Components of Laboratory Biosafety

www.biosecurity.sandia.gov

2006-3687C & 2006-3684C
Sandia is a multiprogram laboratory operated by Sandia Corporation, a Lockheed Martin Company,for the United States Department of Energy's National Nuclear Security Administration under contract DE-AC04-94AL85000

Biocontainment

- **Biocontainment is the control of biohazards through**

  Practices & procedures, including administrative controls
  - **Good lab practices**
  - **Written SOPs for research activities, specialized equipment, etc**
  - **Required training**
  - **Access requirements**

  Primary barriers (safety equipment)
  - **Biosafety cabinets (BSCs)**
  - **Lab equipment (pipetting devices, waste containers, safety centrifuge cups)**
  - **Personal protective equipment**

  Secondary barriers (engineering & architectural controls)
  - **Building & room construction – the floor plan**
  - **HVAC issues – directional airflow, filtration**
  - **Waste treatment**

Biosafety Levels

- **Biosafety Levels**

  Four biosafety levels provide increasing degrees of protection

  What's the right balance of practices & procedures, primary barriers and secondary barriers?

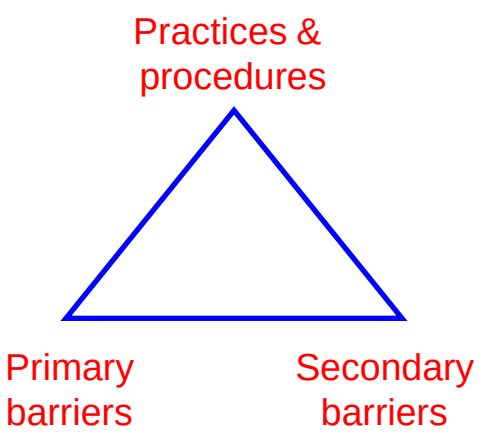
- **Applications:**

  Labs (BSL-1, 2, 3, 4)

  (Small) Animal Containment (ABSL-1, 2, 3, 4)

  Large Animal Containment ("BSL-3 AG")

  Plant Containment (BSL-1P, 2P, 3P, 4P)



Guidance and Regulations

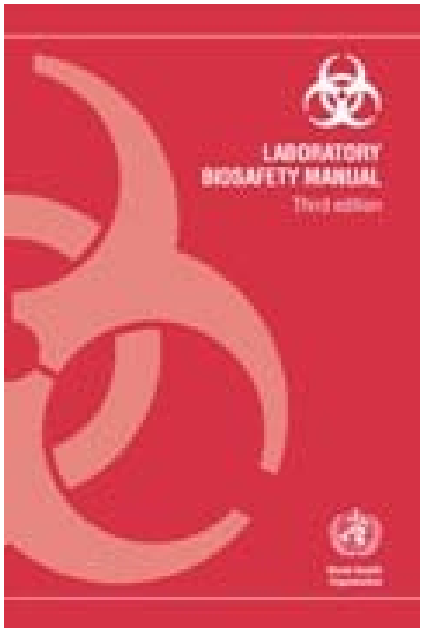
- **Performance-based vs. prescriptive**

  A performance approach generally defines what result is intended, leaving how to achieve the result up to the user.

  Prescriptive approaches outline specific requirements that must be done.

- **WHO Laboratory Biosafety Manual – 3rd edition**

See CD for WHO LBM

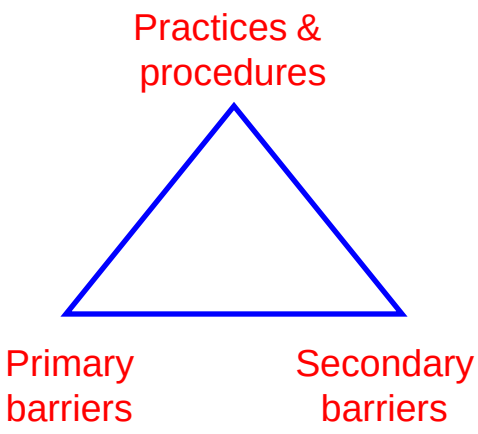


Planning: Risk Assessment is Critical

- **Identify biological agents that will be used**
- **Perform risk assessment**

  Include an evaluation of settings
  - **Labs**
  - **Animal housing**
  - **Patient care**
  - **Autopsy/Necropsy**
  - **Other?**

  And activities:
  - **At the macro scale: research, diagnostics, health care, industry, other?**
  - **At the micro scale: routine manipulations, producing large volumes, aerosolization, animal testing, other?**



- **Determine biosafety level**

  What degree of biocontainment is necessary to mitigate the risk?
  - **What is the appropriate balance of practices & procedures, primary barriers, and secondary barriers?**

Components of Laboratory Biosafety

Biosafety: Engineering Controls

- **Primary barriers – contain the agent at the source**

  Biological safety cabinet

  Animal caging

  Specialized lab equipment (centrifuges, fermenters, etc.)

- **Secondary barriers – contain the agent within the room or facility *in case an agent escapes from the primary barriers***

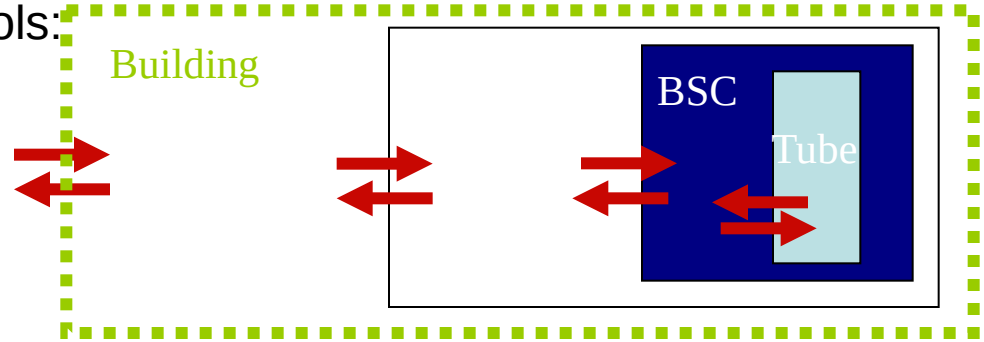
  Building & Room Construction

  HVAC Issues:

  - **Directional airflow**
  - **Exhaust filtration**

  Other Engineering Controls:

  - **Solid waste treatment**
  - **Wastewater treatment**



Biosafety Levels 1 and 2

- **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.

  Examples:

  - ***Bacillus subtilis***
  - ***Naegleria gruberi***
  - **Infectious canine hepatitis virus**
  - ***E. coli***

- **Biosafety Level 2**

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

  Examples:

  - **Measles virus**
  - **Salmonellae**
  - **Toxoplasma species**
  - **Hepatitis B virus**

Biosafety Levels 3 and 4

- **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.

  Examples:

  - ***Mycobacterium tuberculosis***
  - **St. Louis encephalitis virus**
  - ***Coxiella burnetii***

- **Biosafety Level 4**

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

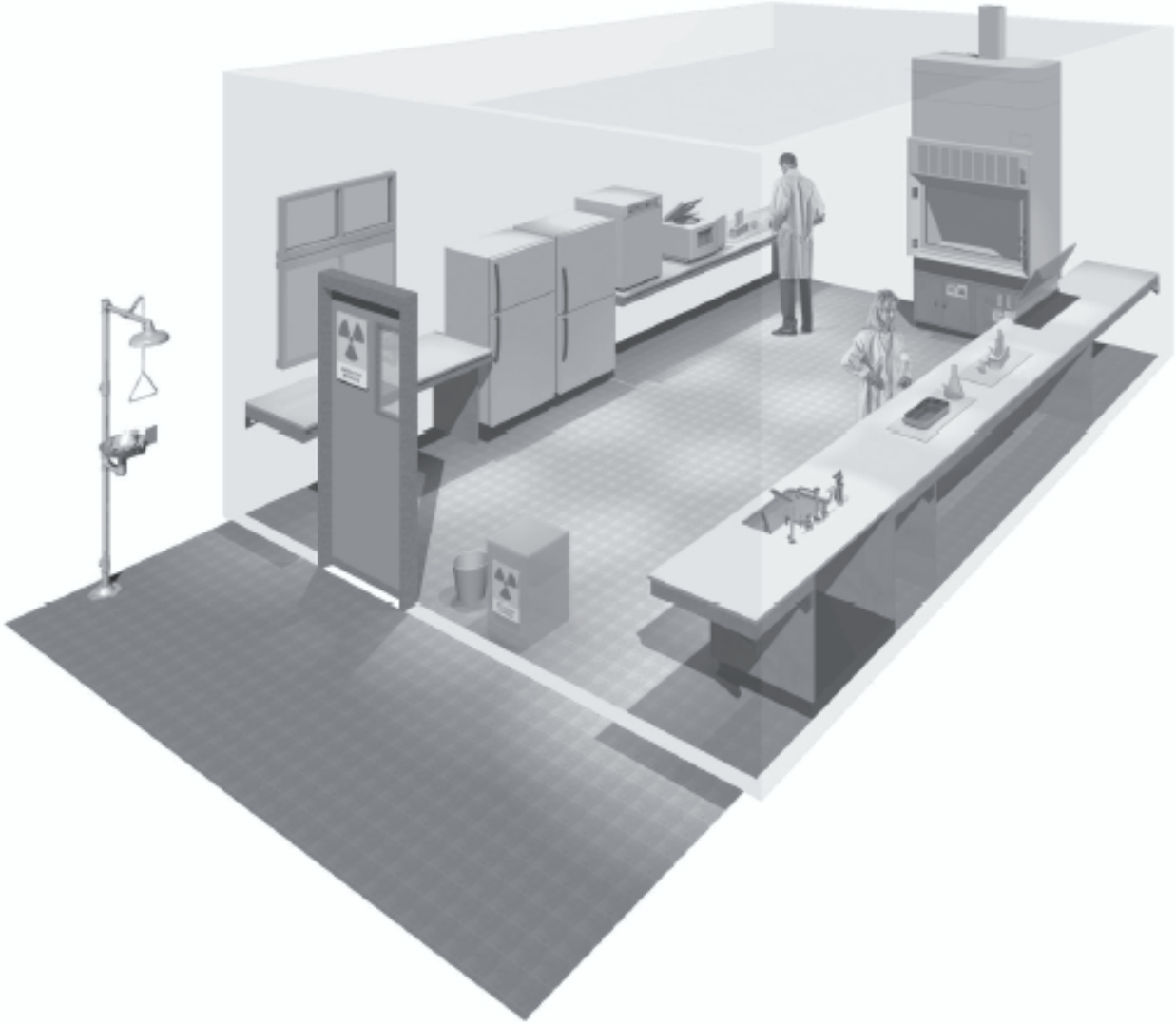
  Examples:

  - **Ebola Zaire virus**
  - **Rift Valley Fever virus**

Biosafety Level 1: Facility Design

- **Requirements:**
  - Laboratories have doors
  - Sink for hand washing
  - Work surfaces easily cleaned
  - Bench tops are impervious to water
  - Sturdy furniture
  - Windows fitted with flyscreens
- **Other design and construction issues:**
  - Location – not separated
  - Structure – normal construction
  - Ventilation – no special requirements

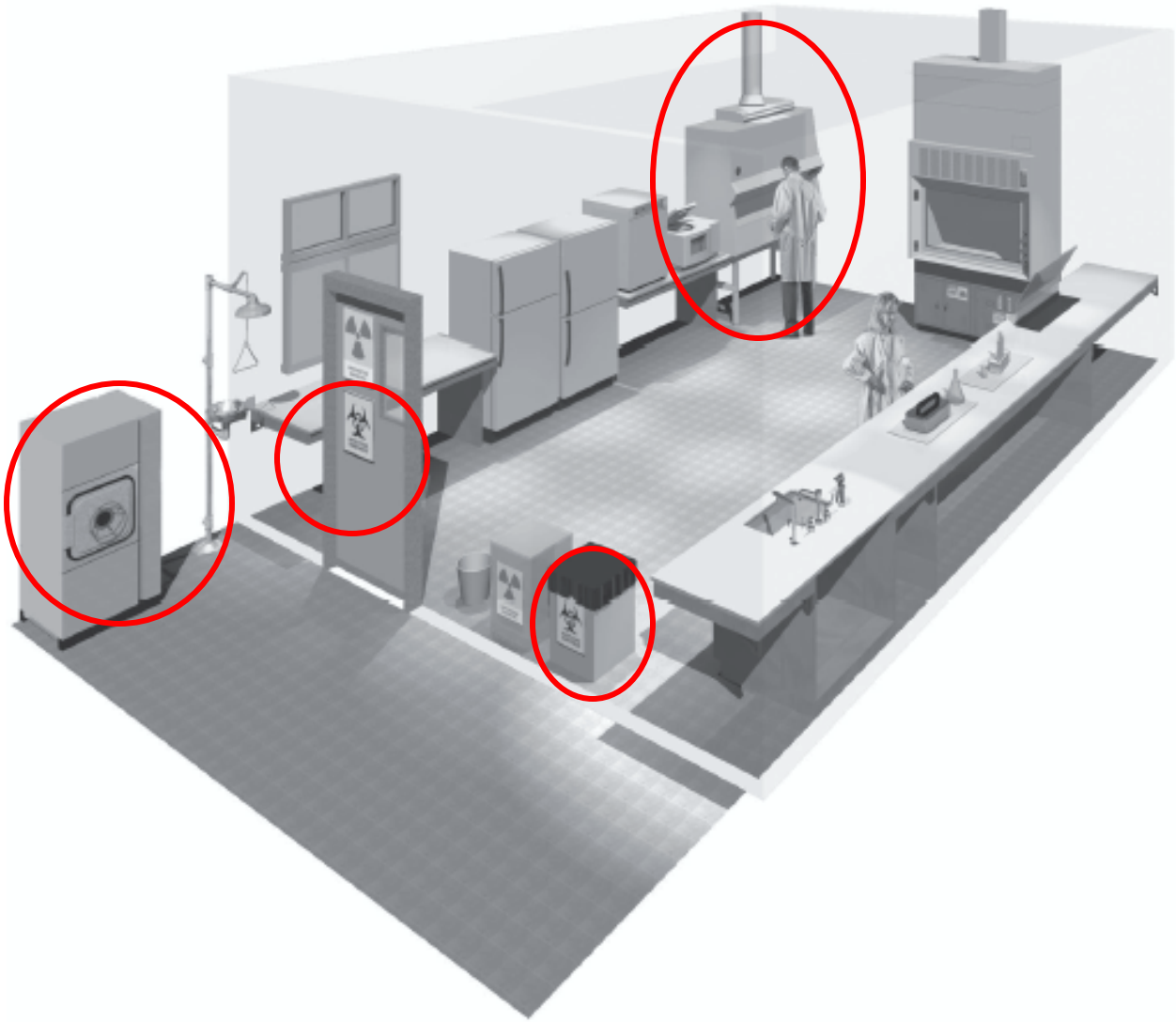
Biosafety Level 1: Facility Design



Biosafety Level 2: Facility Design

- **Requirements:**
  - Laboratories have lockable doors
  - Sink for hand washing
  - Work surfaces easily cleaned
  - Bench tops are impervious to water
  - Sturdy furniture
  - Biological safety cabinets installed as needed
  - Adequate illumination
  - Eyewash readily available
  - Windows fitted with flyscreens
  - Location - separated from public areas
  - Ventilation – directional
    - **Air flows into lab without re-circulation to non-lab areas**

Biosafety Level 2: Facility Design



Biosafety Level 3: Facility Design

- **Requirements:**

  BSL-1 and 2 Facilities PLUS

  - **Enclosures for aerosol generating equipment**
  - **Room penetrations sealed**
  - **Walls, floors and ceilings are water resistant for easy cleaning**
  - **BSC class II or III to manipulate infectious material**
  - **Separate building or isolated zone within a building**
  - **Directional inward airflow**
  - **Single-pass air; can be recirculated if HEPA filtered**
  - **Double door entry**
  - **BSCs mandatory**
- **Additional requirements depending on work and agents:**

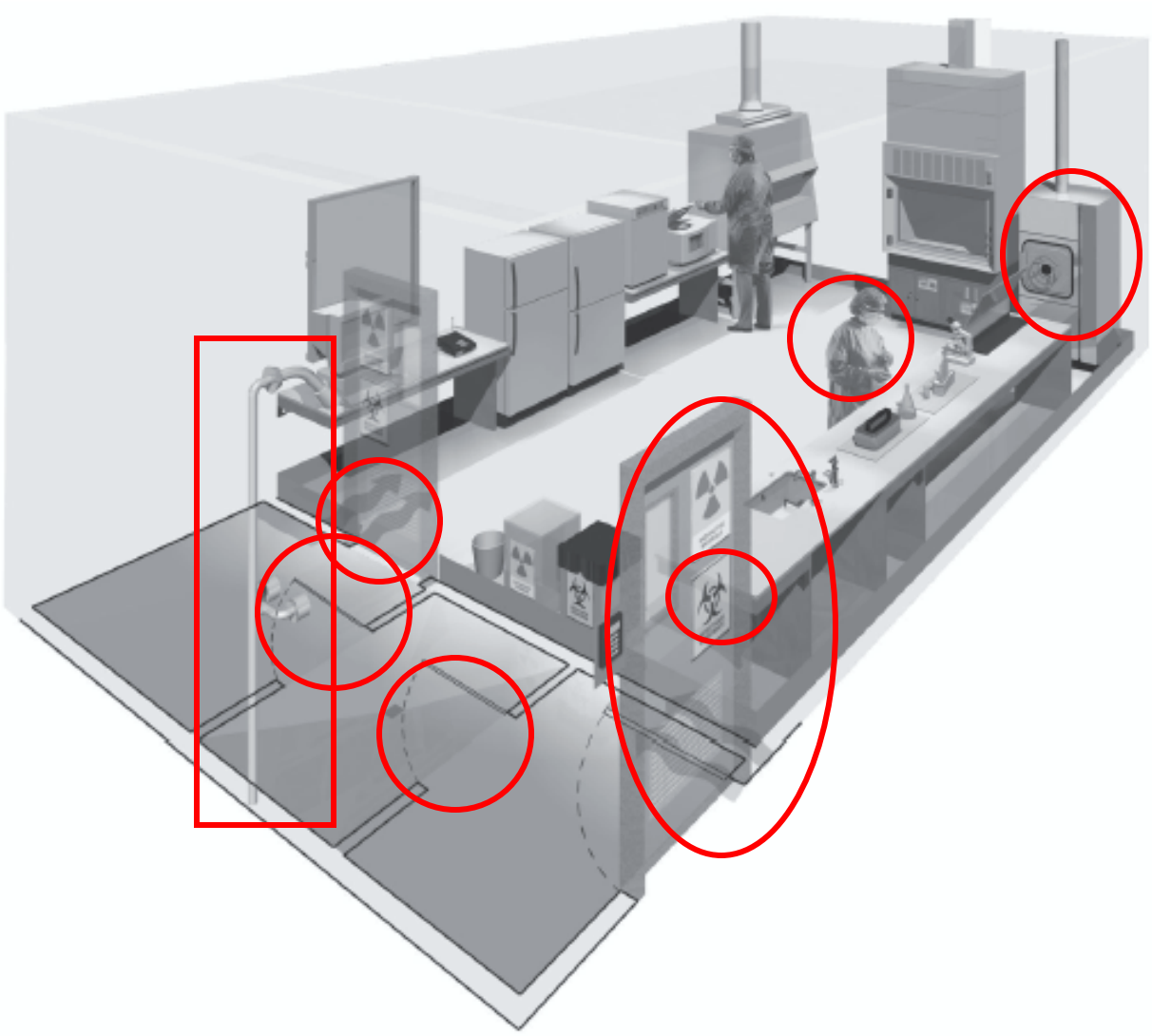
  HEPA filtration of the exhaust

  Effluent decontamination

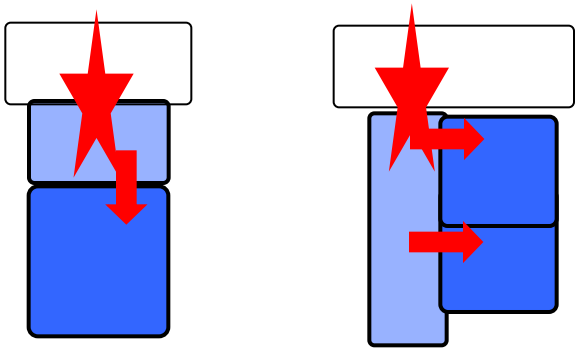
  Personnel showers

Follow the guidelines, regulations, and site-specific risk assessment for your unique situation!

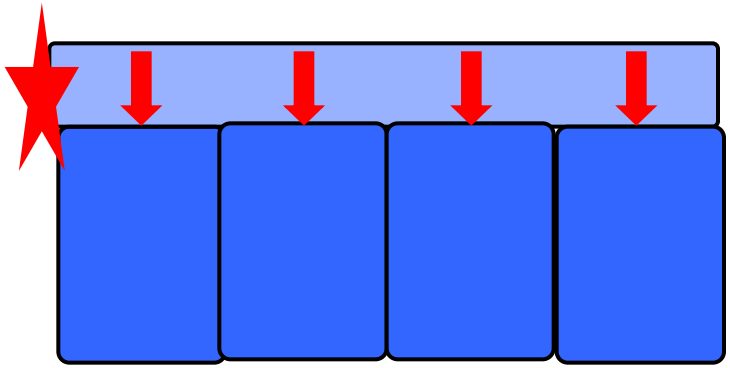
Biosafety Level 3: Facility Design



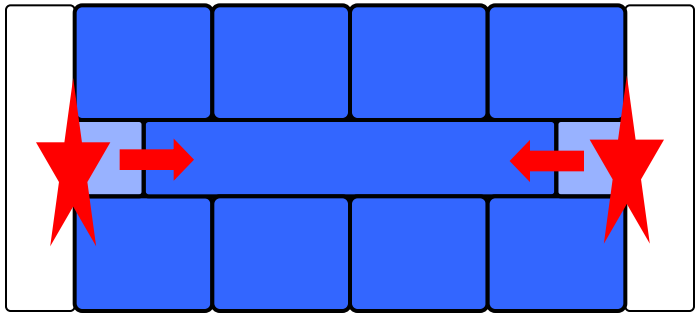
Options for Double Door Entry



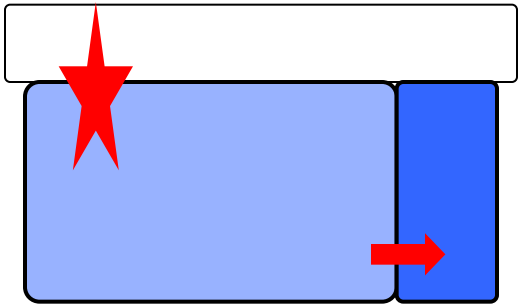
ROOM AS ACCESS ZONE



CORRIDOR AS ACCESS ZONE



SUITE CONCEPT



LAB AS ACCESS ZONE

CORRIDOR ACCESS ZONE BSL3

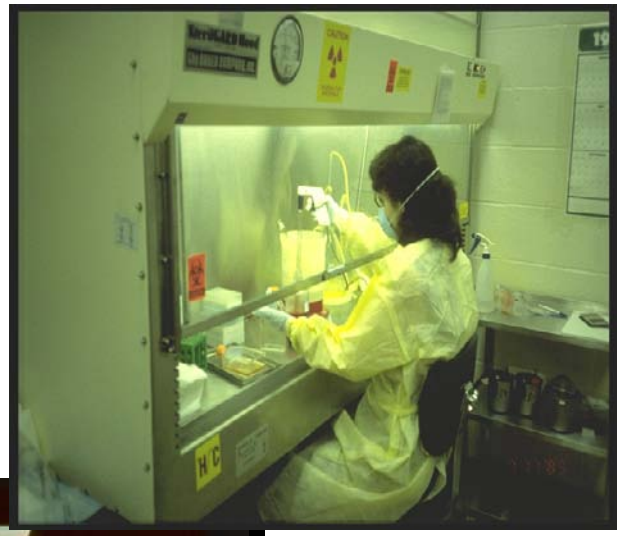
ENTRY DOOR 1

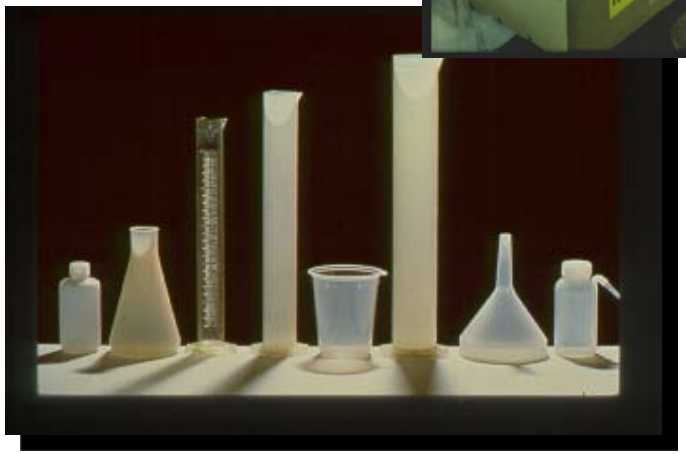
ENTRY DOOR 2

Safety Practices

Standard and Special Laboratory Practices

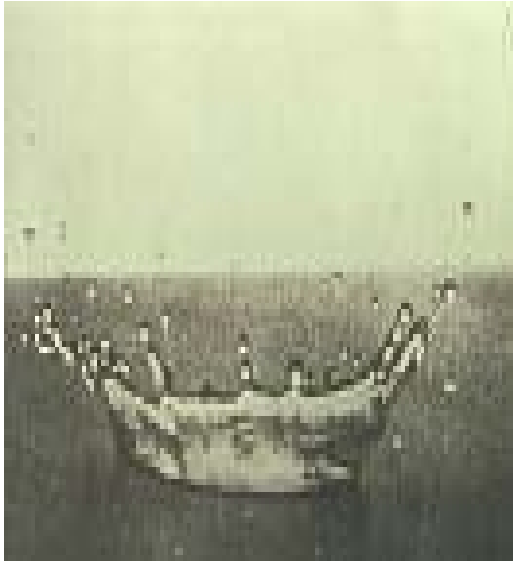
- All procedures with live agents and toxins must be performed inside the biosafety cabinet
- No work with open vessels on open bench
- Use of paper covering on work surface assists clean-up
- Use of appropriate disinfectant in cabinet
- Substitute plastic for glass
- Careful pipetting techniques
- Wash hands often
- No mouth pipeting
- No eating or drinking in lab
- Minimize aerosol generation
- Decontaminate work surfaces
- Safe sharps handling
- Wear applicable PPE





Standard and Special Laboratory Practices: Minimizing Aerosols

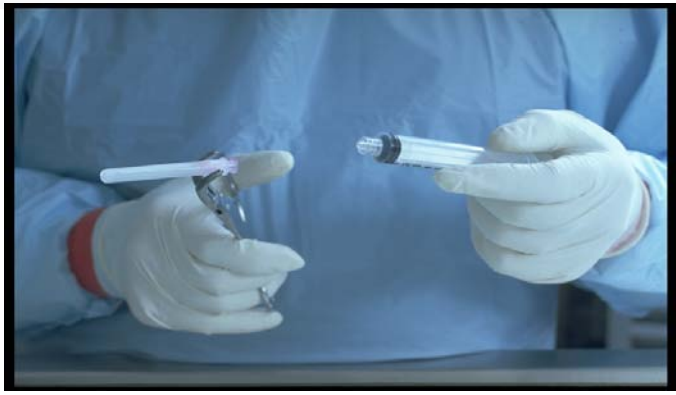
- Use careful pipetting practices
- Avoid drops onto hard surfaces
- Wipe up spills promptly with appropriate disinfectant
- Use capped tubes when mixing, blending, or vortexing
- Pour liquids carefully
- Avoid bubbles



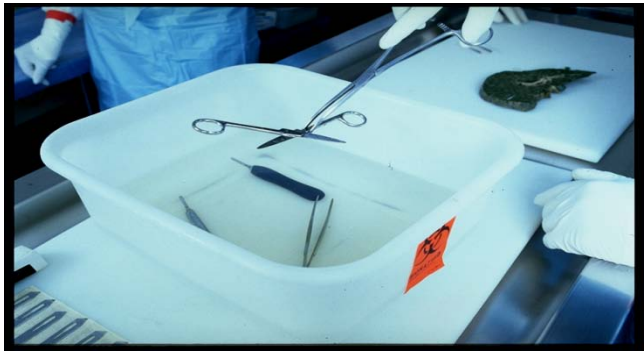
Standard and Special Laboratory Practices: Careful Pipetting Techniques

- Never blow out last drop in pipette
- Use pipetting aids with filters
- Use horizontal pipette collection tubs
- Never mix by suction + expulsion
- Discharge liquid down side of container, using tip-to-wall contact
- Deliver as close as possible to contents
- Work over plastic-backed absorbent matting

Standard and Special Laboratory Practices: Safe Use of Sharps



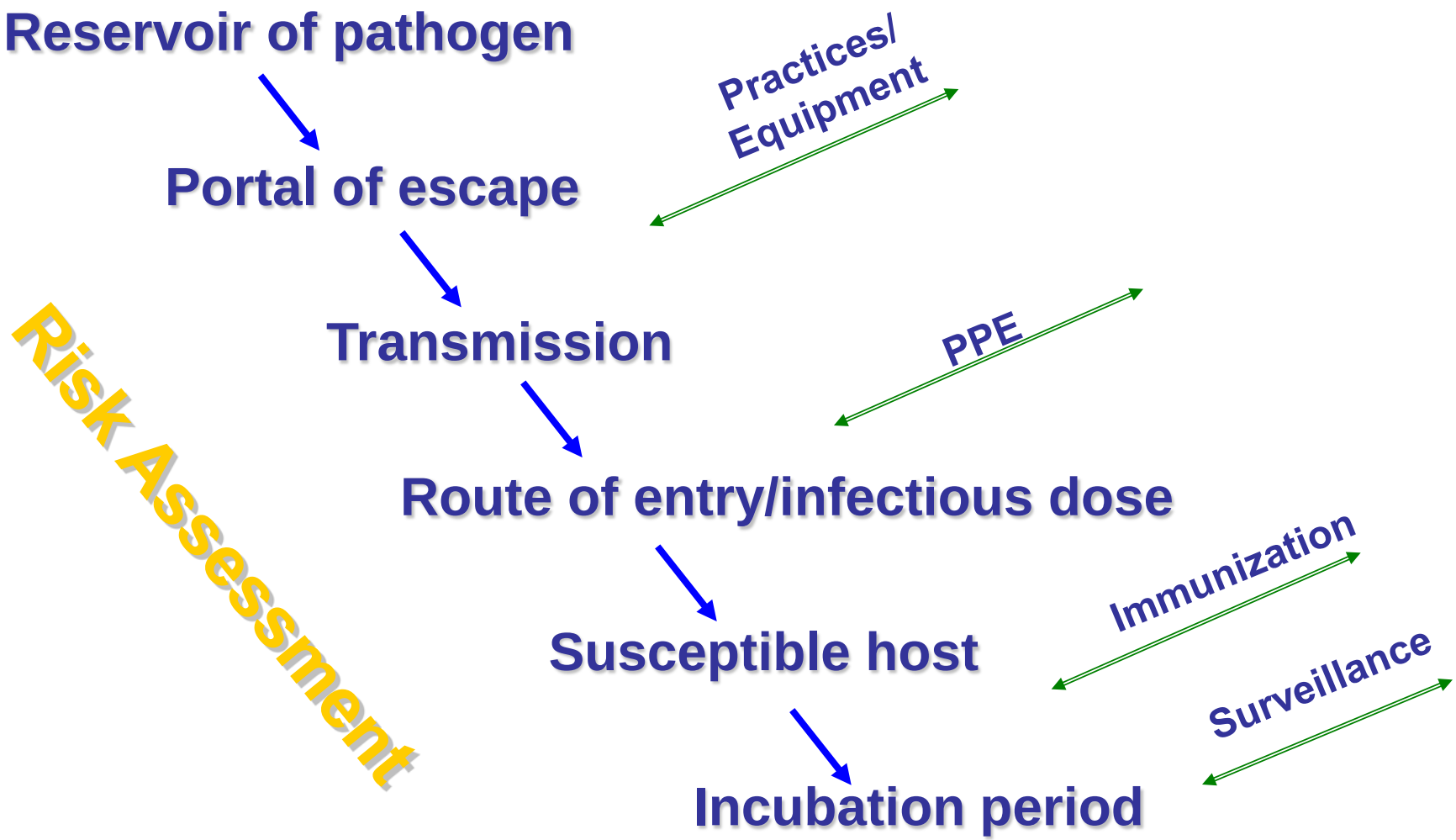
- **Sharps includes needles, scalpels, blades and wires, anything that can cut or puncture**
- **Percutaneous exposure risk**
  - Employ safe work practices
  - Only use sharps if absolutely required as part of a process
- **Aerosol risk**
  - Use biosafety cabinet for removal of air from needle
- **Utilize safe sharp devices**
- **Keep hands away from needles**
- **Use mechanical methods for needle removal**
- **Never bend, recap or manipulate sharps by hand.**
- **Dispose of entire unit into sharps container**
- **Collect reusable sharps in labeled, leak-proof container**



Summary of Biosafety Level Requirements

Table 3. ***Summary of biosafety level requirements***

	BIOSAFETY LEVEL			
	1	**2**	**3**	**4**
Isolation[a] of laboratory	No	No	Yes	Yes
Room sealable for decontamination	No	No	Yes	Yes
Ventilation:				
— inward airflow	No	Desirable	Yes	Yes
— controlled ventilating system	No	Desirable	Yes	Yes
— HEPA-filtered air exhaust	No	No	Yes/No[b]	Yes
Double-door entry	No	No	Yes	Yes
Airlock	No	No	No	Yes
Airlock with shower	No	No	No	Yes
Anteroom	No	No	Yes	—
Anteroom with shower	No	No	Yes/No[c]	No
Effluent treatment	No	No	Yes/No[c]	Yes
Autoclave:				
— on site	No	Desirable	Yes	Yes
— in laboratory room	No	No	Desirable	Yes
— double-ended	No	No	Desirable	Yes
Biological safety cabinets	No	Desirable	Yes	Yes
Personnel safety monitoring capability[d]	No	No	Desirable	Yes

[a] Environmental and functional isolation from general traffic.
[b] Dependent on location of exhaust (see Chapter 4).
[c] Dependent on agent(s) used in the laboratory.
[d] For example, window, closed-circuit television, two-way communication.

Risk Management: Implementation of Biosafety