

*In the name of God*



# *Laboratory Biohazard*



***By:***  
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# Introduction

The purpose of this training session is to familiarize you with the fundamentals of biosafety as it relates to the Office, Vice President of Research and the Department of Environmental Health and Safety.

We will discuss:

- Institutional Biosafety Committee (IBC) Review
- The elements that are reviewed on the application form including but not limited to:
  - Classification of Agents
  - Risk Assessment/Management
  - Work Practices/ Engineering Controls/Personal Protective Equipment

# Biosafety in Academic Research

- Research Universities:

Promoting **safe laboratory** practices, and procedures; proper use of containment equipment and facilities; **provides advice on laboratory design** and risk assessment of experiments involving infectious agents, rDNA *in-vitro* and *in-vivo*.



Bottom Line: Risk & Containment



# Biohazard Symbol

- developed in 1966  
**Charles Baldwin** at  
National Cancer Institute at  
NIH.
- Symbol to be “memorable  
but meaningless” so it could  
be learned.
- Blaze orange – most visible  
under harsh conditions.



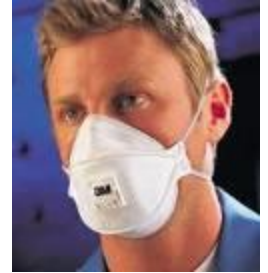
# Biosafety Issues

- Laboratory Safety
- Blood borne pathogens (BBP)
- Recombinant DNA (rDNA)
- Biological waste disposal
- Toxins
- Infectious substance and diagnostic specimen shipping



# Biosafety Issues (cont.)

- Respiratory Protection
- Bioterrorism and Select agents
- Mold and indoor air quality
- Occupational safety and health in the use of research animals
- Biohazards used in animal models



# Recombinant DNA Definition

- In the context of the *NIH Guidelines*, recombinant DNA molecules are defined as either:
  - molecules that are constructed outside living cells by joining natural or synthetic DNA segments to DNA molecules that can replicate in a **living cells**.





# Infectious Agent Definition

Infectious or pathogenic agents capable of causing disease in healthy humans, plants and animals including but not limited to:

- Bacteria
- Virus
- Fungi
- Parasites
- Rickettsia

# Risk Assessment

- Once the investigator has decided **on the agent or recombinant molecule**, then he or she must conduct an assessment of risk. This assessment shall be based on the following:
  - Virulence/pathogenicity/infectious dose
  - Environmental stability
  - Route of spread, communicability
  - Quantity/concentration/volume used
  - Vaccine/Treatment availability
  - Allergenicity

# Risk Assessment



*In-Vitro*



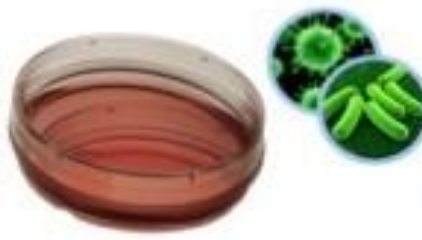
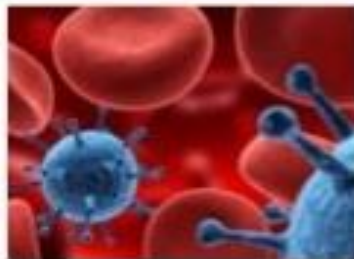
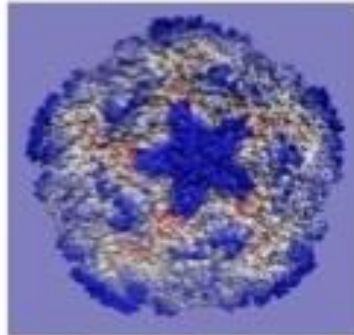
*In-Vivo*



*Human Clinical  
Trial*

# Biohazardous Materials

- Viruses
- Bacteria
- Fungi
- Chlamydiae/Rickettsiae
- Prions
- Toxins
- Recombinant DNA
- Human blood, unfixed tissue
- Human cell lines
- Animal models

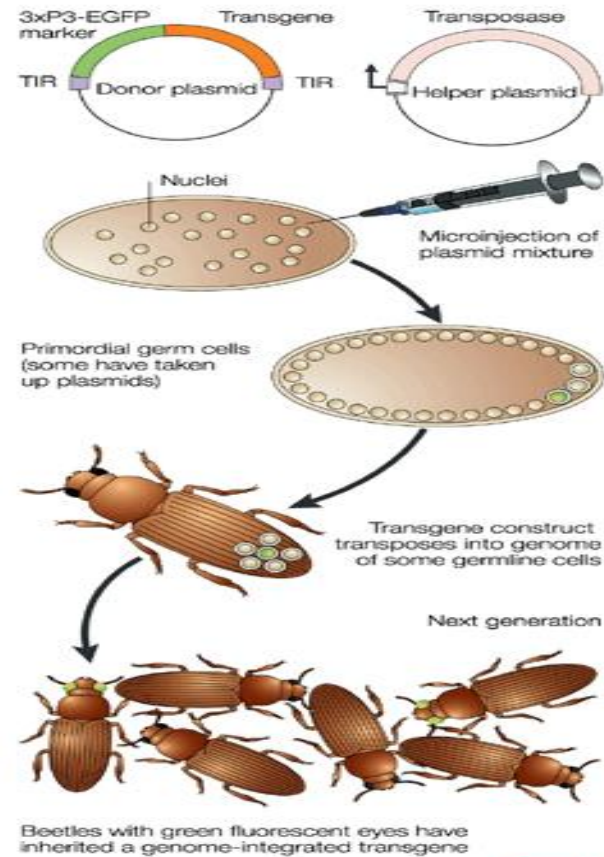


# Biohazardous materials

- Transgenic Plants, Animals and Insects



# Transgenic Insects



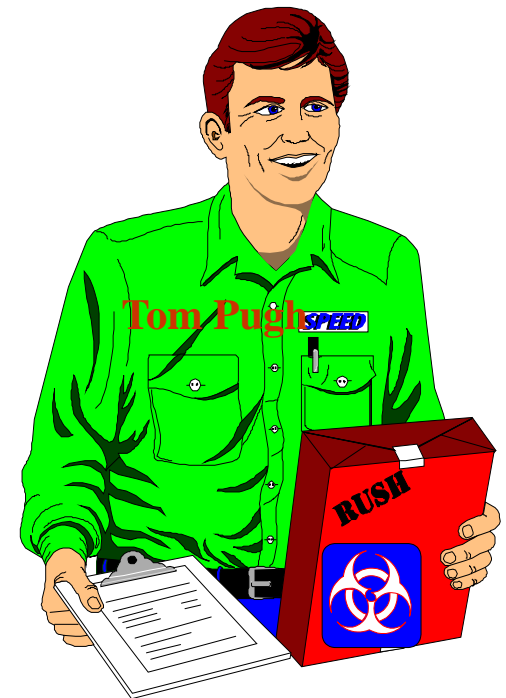


# Addressing Risk Assessments

- What is the organism?
- Is it Wild-type, attenuated, irradiated, or chemically treated?
- What is the max. concentration, volume, infectious dose?
- What is the work space like?
- Aerosolizing procedures? How do they contain their aerosols?

# Risk Assessment, cont.

- **Are personnel trained?** Do personnel understand the organism, infectious dose and symptoms?
- What are their **experimental procedures?**
- Will they be **transporting the material?** Shipping intra, inter-state or international?
- Are they doing **tissue or cell culture?**
- Do they have **adequate containment equipment?**



# Risk Assessment, Cont.

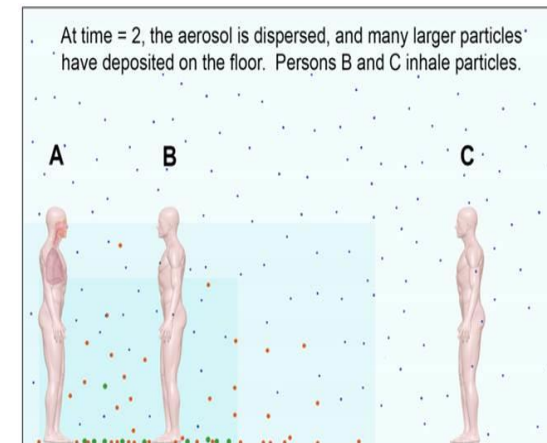
- Are they doing this work *in-vivo*?
- Have you **consulted and discussed this with the Vet** to determine special needs and housing?
- **Waste issues** addressed?
- **Pregnancy** issues with the organisms?



# Risk Assessment

## Routes of Exposure

- The assessment of risk will include common routes of exposure:
  - Parenteral Inoculation (needle stick)
  - Surface Contact (contaminated work area)
  - Ingestion (food in lab)
  - Inhalation (aerosol generating procedures)
  - Mucous Membrane (aerosol droplets in face)
- The IBC will review the agent usage and determine the most likely routes of exposure (e.g. **HIV, blood borne pathogen, percutaneous**)



# WHO-World Health Organization



## Agents Assigned Risk Groups

- **RG-1** Unlikely to cause disease in humans or animals
  - low individual or community risk
- **RG-2** May cause disease but typically not serious
  - individual risk, low community risk, treatable
- **RG-3** May cause serious disease, usually treatable
  - High individual but low community risk, serious respiratory agents
- **RG-4** Serious or fatal, often not treatable,
  - Easy transmission, high individual and community risk

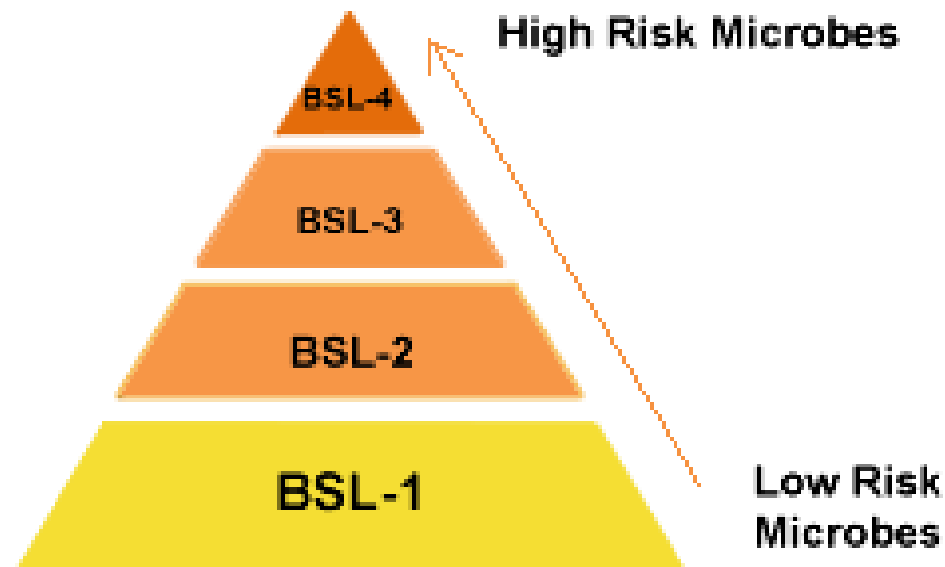
# Biosafety Levels (BSL)

- Different than the Risk Groups!!
  - Risk groups used in risk assessment
  - BSL are used in risk management
- BSL are ways to control the agent
  - facilities, safety equipment, practices, PPE, etc.
- Once risk is assessed then the appropriate BSL is determined



# BIOSAFETY LEVELS

- Basic- biosafety level 1
- Basic- biosafety level 2
- Containment- biosafety level 3
- Maximum containment- biosafety level 4

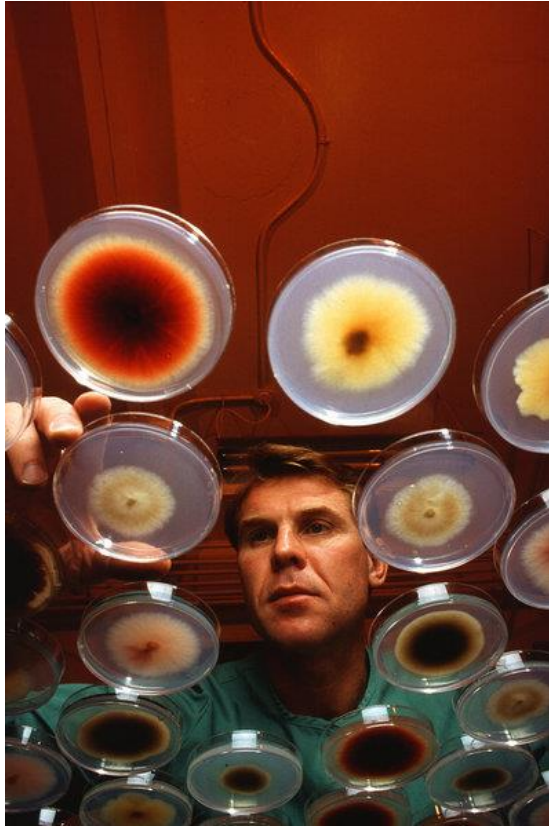


# Biosafety Level 1

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

- *Bacillus Subtilis*
- *E .coli* K-12 strains
- *S. cerevisiae*, polyomavirus
  - Basic laboratory
  - Standard Microbiological Practices

# BioSafety Level 1



- Well characterized, non-pathogenic organisms or agents
- Open bench- no containment
- Use good laboratory practices, waste disposal, and aseptic techniques

# Biosafety Level 1: Safety Equipment

- Laboratory coats
- Gloves
- Eyewear protection for splashes and spills
- Closed-toe shoes
- Special containment equipment or facility design is not required, but may be used as determined by a risk assessment.



# Biosafety Level 2

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

- Measles & herpesvirus virus
- HBV
- Salmonella
- human blood

microorganisms of moderate potential hazard, transmitted by contact, ingestion, puncture

# Laboratory Acquired Infections (LAI)

## **Bacterial:**

76% from clinical labs

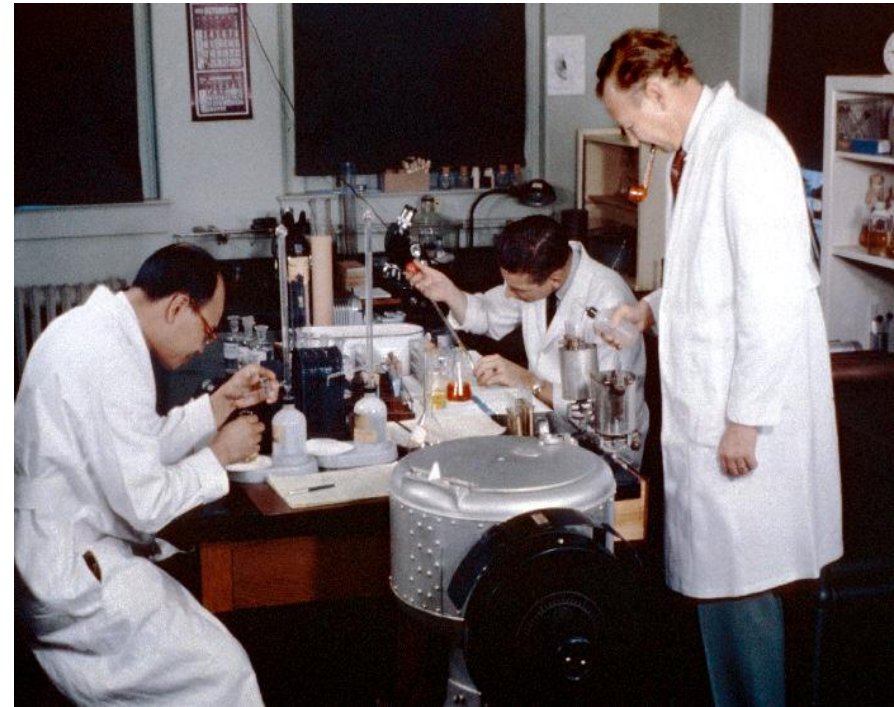
8% from research labs

## **Exposure:**

60% acquired from inhalation

## **Other exposures include:**

digestion, sharps, splashes, direct and indirect contact

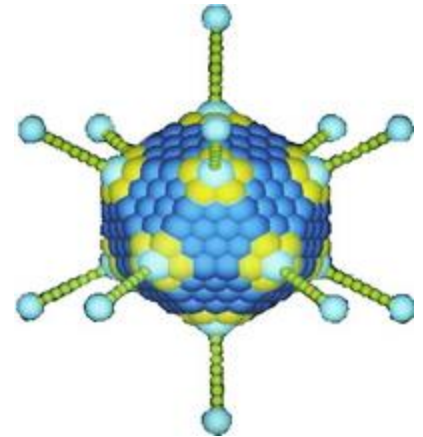




# Laboratory Acquired Infections (LAI)

## Viral

- 16% from clinical labs
- 70% from research labs
  - 32% from animal related activities



# Disinfection

- **10% bleach solution**

- good for general disinfection
- High organics use 20%
- Needs to be made weekly
- Test contact time

- **Ethanol**

- Use 70% solution (most effective)
- Longer contact time and flammable

\* Should research and know effectiveness and contact time for the best disinfectant against your agent!



# Biosafety levels 3

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

- TB
- HIV
- Yellow fever virus

**Containment lab:** double door entry; directional airflow;  
all work in biosafety cabinet

# BSL-3 Gowning Area



# BSL-3 Practices



# Biosafety Level 4

Suitable for work with **dangerous agents** that pose a high individual risk of aerosol transmitted laboratory infectious and life threatening disease.

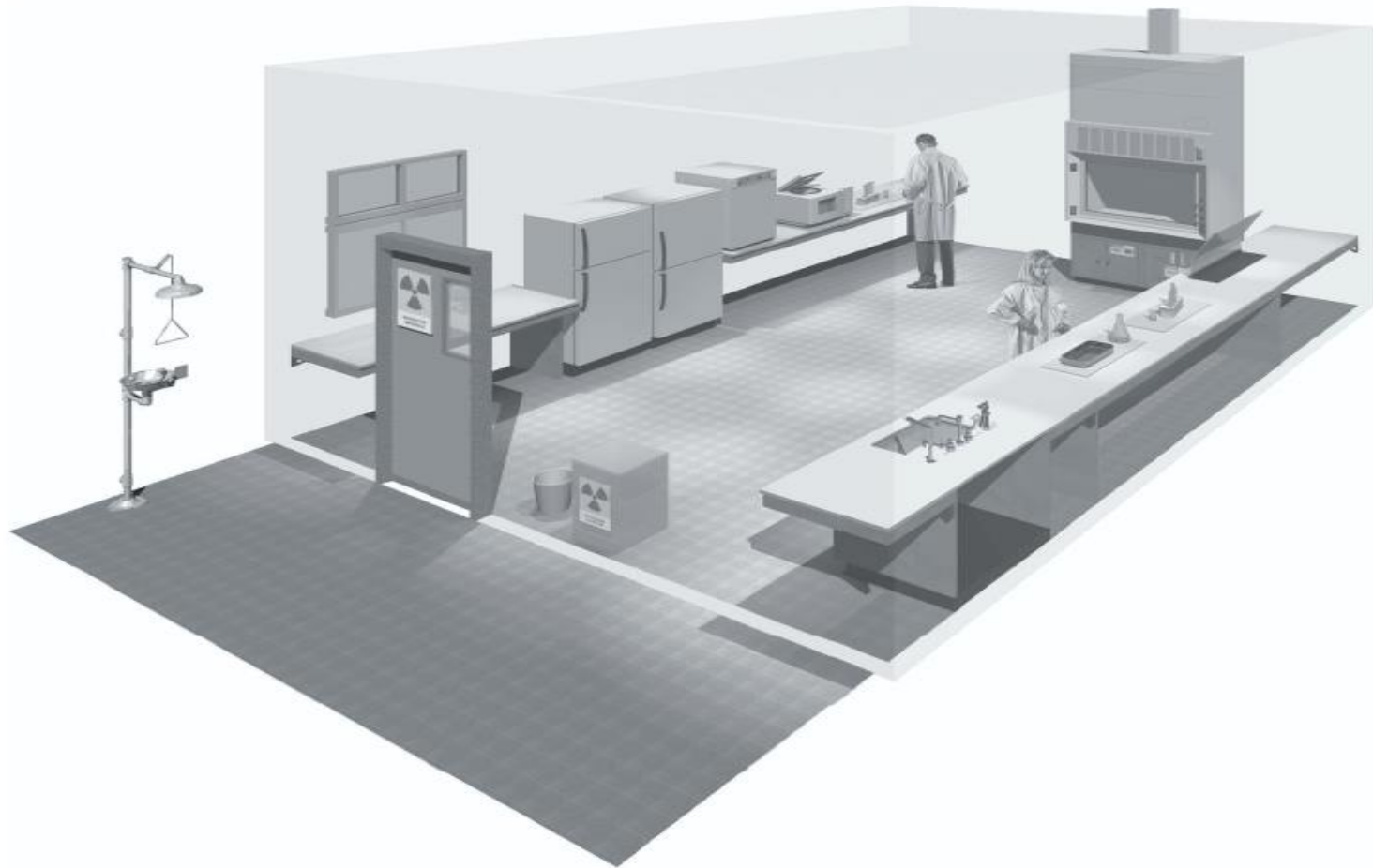
- Ebola Zaire virus
- Rift valley fever virus
- Marburg virus
- Microorganisms that cause lethal disease, with no known treatment or vaccine...

Maximum containment lab; positive pressure ventilated suits (moon suits)

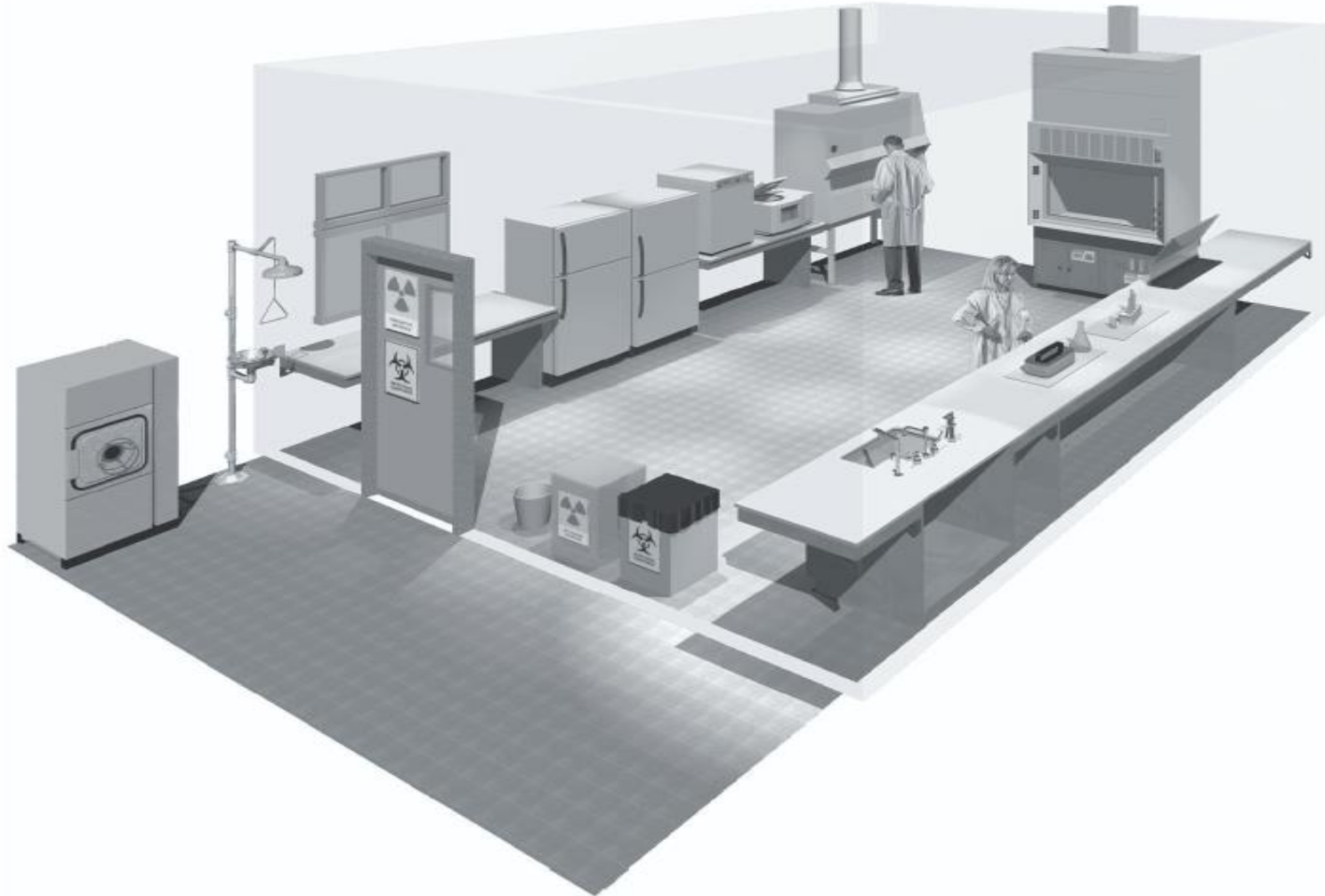




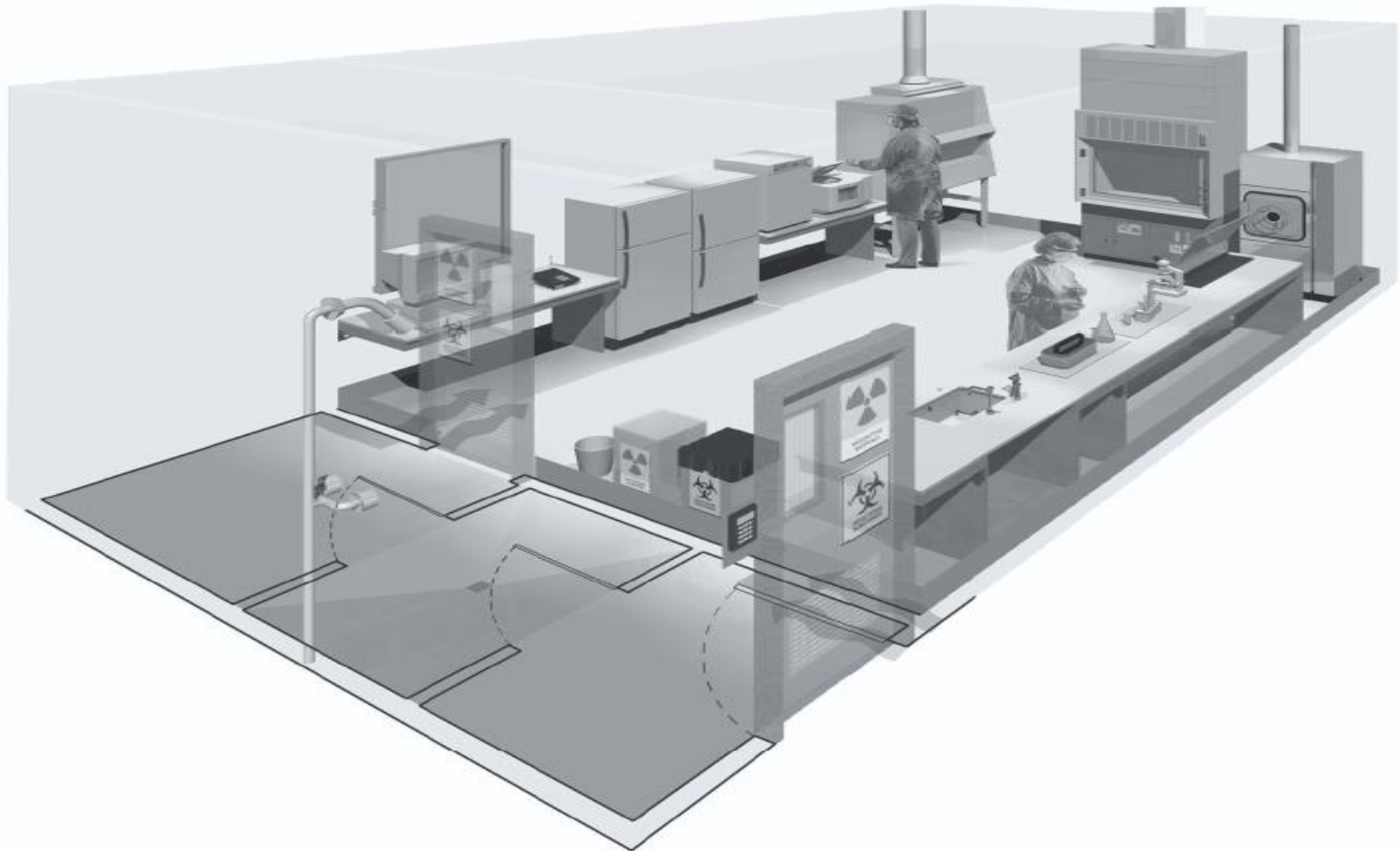
# A typical Biosafety Level 1 laboratory



# A typical Biosafety Level 2 laboratory



# A typical Biosafety Level 3 laboratory



## Biosafety Level 4



# Containment

- What is containment?

The application of combinations of laboratory practice and procedure, laboratory facilities, and safety equipment when working with potentially **infectious microorganisms or toxins**.

# Minimization of Risk

- Once exposure determination has been completed, then ways to minimize risk are evaluated. This includes but is not limited to the following:
  - Work Practices/Engineering Controls
  - Personal Protective Equipment
  - Disinfection/Medical Waste Disposal
  - Medical Surveillance
  - Training (at the laboratory level)
- The investigator must review and determine appropriate measures-it is the reason why the application must be completed in detail.



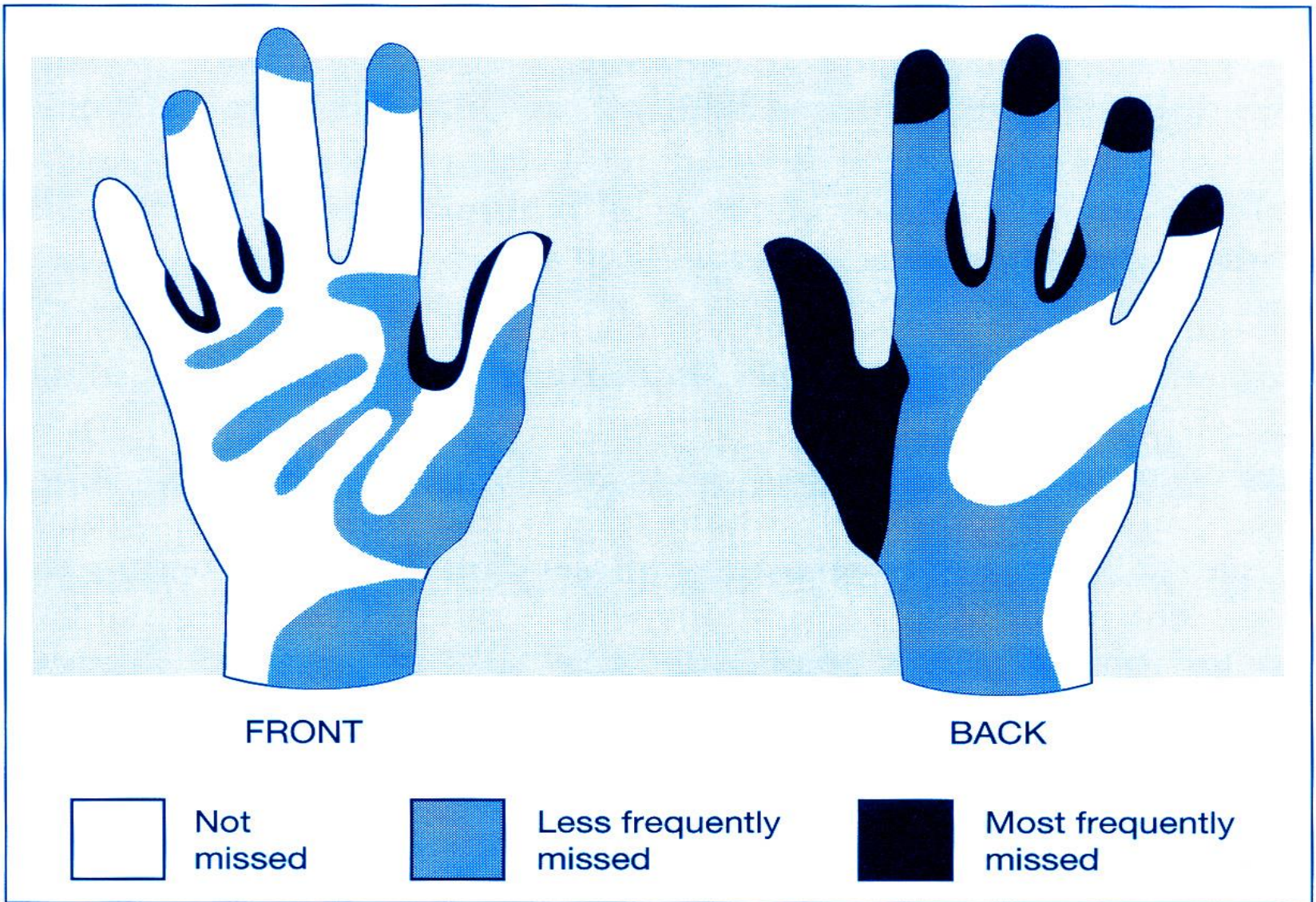
# Minimize Sharps Usage

- Needles **must not** be recapped, bent, sheared or removed from a disposable syringe.
- All used sharps must be placed in a rigid, hard-plastic, puncture-resistant container for disposal.
- Substitute plastic for glass whenever possible.



# Handwashing

- Hands should be washed:
  - immediately and thoroughly with a disinfectant hand soap, if contaminated with biological agents.
  - after gloves are compromised or removed
  - with water after a puncture wound
- If a sink is not available (e.g. equipment room), consider the use of disinfectant towel in these areas.



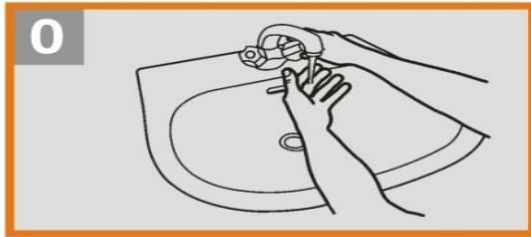
**Figure 12.2** Parts of the hands most frequently missed during hand washing.

Reproduced with permission from Taylor LJ. An evaluation of handwashing techniques. *Nursing Times* 1978; 74: 54-55.





## Duration of the entire procedure: 40-60 seconds



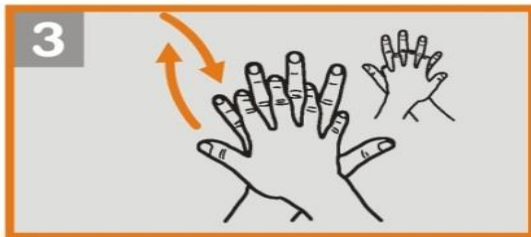
ابتدا دست ها با آب خیس شود



مابع صابون به اندازه کافی روی دست ها ریخته شود



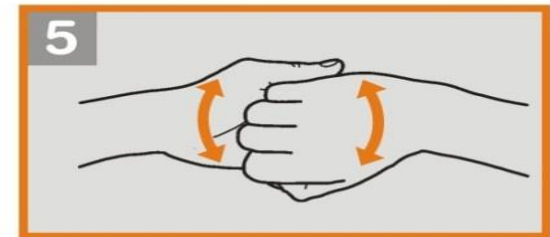
کف دست ها را به هم بمالید



کف دست راست را روی پشت دست چپ گذاشته و بین انگشت ها را اسکراب کنید و بر عکس



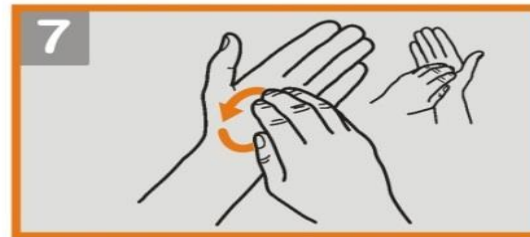
کف دست ها روی هم قرار گرفته و مابین انگشتان را مالش دهید



انگشتها را در هم تابیده به حالت قفل شده و پشت انگشت ها به کف دست مقابل مالش داده شود



انگشت شصت دست چپ را با کف دست راست احاطه کرده به صورت دورانی مالش دهید و بر عکس



انگشتان را جمع کرده و به صورت چرخشی جلو و عقب در کف دست مقابل حرکت دهید و برعکس



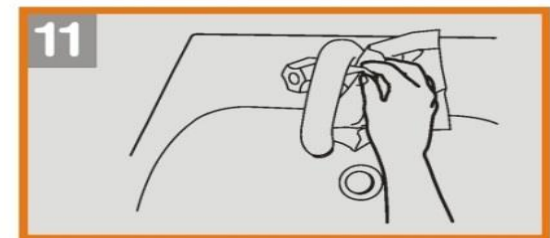
تمام سطح مچ دست چپ را با کف دست راست مالش دهید و بر عکس



دست ها را با آب شستشو دهید



دست ها را با حوله یکبار مصرف خشک کنید



برای بستن شیر آب از همان حوله استفاده کنید . اینک دستان شما کاملا تمیز است.

# Minimize Aerosol Generation

- **To avoid inhalation exposure** to agents, workers must minimize the potential generation of aerosols. These procedures include but are not limited to:
  - sonicating
  - centrifuging
  - grinding
  - blending, mixing
  - Vortexing
- Work on the open bench top should be limited whenever conducting these procedures.



# Use Biosafety Cabinets

- Biological safety cabinets are:
  - used for product/personnel protection
  - used for aerosol generating procedures
  - disinfected after use
  - certified annually
  - not recommended for chemical or radioisotope usage.



# Use Mechanical Pipettes

- Mechanical pipetting devices should be used for manipulating all liquids in the laboratory.
- Never mouth pipet.





# Decontaminate Work Surfaces

- Laboratory work surfaces should be decontaminated with an appropriate chemical disinfectant after a spill of biohazardous materials and when work activities are completed.

# Disinfectant Selection

- Disinfectants must be selected on a case by case basis to ensure efficiency.
  - Quaternary Ammonia Compounds
  - **Chloride Compounds (Bleach)**
  - Iodophores (Wescodyne)
  - Phenolics (Amphyl))
  - **Alcohols (70% Ethanol)**
  - Formaldehyde/Glutaraldehyde
  - **Deconex**



# No Eating, Drinking, or Smoking

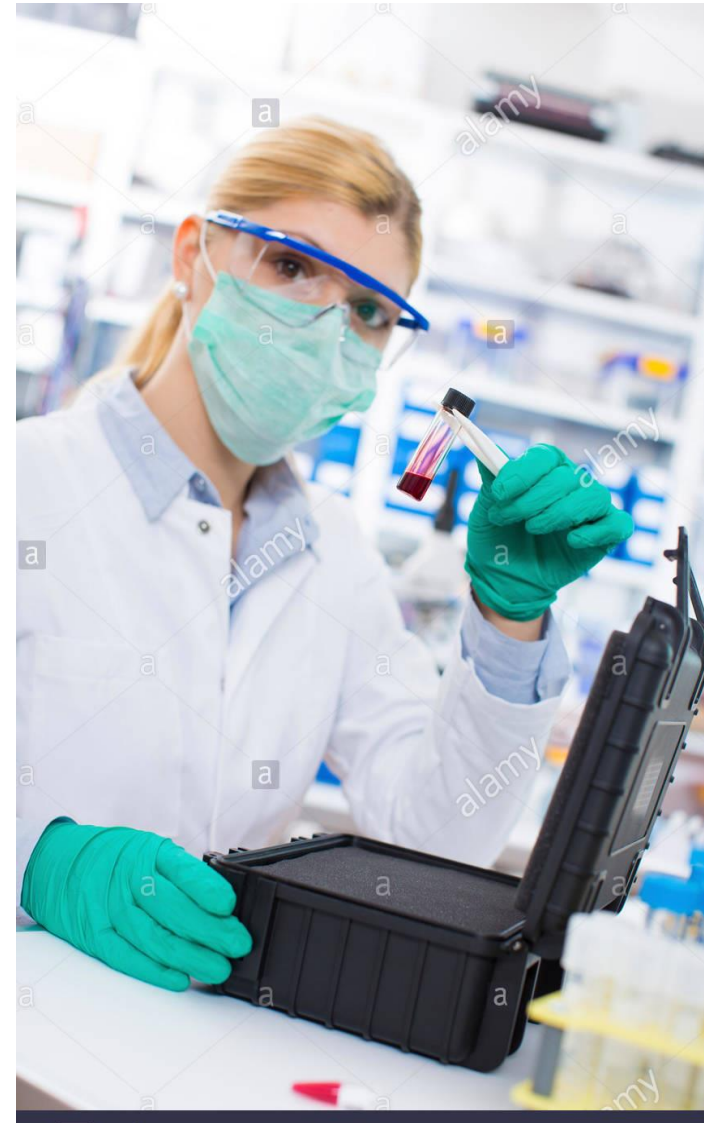
Hand to mouth transmission of disease is a common route of exposure while handling biological agents.

- Avoid eating, drinking, or smoking in laboratory work areas.
- Wash hands with disinfectant soap prior to leaving the work area.
- Do not store or heat/chill food or beverages in the lab.



# Personal Protective Equipment

- Use personal protective equipment to prevent skin/mucous membrane exposure during agent use, such as:
  - Gloves
  - Safety glasses/face shield
  - Lab coat
  - Closed toe shoes/foot covers
  - Respiratory protection (BL-3 agents)



# Hazard Communication



- **Biohazard labels shall be placed on:**

- The surface of all equipment (freezers, incubators, refrigerators) which may be contaminated with biohazardous materials.
- sample transport outer containers.
- medical waste bins

- **Biohazard signs shall be placed on:**

- The **outer door of BL 2 labs**
- **Medical waste** storage areas



# Medical Surveillance Requirements- Vaccinations

- Vaccinations are available for some organisms and should be offered when feasible.
- However, some members of the population may be at risk for medical complications from the vaccination (e.g., excema-vaccinia vaccinations).

# Medical Surveillance

## Some Available Vaccinations

### Organism

**Bordetella pertussis**  
**Clostridium tetani**  
**Corynebacteria diphtheriae**  
**HBV**  
**Influenza**  
**Mycobacterium tuberculosis**  
**(Bacille Calmette Guerin or BCG)**  
**Neisseria meningitidis**  
**Polio Virus**  
**Rubivirus**  
**Salmonella typhi**  
**Varicella Zoster**  
**Variola major**

### Disease

**Whooping Cough**  
**tetanus**  
**diphtheria**  
**hepatitis**  
**Flu**  
  
**TB**  
**meningococcal disease**  
**Poliomyelitis**  
**Rubella**  
**Typhoid Fever**  
**Chicken Pox**  
**Smallpox**





# Accidental Spills

- Evacuate area, alert personnel and cordon off so that aerosols may settle
- Don PPE; Cover with paper towels and apply bleach (1 part bleach : 9 parts water)
- Allow 15 – 20 min contact time
- Wipe up working towards center
- Use tongs if broken glass is involved
- Is Recombinant DNA involved?



## Eliminate accidental routes of entry

Workers not wearing closed-toed shoes at BSL-2 is evidence of a lack of training in standard microbiological practices.



No sandals or open-toed shoes in the BSL-2 (or any) laboratory.



Appropriate footwear

# First Aid Measures



- Splash to Eye or Needle stick Injury
  - Rinse thoroughly for 15 minutes at the eyewash or sink...

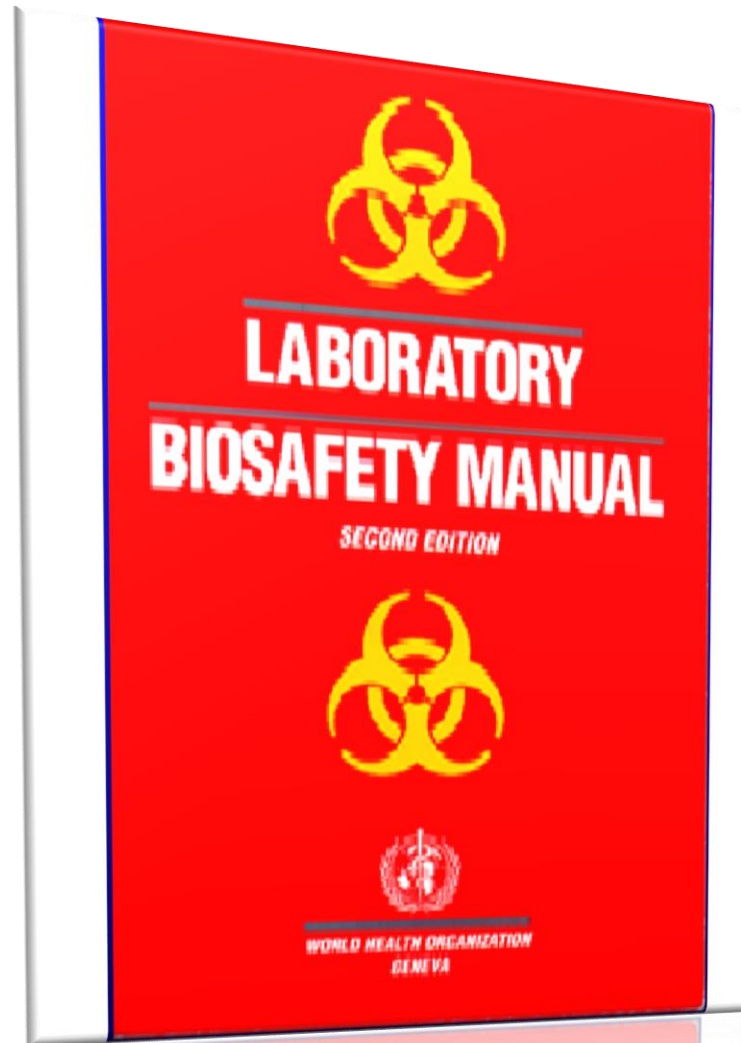
# Managing All That Other Waste...

- Do NOT discard medications in the trash.
- Return to source for disposal or seek assistance from your campus waste group.





# REFERENCE



საბიოლოგიური  
საფრთხოების მართვა



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FOR YOUR  
TIME

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MEET TED ECHOLS  
AM DEPART  
LAX 11  
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5,