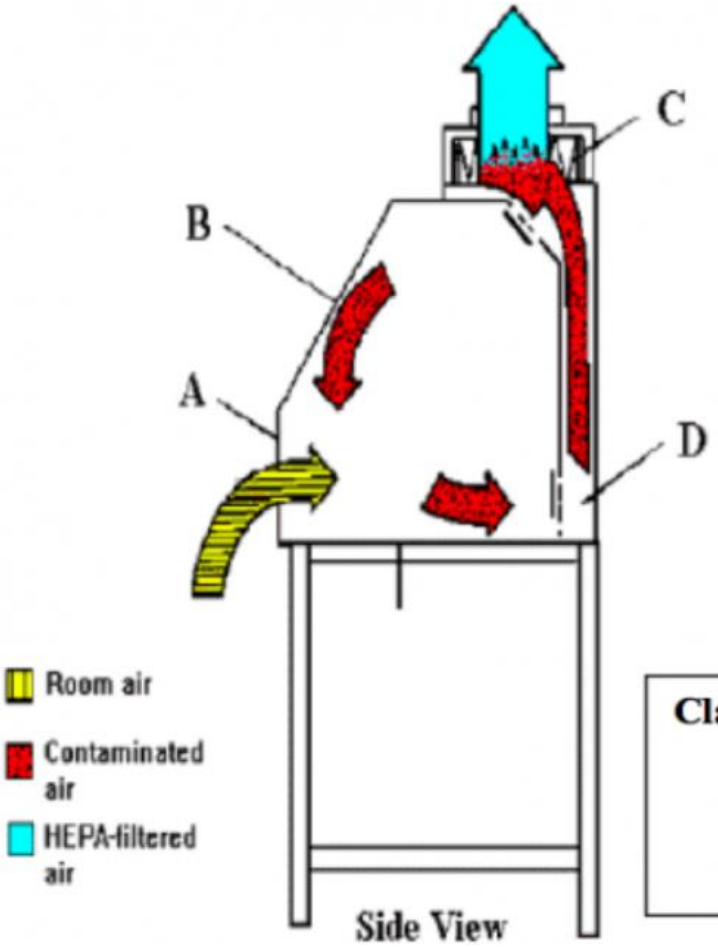
Class I Biological Safety Cabinet

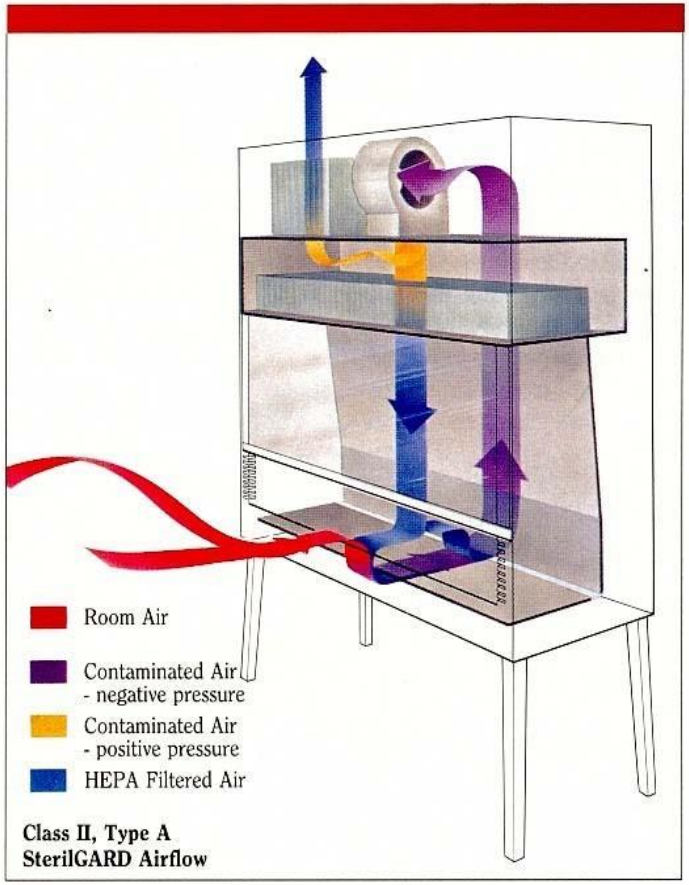


- It protects people and environment but does not protect the simples inside, hence it is not often used.

Class I BSC

A. front opening
B. sash
C. exhaust HEPA
D. exhaust plenum

Class II (Type A1, A2) Biological Safety Cabinet



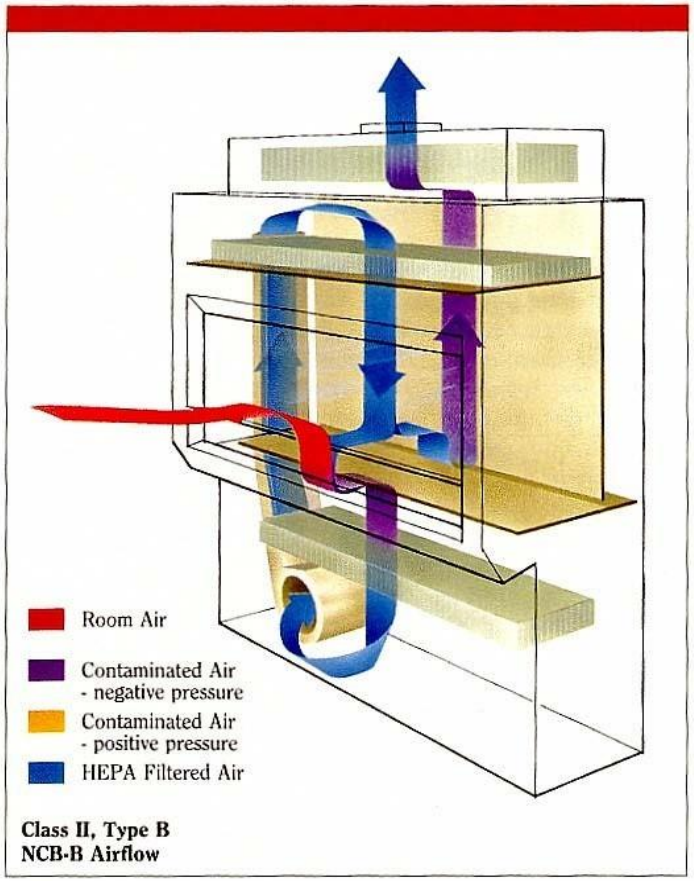
Class II (Type A1)

- Face velocity ≥ 0.40 m/s
- Cannot be used for testing with volatile toxic chemicals and volatile radionuclides

Class II (Type A2)

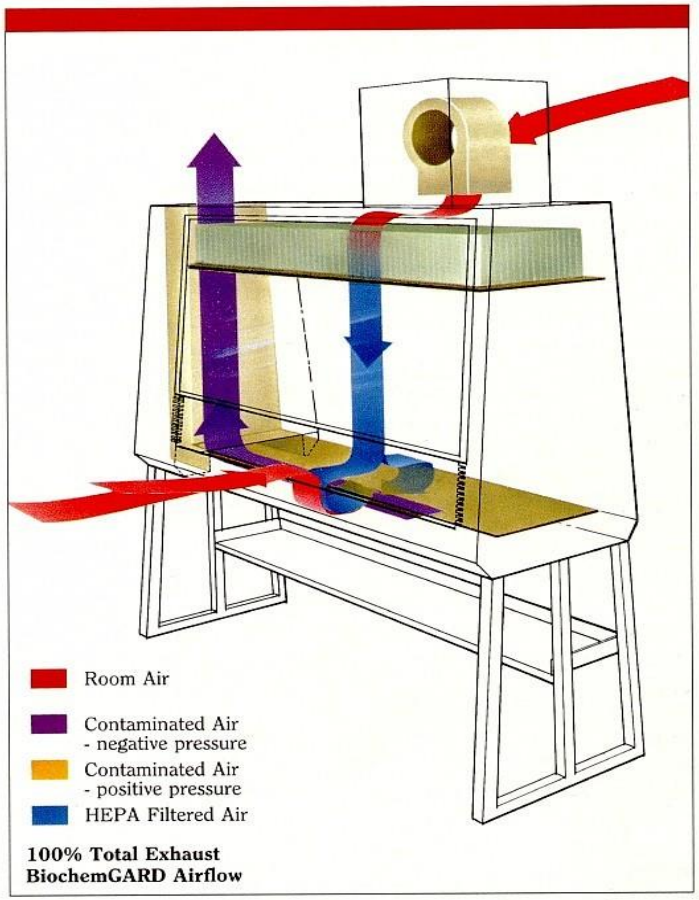
- Face velocity ≥ 0.50 m/s
- Can be used for microbial experiments with trace volatile toxic chemicals and trace radionuclides as auxiliary agents, a properly functioning exhaust hood must be connected

Class II (Type B1) Biological Safety Cabinet



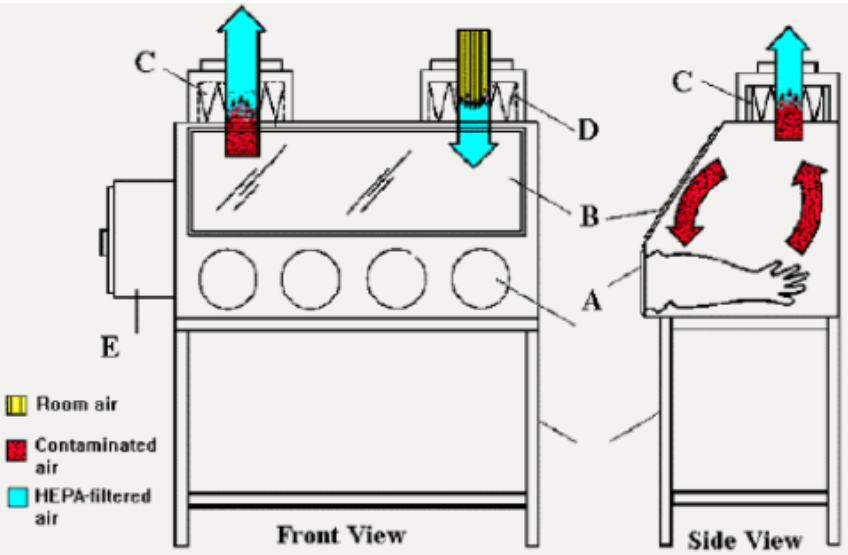
- Face velocity ≥ 0.50 m/s
- If the volatile toxic chemicals or radionuclides circulate with the air without affecting the experimental operation or the experiment is conducted in the direct exhaust area of the safety cabinet, it can be used for microbial experiments with trace volatile toxic chemicals and trace radionuclides as auxiliary agents

Class II (Type B2) Biological Safety Cabinet



- Face velocity ≥ 0.50 m/s
- Can be used for microbial experiments with volatile toxic chemicals and radionuclides as auxiliary agents

Class III Biological Safety Cabinet



A. glove ports with O-ring for attaching arm-length gloves to cabinet
B. sash
C. exhaust HEPA filter
D. supply HEPA filter
E. double-ended autoclave or pass-through box

- Fully enclosed, no leakage
- Operate in the operating area of the biosafety cabinet through gloves connected to the cabinet
- The negative pressure in the biosafety cabinet to the laboratory ≥ 120 Pa

BSCs Need Periodic Testing

- Annual testing
  - Appearance
  - Integrity of HEPA filters
  - Downdraft flow rate
  - Flow rate of inflow air
  - Air flow pattern
- Tests also required after each relocation or filter change
- Fumigation to decontaminate unit before servicing or filter change

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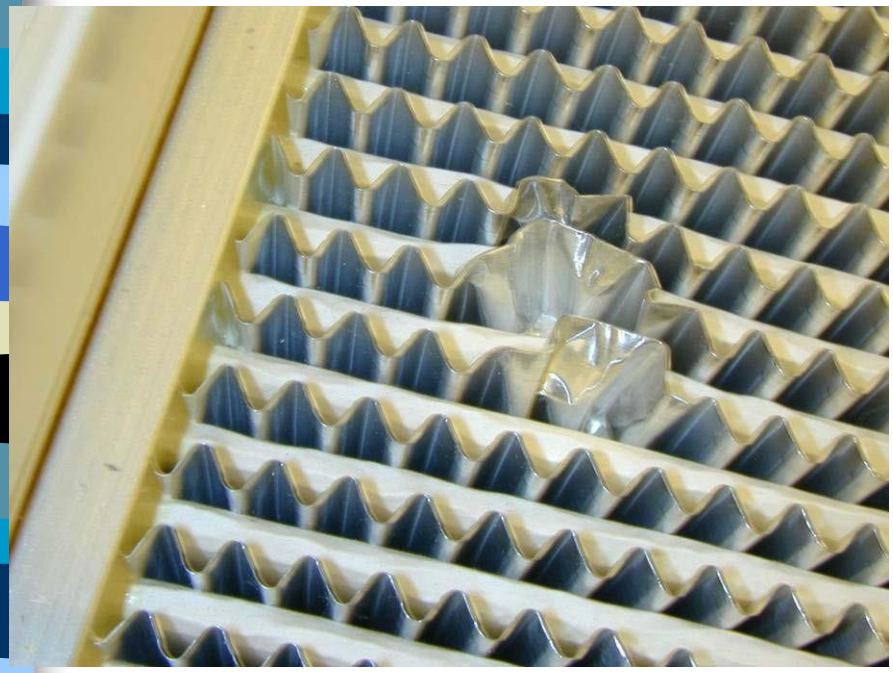
Certification of BSC



Safe Use of BSCs—Before You Begin…(1)

- Check type and certification
- UV light is off
- Block opening when UV light is on
- Let BSC run 2-3 mins when first turned on, or after disturbing airflow inside
- Use proper disinfectant
- Check opening and drain position

Safe Use of BSCs—Before You Begin…(2)

- Do not place contaminated items inside the cabinet
- Place items as far back as possible
- NEVER block the grilles
- Segregate clean items and those that will become contaminated

Safe Use of BSCs—Working in the Cabinet…(1)

- Minimize people movement near BSC,
- Minimize interruption of work, put up sign, etc
- One person at a time
- Armpit level with bottom of window
- Use good aseptic techqniue…ALWAYS
- Slow movement, minimize entering and exiting

Safe Use of BSCs—Working in the Cabinet…(2)

- Enter and exit straight on, allow cabinet to stabilize
- Put clean items upwind from dirty items
- Minimize moving dirty items near clean ones
- Do not use open flame in cabinet
- Post and read spill handling procedures
- Never eat or drink near the cabinet

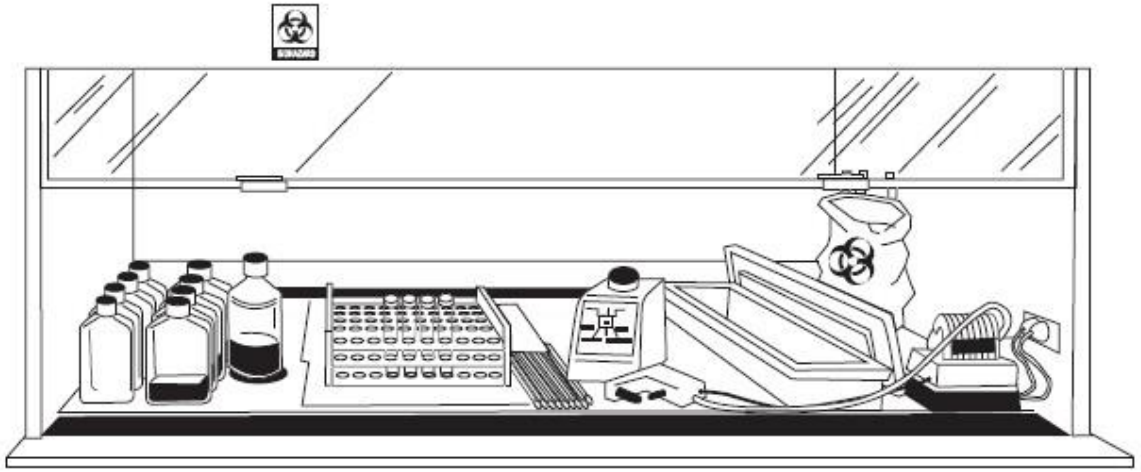


Figure 11. A typical layout for working "clean to dirty" within a Class II BSC. Clean cultures (left) can be inoculated (center); contaminated pipettes can be discarded in the shallow pan and other contaminated materials can be placed in the biohazard bag (right). This arrangement is reversed for left-handed persons.

Source: CDC & NIH, Sep 2007

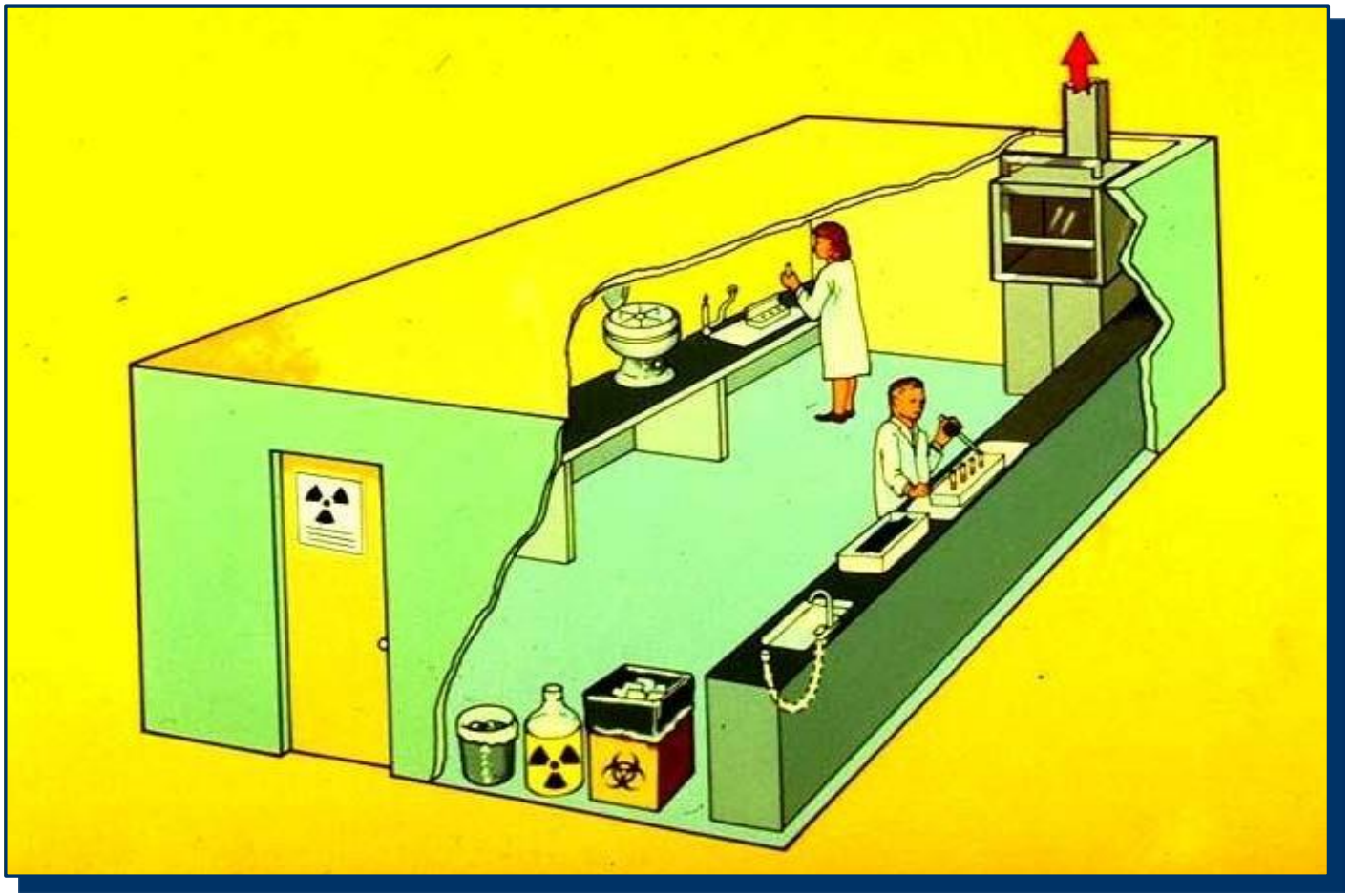
Safe Use of BSCs—After Using the Cabinet…

- Contaminated items should be surface-disinfected or be enclosed before removal
- Remove items and disinfect cabinet inner surfaces
- Do not store equipment or supplies in cabinet
- If possible, leave cabinet running
- Before shutting off, purge for 2-3 minutes

Secondary Barrier: Facility Requirements

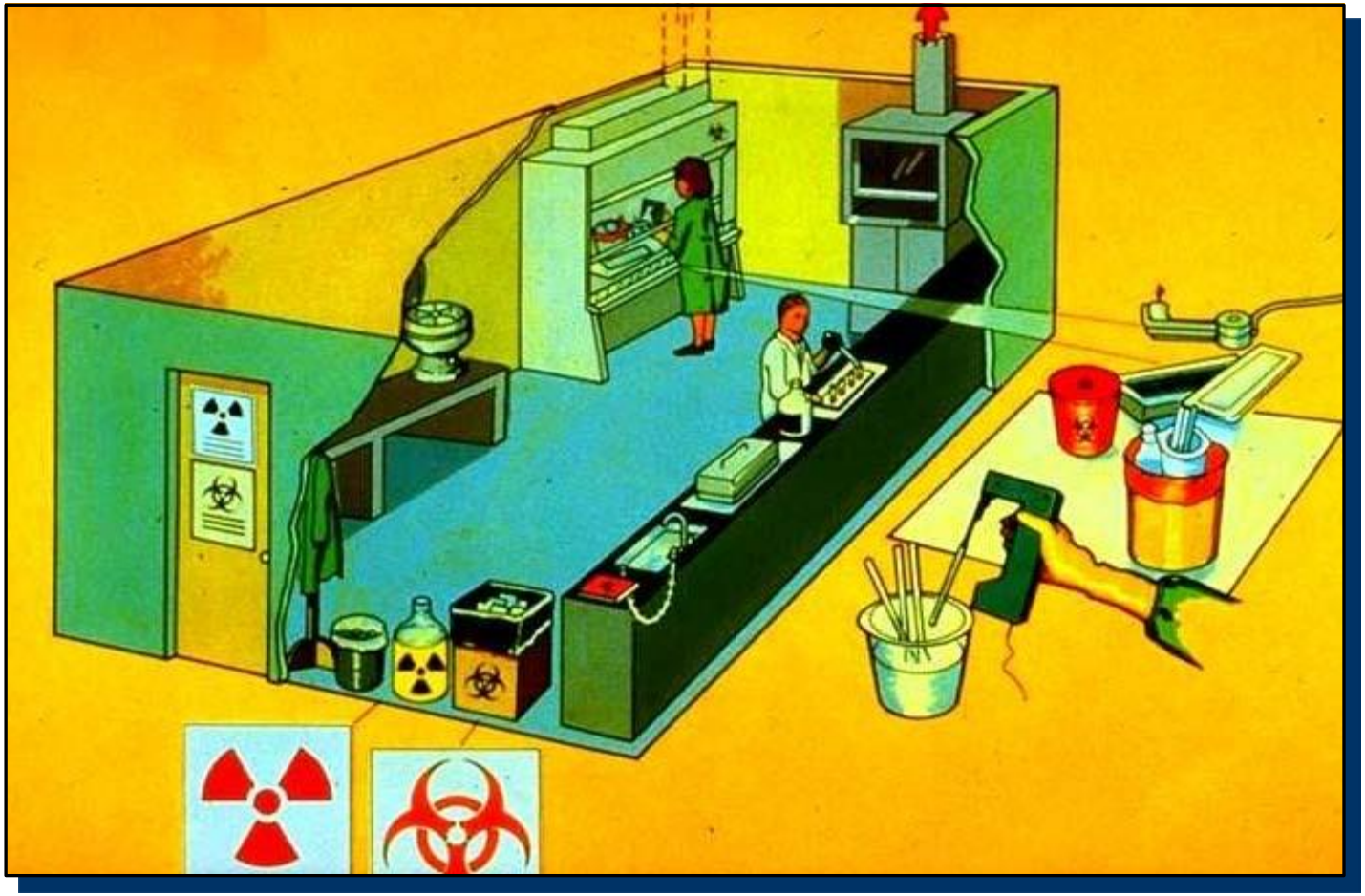
Biosafety Level 1

Laboratory Facilities (Secondary Barriers)



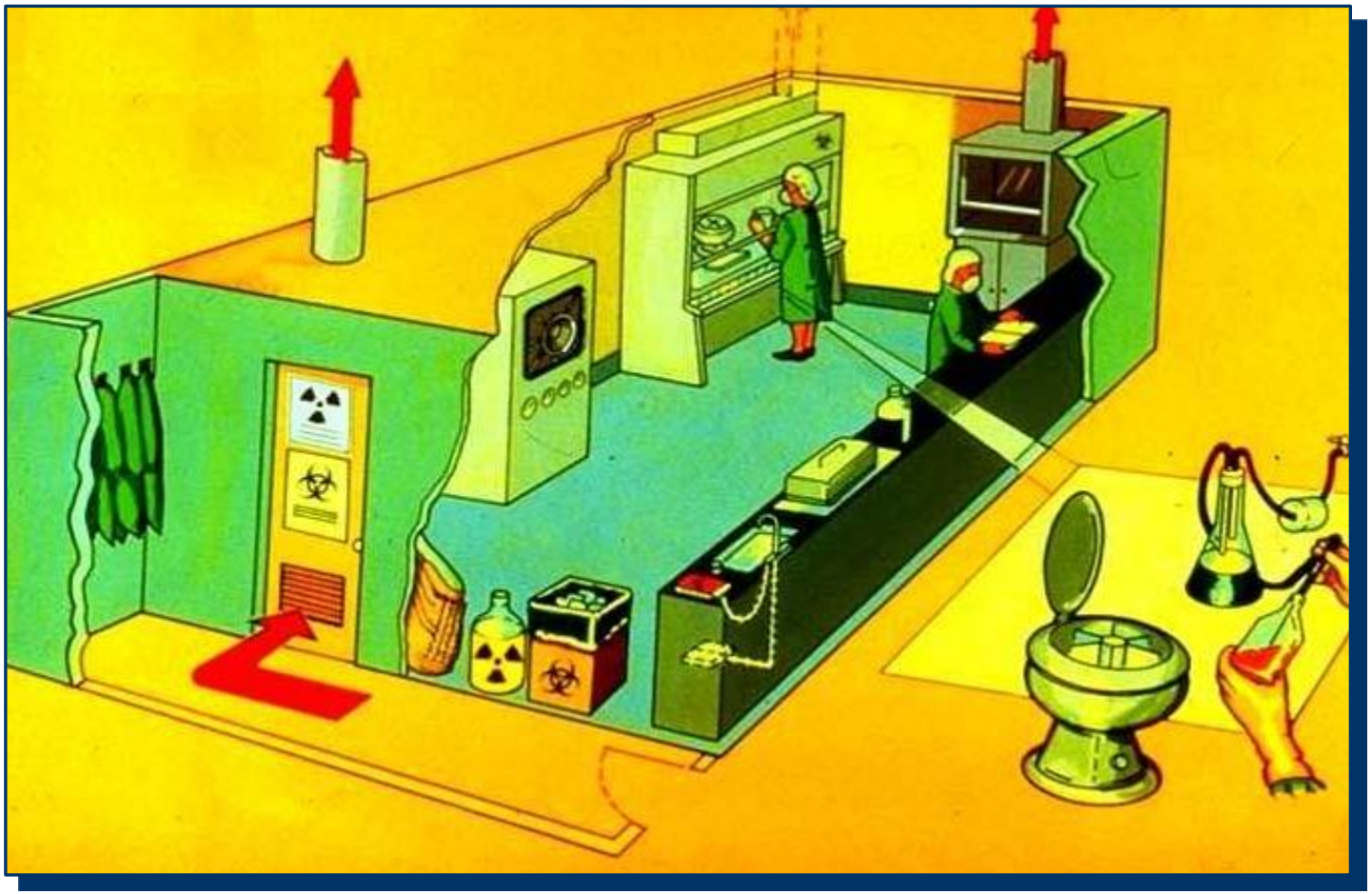
Biosafety Level 2

Laboratory Facilities (Secondary Barriers)



Biosafety Level 3

Laboratory Facilities (Secondary Barriers)



Administrative Controls and Personal Protective Equipment (PPE) for Biohazards

Administrative Controls for Biohazards

- Work procedures: aseptic techniques, use of BSC, Universal Precautions, etc
- Waste management procedures, include disinfection and sharps management
- Access control and warning signs
- Awareness and hands-on training
- Occupational health: vaccination, consultation, medical surveillance (serum banking, regular check)

Standard Microbiological Practices

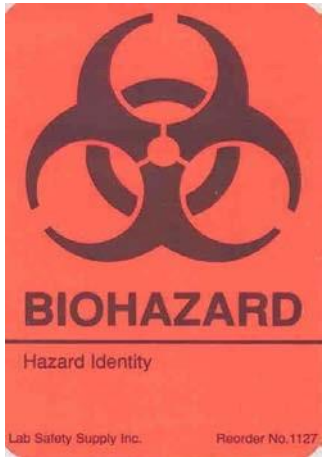
- No eating, drinking, smoking or application of cosmetics
- No mouth pipetting
- Minimize splashing or aerosol generation
- Wash hands after handling viable organisms and before leaving lab
- Decontaminate work surface daily
- Decontaminate wastes prior to disposal
- Insect and rodent control

Biological Safety Level 1 Requirements

- Most HKUST(GZ) biological research labs and all undergrad labs are BSL-1
- Allows public access
- No special facility requirements
- Lab coats recommended
- Allows open bench operations
- Requires good laboratory practice
- Requires Standard Microbiological Practices

Biological Safety Level 2 Requirements

- A few HKUST(GZ) biological research labs are BSL-2
- All level 1 requirements
- Limit access to lab personnel
- Post biohazard sign at entrance
- Surfaces can be easily cleaned
- Wear lab clothing in lab only
- Use gloves, when appropriate, to avoid skin contamination



Biological Safety Level 2 Requirements (Continued)

- Use hypodermic needle only if necessary
  - Use only needle-locking syringes
  - Use disposable syringes whenever possible
  - Exercise caution to avoid auto-innoculation
  - Avoid aerosol generation
  - Do not bend or clip needle
  - Do not recap needle into sheath
  - Place syringe/needle in puncture-resistant container for decontamination and disposal

Biological Safety Level 2 Requirements (Continued)

- Confine all aerosol generation procedures in biological safety cabinets:
  - Centrifuging
  - Grinding
  - Blending
  - Shaking
  - Mixing
  - Sonicating
  - Opening containers

Biological Safety Level 2 Requirements (Continued)

- Report all spills and accidents to supervisor and HSE
- Decontaminate biohazardous waste prior to disposal
- Require on-site autoclave for decontamination of infectious waste

Biological Safety Levels 3 and 4

- BSL-3 or 4 facilities are NOT available at HKUST(GZ)
- BSL-3 requires specially isolated labs that can be fumigated
- All BSL-4 work must be done in Class III BSCs (glove boxes); may require personal protective equipment, such as full body suit, supply-air respirator; and chemical disinfectant showering facility

For More Info on Biohazard Controls and Biosafety Levels...

- Biosafety in Microbiological and Biomedical Laboratories (BMBL) 5th Ed. US CDC & NIH, Feb 2007
- http://www.cdc.gov/od/ohs/biosfty/bmbl5/bmbl5toc.htm

Personal Protective Equipment for Biohazards

- Hand protection--gloves (selection, proper use)
- Eye/face protection--glasses, goggles or face shields
- Respiratory Protection
  - Masks
  - Respirators with HEPA filter cartridges
  - Supply air respirators
- Body protection--lab coats

Removing Gloves (1)

- With right hand, pinch palm of glove on left hand and pull left glove down and off fingers.

Removing Gloves (2)

- Form left glove into a ball and hold in fist of right hand.
- Insert one or two fingers of left ungloved hand under inside rim of right glove on palm side.

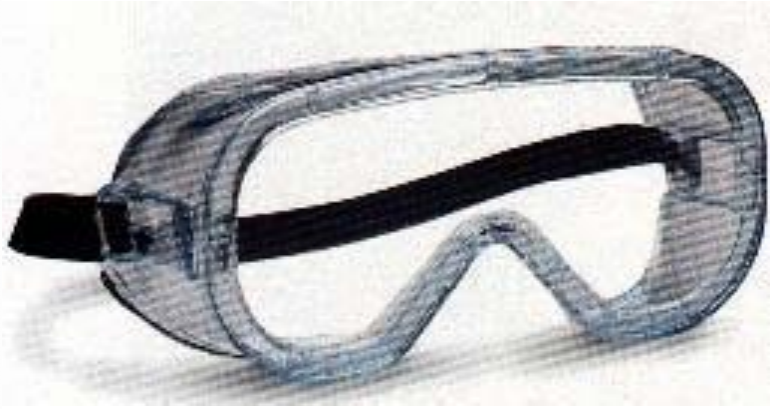
Removing Gloves (3)

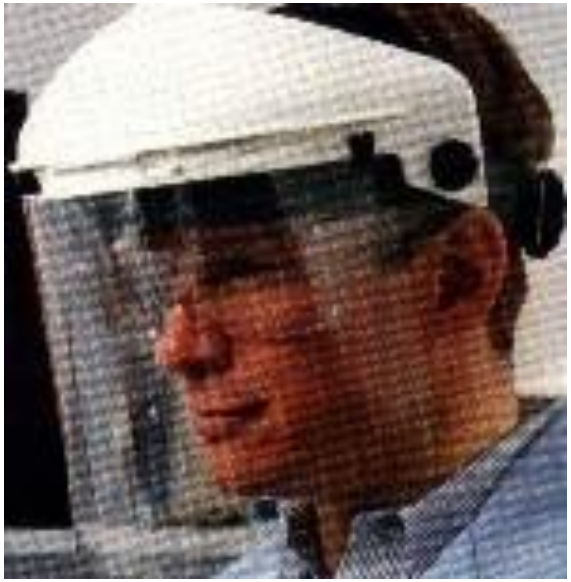
- Push glove inside out and down onto fingers and over balled left glove.
- Grasp gloves, which are now together and inside out, with left hand and remove from right hand.

Remember...

- Do not touch uncontaminated areas when wearing gloves.
- Remove gloves upon exit of hazardous work area.
- Avoid contact of skin with outside surface when removing gloves
- DO NOT SNAP!

Face and Eye Protection



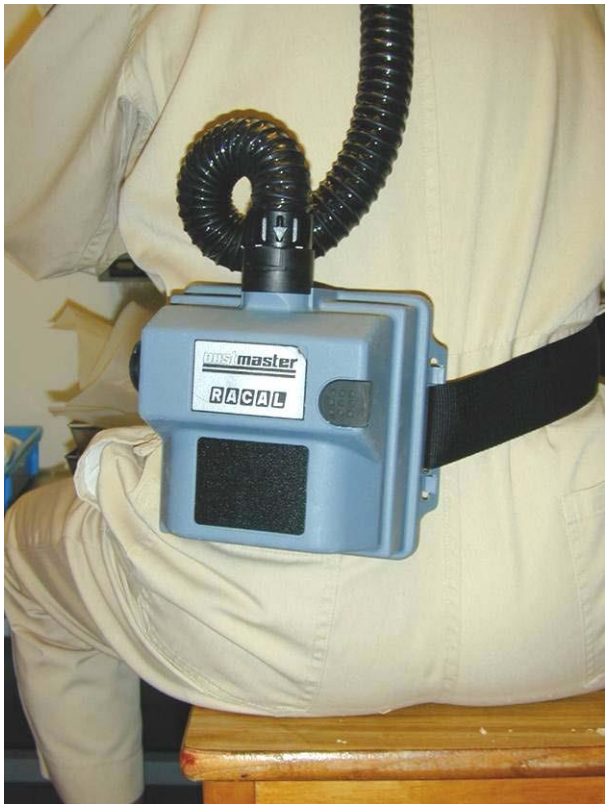


Different Negative Pressure Air-purifying Respirators

Full-face Air-purifying Respirator

Half-face Air-purifying Respirator

Powered Air-purifying Respirator



Protection Factors of Respirators

Type of Respirator	Assigned Protection Factors
Half facepiece, negative pressure air purifying	10
Full facepiece, negative pressure air purifying	50
Powered air purifying, hood or helmet & visor	25
Powered air purifying, half facepiece	50
Powered air purifying, full facepiece	100
SCBA, demand, full facepiece	1000+

Filtering Facepiece Respirator

Mask versus Respirator

Mask

- Comfortable
- No sealing
- Only good for large particles
- No physical requirements

Respirator

- Breathing resistance
- Sealing to face
- Can filter small particles, chemicals
- Requires lung function test, fit testing

Controls of Specific Biohazards

Controlling Infectious Agents in Clinical Specimens

- Main concern is Blood-Borne Pathogens in human bodily fluids and tissues
- Major route of exposures: percutaneous, e.g. needle sticks contamination of wounds and lacerations; and mucous membrane exposure
- Practice *Universal Precautions*

Universal Precautions for Clinical Specimens

- Consider all blood and body fluid infectious
- Always use PPE
- Wash hands thoroughly & frequently
- Take special care with needles & sharps
- Work in a BSC when needed
- Follow appropriate routine cleanup and spill handling procedures

Controlling Infectious Agents in Cell Cultures

- Cell lines should only be obtained from recognized cell banks or reliable sources
- New cell lines should be treated as BSL-2 until confirmed to be non-infectious
- Be careful with genetic material transfer
- Use replication-defective viral agents
- Measures to prevent contamination of cell cultures many times also protect workers
- The overall risk of cell culture work may be considered low

Controlling Infectious Agents in Experimental Materials

- Environmental samples may contain indigenous microbes, many of these should be non-infectious
- Some samples, e.g. sewage, may contain excreted materials and potentially infectious agents, however the actual risk varies and usually low
- The most important control is strict personal hygiene

Controlling Infectious Agents in Indoor Air

- Legionnaire's Disease and other biological indoor air quality problems are mostly due to poorly maintained buildings, especially ventilation systems
- Moisture and substrate always control microbial growth
- The control principles are to eliminate microbial reservoirs and to properly maintain building systems

Controlling Biohazards of Laboratory Animals

- Engineering controls:
  - facility design, e.g. isolation and ventilation
  - ventilated cages, isolators
  - biosafety cabinets
- Administrative controls:
  - entry restriction
  - medical surveillance
  - safe procedures of handling animals
  - hands-on training

Controlling Biohazards of Laboratory Animals (Continued)

- Personal protective equipment
  - clothing
  - eye/face protection
  - gloves
  - respirators
- Animal carcasses must be properly handled and disposed of
- Ethics in rearing, using, and sacrificing lab animals must also be considered

Controlling Biohazards of Biological Toxins

- Microorganisms producing potent toxins are usually in Class 3 or higher, and therefore should be handled under equivalent BSL
- Concentrated biological toxins produced naturally or by genetic engineering should be handled the same as toxic chemicals
- Remember common biological safety cabinets (Class 2 Type A) which recirculate air *cannot* handle toxic chemicals

Control of Biohazards of Recombinant DNA Molecules

- Genetically modified microorganisms may be considered *potential* pathogens
- The risk depends on nature of
  - the host (its Class, survivability outside the lab)
  - the vector (ability to transfer to other organisms and ability to replicate)
  - the genetic insert (possibility of gene expression and hazard posed by the gene product)

Control of Biohazards of rDNA Molecules (Continued)

- UK regulations assess three parameters
  - *Access*: probability a rDNA could enter the environment, including human body, and survive
  - *Damage*: biological activity of the DNA or the gene product, and the levels and nature of the product required to elicit this activity
  - *Expression*: level of expression of the insert
- Product of the three parameters determines the containment level (equivalent to BSL)

Control of Biohazards of rDNA Molecules (Continued)

- Examples
  - over-expression of harmless proteins using standard vectors in *E. coli* K12, LEVEL 1
  - over-expression of harmless proteins in eukaryotic cell lines (no infectious virus involved), LEVEL 1
  - over-expression of a biologically active protein such as interleukin-2 using a mobilization-defective vector in *E. coli* K12, LEVEL 2

Control of Biohazards of rDNA Molecules (Continued)

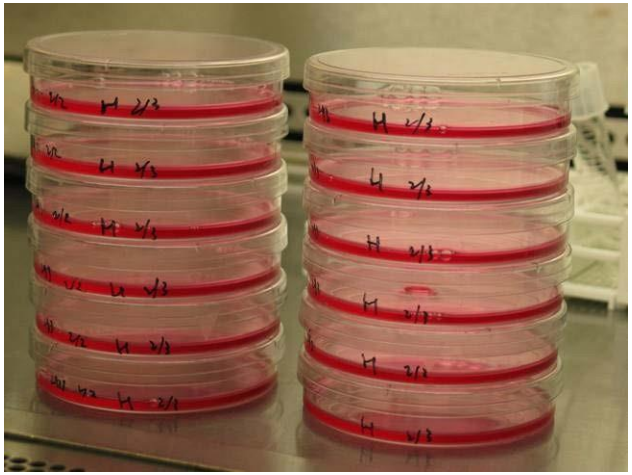
- Examples (continued)
  - a replication-defective retroviral particle carrying the interleukin-2 gene and capable of infecting human cells, LEVEL 2
  - a replication-defective retroviral particle carrying an oncogene and capable only of infecting murine cells, LEVEL 2
  - a replication-defective retroviral particle carrying an oncogene and capable of infecting human cells, LEVEL 3

Disinfection and Biological Wastes

Typical Solid Biological Wastes from Laboratory

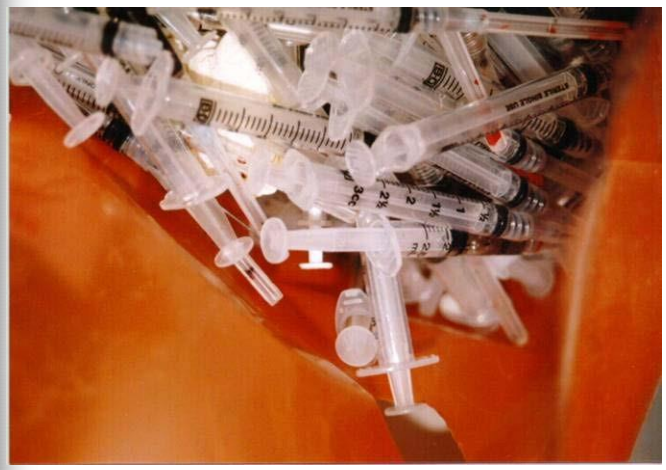
- Pipettes, pipette tips,
- Petri dishes, flasks, tubes,
- papers, plastic-backed diapers
- Gloves

Typical Liquid Biological Wastes from Laboratory



- Cell culture media/broth
- Human tissues, blood, blood elements, bodily fluids
- animal blood and related products

Typical Sharp Biological Wastes from Laboratory



- Hypodermic needles, syringes
- Blades (scalpels, razors)
- Glass slides, glass vials
- Pasteur pipettes

There Are Three Levels of Biological Decontamination

- Sanitizing - overall reduction of microbial population
  - Examples: general cleaning agents
- Disinfection - destruction of target organisms
  - Examples: liquid disinfectants, pasteurization
- Sterilization - complete destruction of all microbes
  - Examples: autoclaving, ionizing radiation

Decontamination of Biological Agents

- Physical agents:
  - Heat - dry and wet
  - Non-ionizing radiation - UV, microwave
  - Ionizing radiation - cobalt-60, X-ray
- Chemical agents:
  - Liquid - alcohols, halogens, phenolics, quaternary ammonium compounds
  - Gaseous - ethylene oxide, formaldehyde, hydrogen peroxide vapor

Autoclave

- An automatic pressure cooker
- 1 atmosphere above normal pressure
- 121^{o}C (boiling point of water at 2 atm)
- Maintain temperature and pressure for 15 minutes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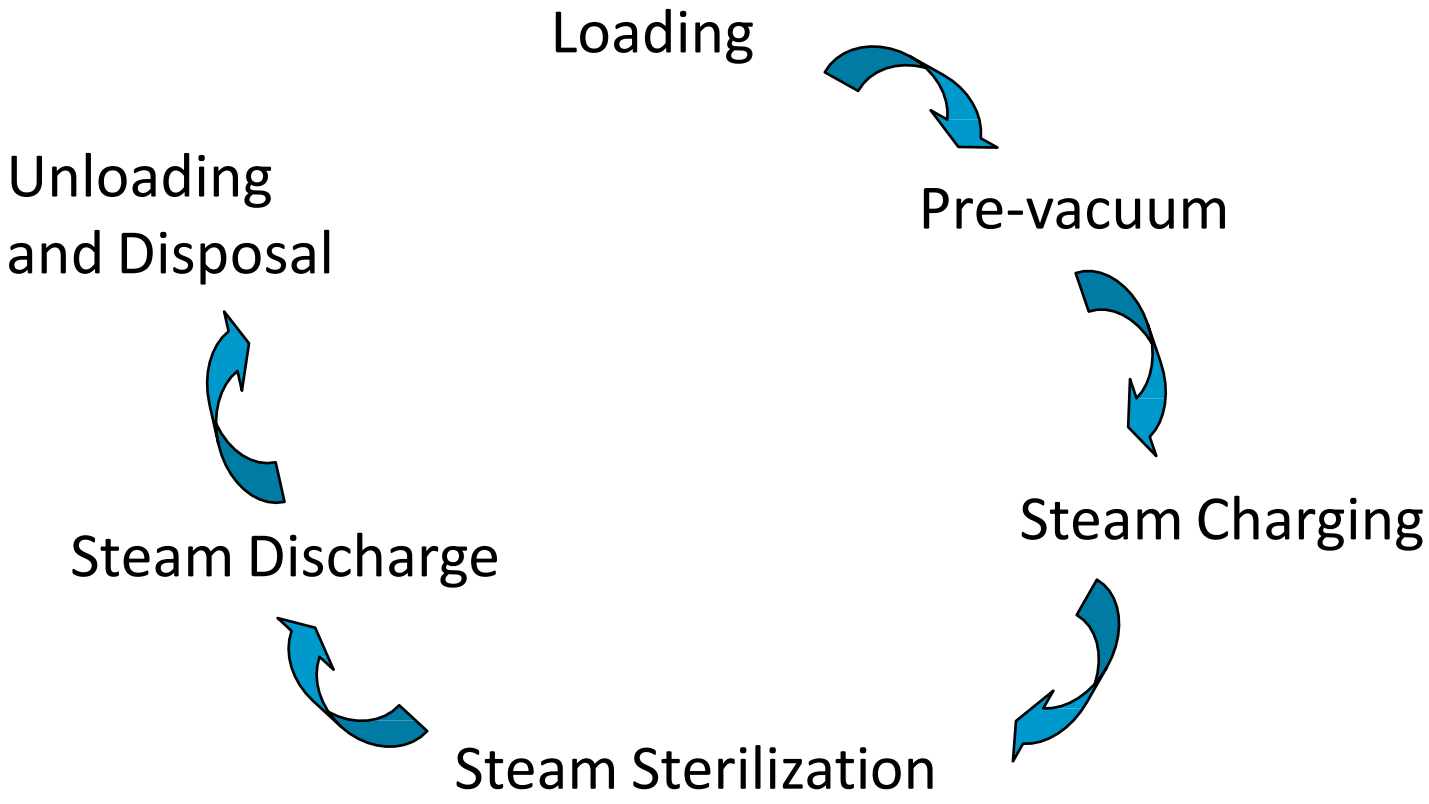
Market Forge Sterilmatic

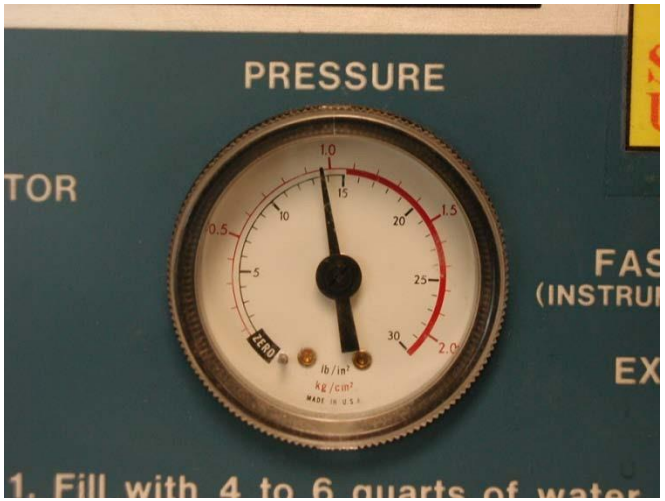

HIRAYAMA

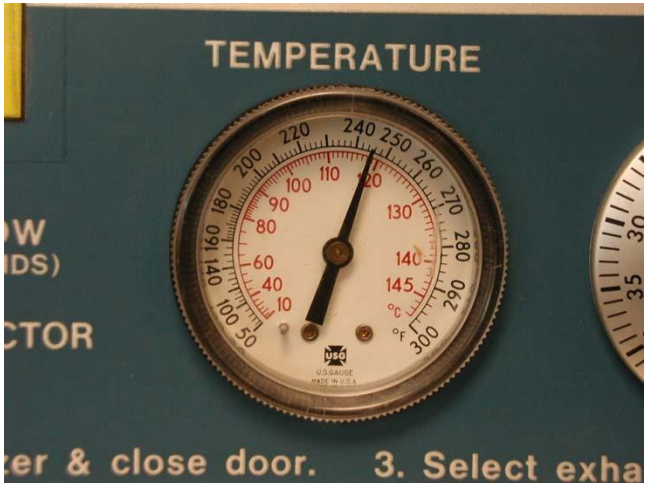
Autoclave Operation Cycle



Critical Parameters

- Pressure
- Temperature
- Exposure Time
- Access of steam





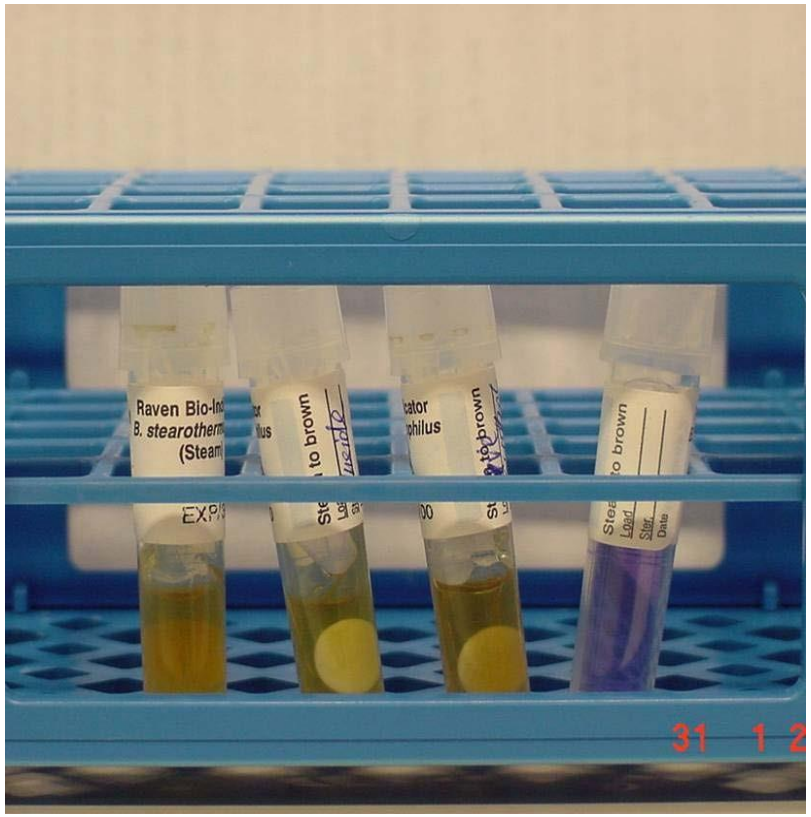
Precautions for Autoclave Operation

- Do not put in mix wastes (toxic chemicals, radioisotopes)
- Do not overload autoclave
- Allow easy penetration of steam
- Monitor pressure and temperature
- Allow pressure to drop before opening
- Be careful when handling hot items
- Conduct periodic tests to verify sterility

Sterilization Performance Check (1)

- Biological performance indicator
- *Bacillus stearothermophilus* spores
- Represent worst case: spores of thermophilic bacteria

Sterilization Performance Check (2)



- Autoclaved together with waste load
- Incubated at 55^{o}C-60^{o}C for 24 hours
- Color change from purple to yellow indicates bacterial growth

Physical Disinfectant—Ultraviolet (UV)

- Three ranges:
  - UVA: 315 - 400 nm
  - UV-B: 280 - 315 nm
  - UV-C: 100 - 280 nm
- Most effective for killing or inactivating microbes is UV-C, with 260 nm being the best
- Applying UVC for control of microbes aka Ultraviolet Germicidal Irradiation (UVGI)
- UVGI kills or inactivates microbes by damaging their DNA

Applications

- Healthcare
- Commercial buildings
- Residential
- Laboratories
- Pharmaceutical
- Food industry

UVGI lamp array installed inside the air handling units

Killing Performance

- Typical kill rates
- Using common microorganisms, e.g. Anthrax, Influenza, small-pox and TB
- From W.J. Kowalski, Proposed Standards Guidelines for UVGI Air Disinfection

UV Dose $\mu J/cm^2$	Anthrax Kill, %	Influenza Kill, %	Small-pox Kill, %	TB Kill %
1	0	0	0	0
10	0	1	2	2
20	0	2	3	4
30	0	3	4	6
50	1	6	7	10
75	1	9	11	15
100	2	11	14	19
150	2	16	20	27
250	4	26	32	41
500	8	45	53	66
1000	15	69	78	88
1500	22	83	90	96
2000	28	91	95	99
3000	39	97	99	100
4000	49	99	100	100
5000	57	100	100	100
6000	63	100	100	100
8000	74	100	100	100
10000	81	100	100	100
20000	96	100	100	100
k ($cm^2\ \mu J^{-1}$)	1.67×10^{-4}	1.187×10^{-3}	1.1528×10^{-3}	2.132×10^{-3}

Advantages of UV

- Proven technology for effective killing of microbes, in use for decades for air and surface sterilization in hospital & laboratories
- No airflow resistance (unlike HEPA filter)
- Can be a good substitute to chemical disinfectants, to avoid fumigation, soaking or wiping
- A physical agent, will not disperse like chemical disinfectants, therefore more controllable and pose less risk to human if properly isolated

Disadvantages of UV

- Variable effectiveness for different microbes, may need to couple with other disinfecting agent
- To be effectively killed by UVC, germs must be irradiated directly, i.e. shading effect
- Lamp soiling and aging: Dust can accumulate on UV lamp, usage also decreases lamp efficiency
- Adverse health effect: exposure to UV rays can burn skin and eyes
- Material deterioration, VOC off-gasing under prolonged UV irradiation

Liquid Chemical Disinfectants

- Chlorine compounds, including hypochlorites (bleach)
- Other halogen compounds (e.g. iodine, bromine compounds)
- Alcohols
- Phenols (e.g. Lysol, Dettol)
- Quaternary ammonia compounds

Gaseous Chemical Disinfectants

- Ethylene oxide
- Formaldehyde
- Ozone
- Peroxiacetic acid mist
- Hydrogen peroxide vapor

Chemical Disinfectants Must Be Used with Caution (1)

- Many disinfectants are highly toxic:
  - Phenol splashed on eyes and skin can cause blindness and systemic poisoning
  - Formaldehyde and ethylene oxide are carcinogens
- Compatibility issues:
  - Chlorine-based disinfectants can displace radioactive iodine

Chemical Disinfectants Must Be Used with Caution (2)

- Compatibility issues (continued):
  - Formaldehyde + hydrochloric acid = bis(chloro-methyl) ether
- Proper concentration and contact time

Disinfection by Gases

- Good penetration of hard-to-reach areas
- Must have sealable facility to prevent leakage and potential harmful effects to human
- Need neutralization or aeration to remove residual disinfectants afterwards

Disinfection with Formaldehyde

- Fumigation with formaldehyde (HCHO) was conventional practice for HEPA filter disinfection, including biological safety cabinets
- Formaldehyde was classified as human carcinogen since 2004
- Should be substituted with alternatives

One Promising Alternative: Hydrogen Peroxide Vapor

- H_2O_2 is a colorless, strong oxidizing agent
- Aqueous H_2O_2 has a long history of use as a sterilant
- Vapor-phase H_2O_2 (VHP) sterilization has been developed within the last two decades

Vaporized H_2O_2 Disinfection of BSC and HEPA Filters

- HKUST acquired a VHP unit in Nov 08 for disinfection of biological safety cabinet, in-line HEPA filter, and animal cage washer
- Disinfection efficiency was verified by *Geobacillus stearothermophilus* spore stripes
- Avoid use of formaldehyde, which is now a confirmed human carcinogen

Solid Chemical Disinfectants

- Stainless steel surface
- Silver particles
- Titanium dioxide coating

Biohazardous Waste Management

- Class IV agents - Add disinfectant and dispose as municipal waste
- Class III agents - Sterilize (autoclave) and dispose as municipal waste
- Contaminated sharps - store in non-penetrating containers, autoclave with the container, and dispose as municipal waste
- Clinical wastes must follow government regulations


VACUTAINER
BIOHAZARD
FILL TO THIS LEVEL
Fisherbrand
CAUTION: INFECTIOUS WASTE

Biohazardous Waste Management (Continued)

- Biological waste containing radioactive or toxic chemicals - *Do not autoclave!*
- If the toxic chemical can be deactivated, do so, then autoclave
- Waste containing radioactive or toxic chemical that cannot be deactivated: mix with disinfectant (*do not use chlorine*) and dispose as radioactive or chemical waste

Animal Carcasses

- Animal Carcasses are classified as clinical waste.
- Generators need to pack carcasses in double plastic bags
- Transfer carcasses to designated freezers at Animal & Plant Care Facility
- HSE retains a licensed contractor to dispose of carcasses

Response to Biological Spills

- First priority - protection of personnel
- Evaluate nature of spill to determine level of response
- Call for help when necessary
- Don appropriate protective equipment
- Use appropriate disinfectant to contain spill
- Proper documentation of incident

Spill of Biological Material in Laboratory

- Warn others in the lab to leave ASAP
- Leave contaminated clothing behind
- Get clean up material and don appropriate personal protective equipment
- Allow 30 min for aerosol to settle (do NOT activate Emergency Ventilation)
- Pour disinfectant around and into the spill
- Allow minimum of 20 minutes contact time
- Collect material on absorbant material and send to autoclave

Spill of Biological Material Inside a Biosafety Cabinet

- Keep cabinet running
- Don protective clothing (gloves and gown)
- Cleanup spill with suitable disinfectant
- Wipe all reachable cabinet surfaces with a germicidal detergent
- Flood catch basin with disinfectant, allow 20 min contact time
- Pack contaminated lab ware for sterilization
- Remove protective clothing for decontamination
- Consider fumigation of entire cabinet

HKUST Biological Safety Program

General Biological Safety Related Services

- Biological Safety Training, and periodic refresher training
- Biological Safety Cabinet annual testing and certification
- Trouble-shooting BSC (with FMO/LS)
- Regular comprehensive health and safety inspections, covering biological safety items
- Emergency response to biohazard accidents
- Consultation on biological safety issues

Biohazard Workers

- Biohazard Workers are those HKUST(GZ) lab workers who handle:
  - Class 3 (or above) infectious agents
  - lab animals
  - clinical specimens
  - biological toxins
  - cell and tissue cultures that may harbor infectious agents
  - recombinant DNA molecule that may pose health hazards

Biohazard Worker Requirements

- Pass Biological Safety Training
- Register as Biohazard Workers
- Subscribe to occupational health programs provided through HSE:
  - pre-placement, periodic and exit medical examination
  - serum banking
  - vaccinations
  - consultation with Occupational Physician

Biohazard Project Requirements

- All labs involved with BSL-2 work are identified by a unique hazard placard sign
- All spills and accidents in BSL-2 labs must be reported to HSE
- All BSL-2 work requires a Biohazard Control Plan
- Labeling of equipment designated for BSL-2 work; declaration and decontamination before servicing

Most Accidents Are Caused By

- Improper technique and procedure
- Failure to employ proper protective measures and equipment
- Equipment failure
- Lack of training
- Job done in a hurry

Human Factors Play An Important Role

- Proper attitude towards safety
- Safety techniques and equipment
- "Defensive" work habits help to avoid accidents
- Don't place excessive reliance on previous experience
- Develop an accident perception ability
- Avoid taking excessive risk

For More Information...

- HSE webpage at https://hse.hkust-gz.edu.cn
- HKUST(GZ) Safety Manual (Chapter 9, Biological Safety)
- HKUST(GZ) Emergency Procedures

Reference

- 《实验室 生物安全通用要求》（GB 19489-2008）
- 《生物安全实验室建筑技术规范》（GB 50346-2004）
- 《生物安全柜》（GB 41918-2022）
- 《病原微生物实验室生物安全管理条例》（2018年修订版）
- 《人间传染的病原微生物名录》（2006年版）
- 《动物病原微生物分类名录》（2005年版）

THE END

Thank you!