



Laboratory Biosafety 101



Biosecurity Inspector Training

Staten Serums Institut

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www.biosecurity.sandia.gov

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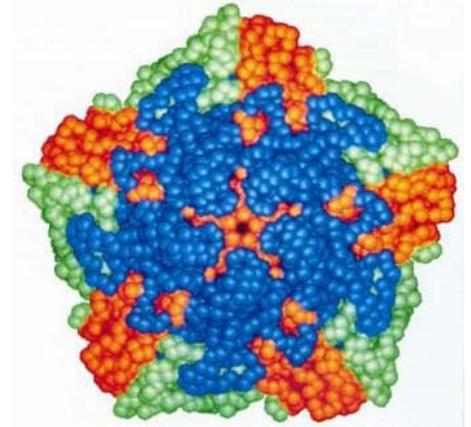
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The Problem of Terminology and Language



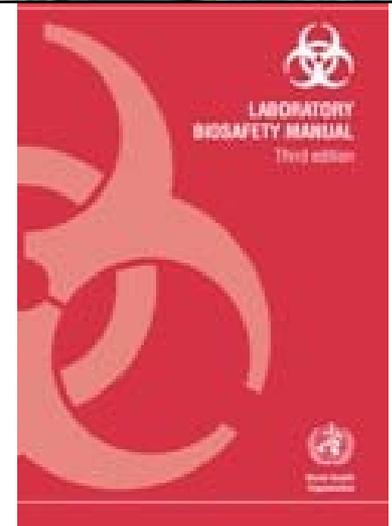
- **Biosafety**
 - Cartagena Protocol on Biosafety seeks to protect biological diversity from the potential risks posed by living modified organisms resulting from modern biotechnology
- **Biosecurity**
 - Prevention of the entry of a pathogen or pest into a susceptible population of plants or animals
- Fundamentally, the issue is containment of pathogens





A Focus on the Laboratory

- **Laboratory Biosafety**
 - A set of preventive measures designed to reduce the risk of accidental exposure to or release of a biological agent
- **Laboratory Biosecurity**
 - A set of preventive measures designed to reduce the risk of intentional removal (theft) and misuse of a biological agent – intent to cause harm
- Fundamentally, the issue is a concern that there are risks to working with pathogens and toxins in a laboratory setting





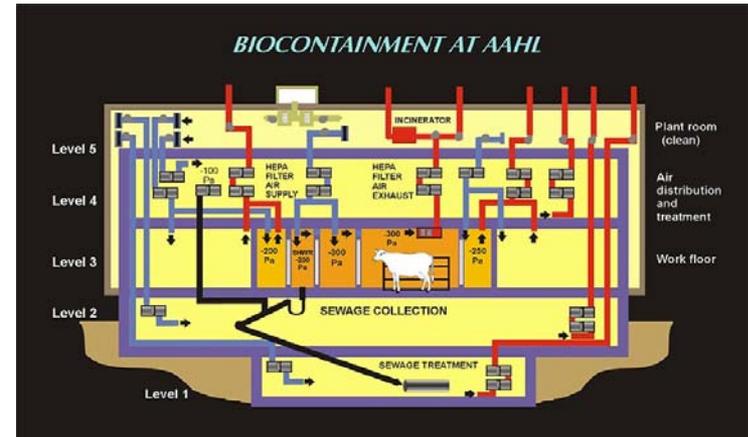
Biocontainment

- 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

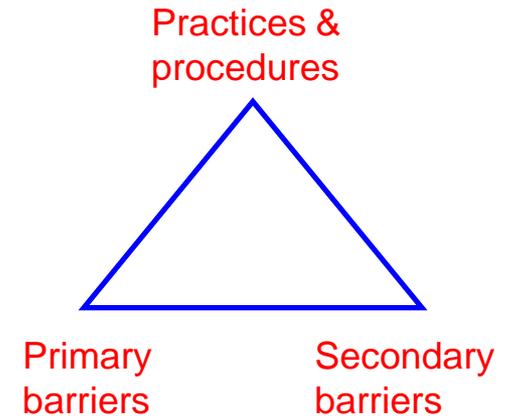
- **Biosafety Levels**
 - Four biosafety levels provide increasing degrees of protection
 - What's the right balance of practices & procedures, primary barriers and secondary barriers?
- **Applications:**
 - Labs (BSL-1, 2, 3, 4)
 - (Small) Animal Containment (ABSL-1, 2, 3, 4)
 - Large Animal Containment (“BSL-3 AG”)
 - Plant Containment (BSL-1P, 2P, 3P, 4P)





Planning: Risk Assessment is Critical

- Identify biological agents that will be used
- Perform risk assessment
 - Include an evaluation of settings
 - Labs
 - Animal housing
 - 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?





Biosafety Risk Assessment: Safety Risk Groups

- Risk Group 1
 - No or low individual and community risk
 - Unlikely to cause human or animal disease
- Risk Group 2
 - Moderate individual risk, low community risk
 - Can cause disease but unlikely to be a serious hazard. Lab exposures may cause serious infection, but effective treatment and preventative measures are available and risk of spread of infection is limited
- Risk Group 3
 - High individual risk, low community risk
 - Usually causes serious human or animal disease but does not ordinarily spread. Effective treatment and preventative measures are available.
- Risk Group 4
 - High individual and community risk
 - Usually causes serious human or animal disease and can be readily transmitted. Effective treatment and preventative measures are not usually available





Safety Risk Group Examples

- Risk Group 1

- *Bacillus subtilis*
 - Ubiquitous bacterium found in water, soil, air
 - Not considered pathogenic or toxigenic to humans, animals, or plants
- *Escherichia coli* K-12
 - E. coli is normal inhabitant of colon of almost all mammals
 - K-12 is debilitated strain – does not normally colonize human intestine
 - History of safe commercial use

- Risk Group 2

- Measles virus
 - Pathogenicity: acute disease, fatality <0.5% - 25%
 - Host range: Humans
 - Transmission: primarily droplet spread
 - Vaccine available
- Hepatitis B virus
 - Pathogenicity: asymptomatic and symptomatic infections, long-term fatality = 2-3%, 95% of adult infections self-limiting
 - Host range: Humans (chimpanzees are susceptible)
 - Vaccine available



Safety Risk Group Examples

- Risk Group 3

- *Mycobacterium tuberculosis*
 - Proven hazard to laboratory workers (3x higher rate of infection)
 - Low aerosol infectious dose ($ID_{50} < 10$ bacilli)
 - Host range: Primarily humans, cattle, primates, other animals (rodents)
- St. Louis encephalitis virus
 - Pathogenicity: Fatality rate of 2-22%, 30-50% of severe cases have prolonged convalescence
 - Host range: Humans, wild birds, other mammals
 - Supportive care is only treatment

- Risk Group 4

- Ebola virus
 - Pathogenicity: Sudden onset, 50 – 90 % fatality
 - Host range: Humans, monkeys, chimpanzees, domestic guinea pigs
 - BSL4 laboratory recommended even for clinical work (Public Health Agency of Canada)



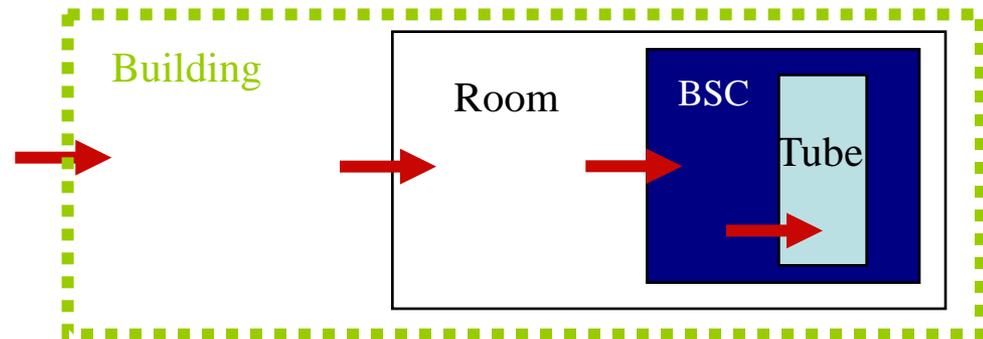
Components of Laboratory Biosafety





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*
 - *E. coli*
- Biosafety Level 2
 - Suitable for work involving agents of moderate potential hazard to personnel and the environment
 - Examples:
 - Measles virus
 - *Salmonellae* species
 - Toxoplasma species
 - Hepatitis B virus



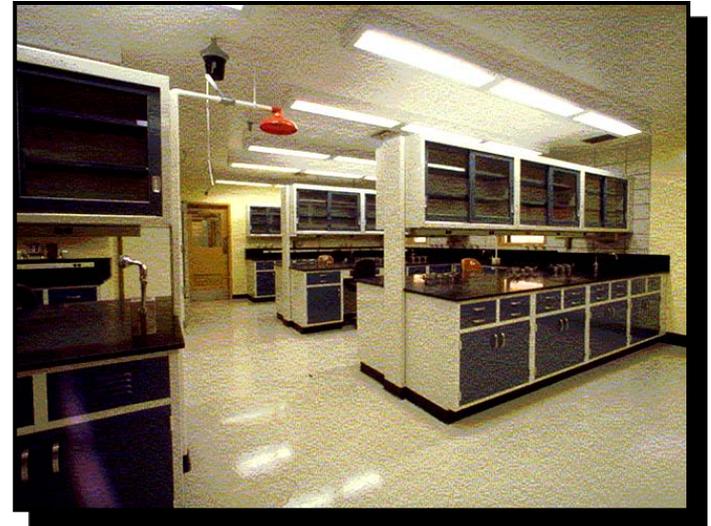
Biosafety Levels 3 and 4

- **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



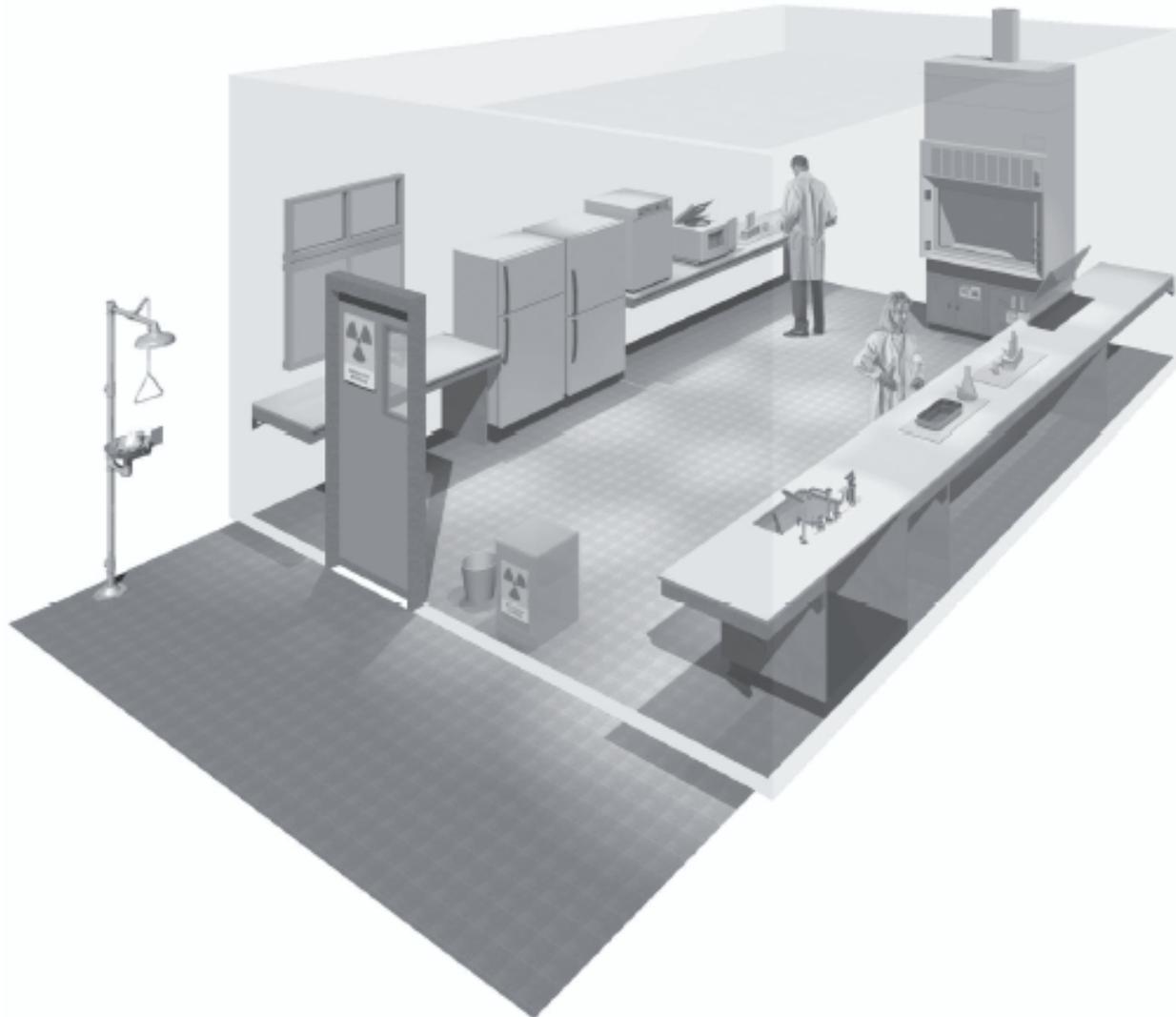
Biosafety Level 1: Facility Design

- 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

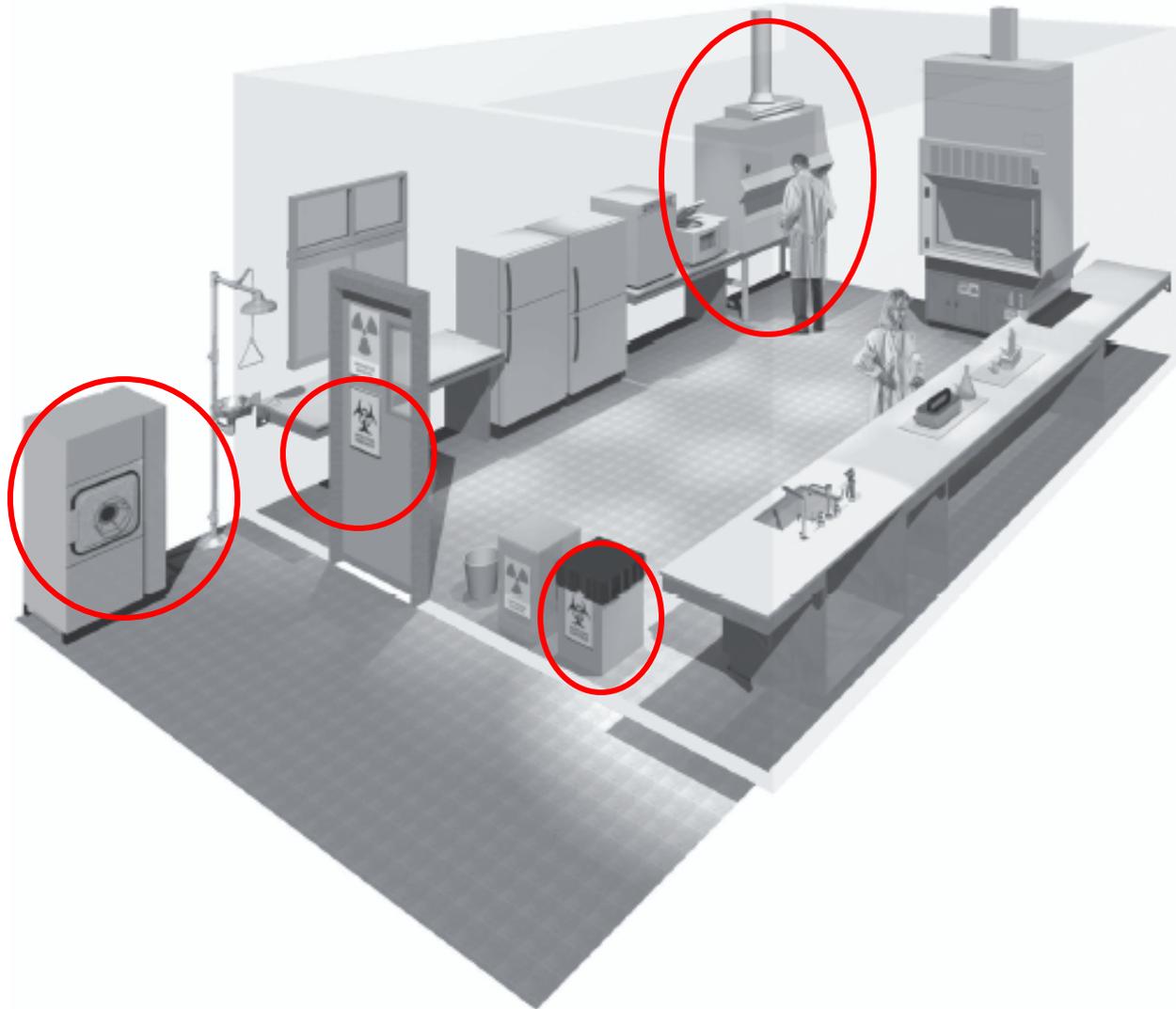
- Requirements:
 - Laboratories have lockable doors
 - Sink for hand washing
 - Work surfaces easily cleaned
 - Bench tops are impervious to water
 - Sturdy furniture
 - Biological safety cabinets installed as needed
 - Adequate illumination
 - Eyewash readily available
 - Windows fitted with flyscreens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



Restricted access
when work in progress



Biosafety Level 2: Facility Design





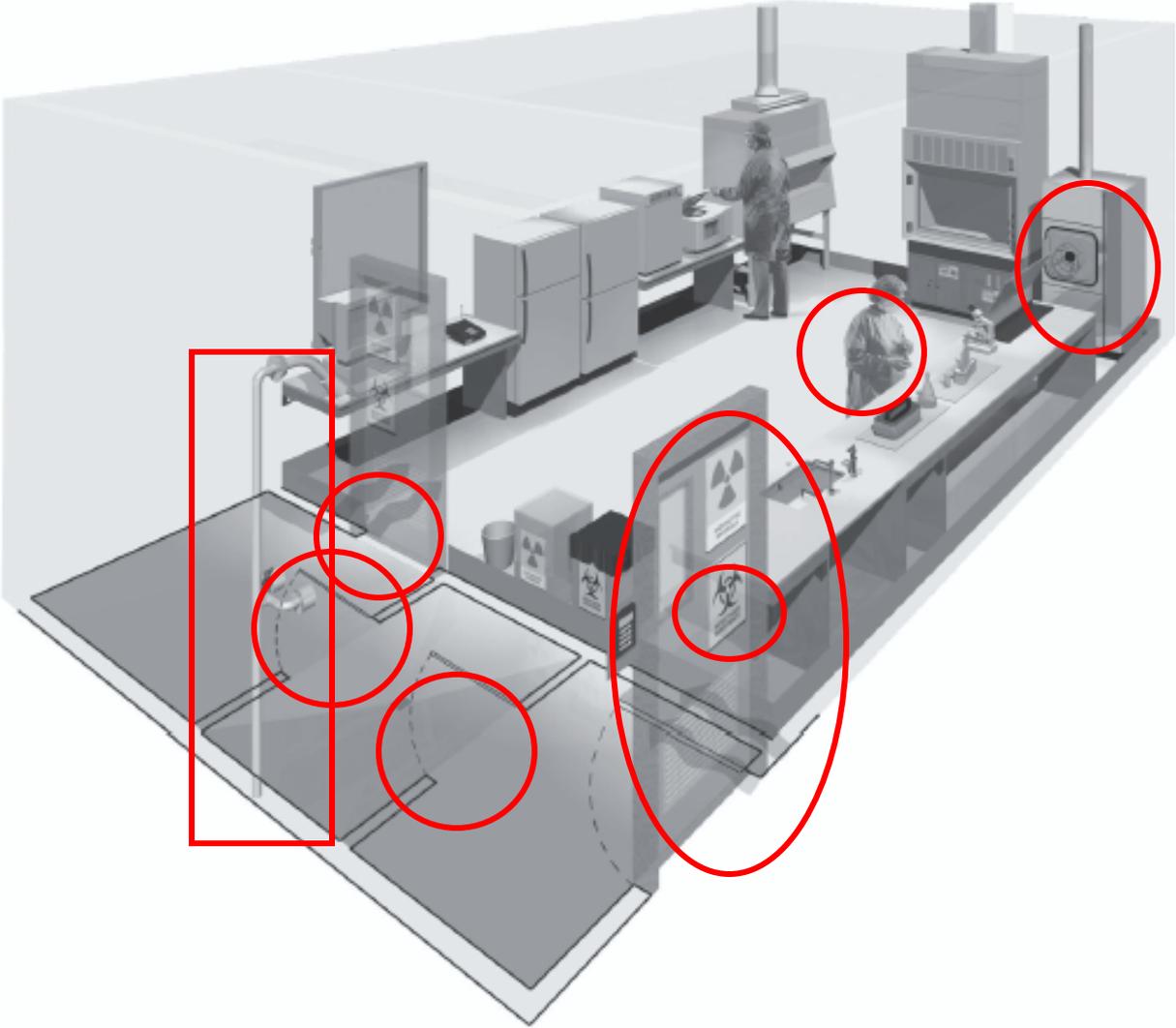
Biosafety Level 3: Facility Design

- Requirements:
 - BSL-1 and 2 Requirements 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





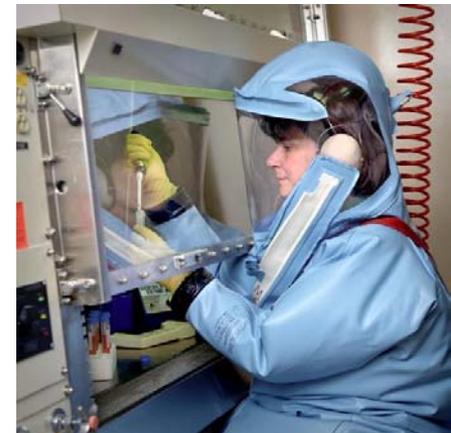
Biosafety Level 3: Facility Design





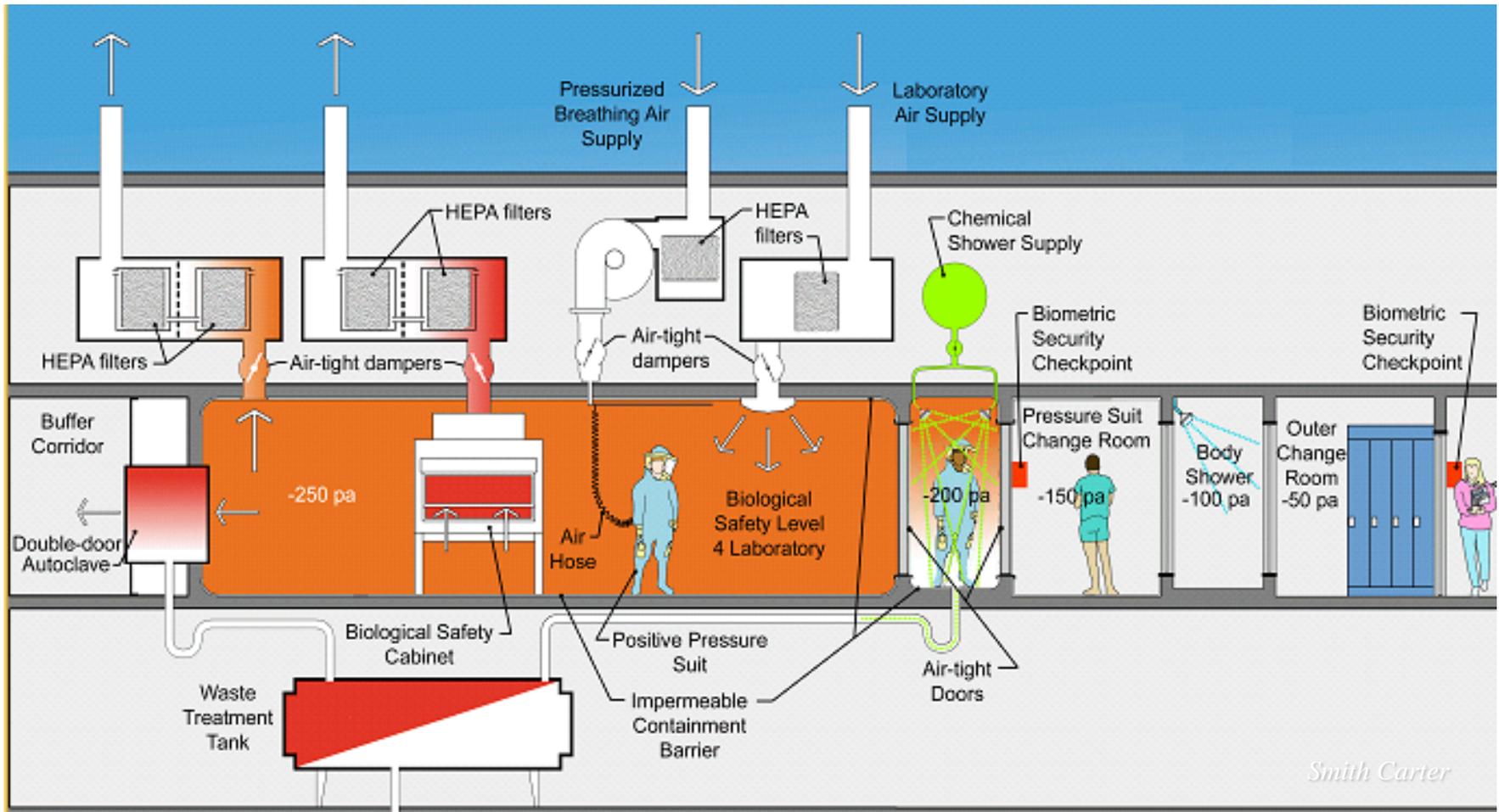
Biosafety Level 4: Facility Design

- BSL3 enhanced requirements plus:
 - Either:
 - Class III biosafety cabinet
 - Personnel shower required to exit room with cabinet
 - Or
 - Positive pressure suit with self-contained breathing apparatus
 - Suit decontamination shower
 - Personnel shower to exit
 - Double HEPA-filtered exhaust
 - HEPA-filtered supply air
 - Decontamination of all effluents
 - Sterilization of waste and materials
 - Redundant back-up systems
 - Pass through autoclave





Biosafety Level 4: Facility Design





Standard and Special Laboratory Practices

- Use of paper covering on work surface assists clean-up
- Use of appropriate disinfectant
- Substitute plastic for glass
- Careful pipetting techniques
- Wash hands
- Minimize aerosol generation
- Wear appropriate PPE





Standard and Special Laboratory Practices: Safe Use of Sharps

- Percutaneous exposure risk
 - Employ safe work practices
 - Only use sharps if absolutely required as part of a process
- Utilize safe sharp devices
- Keep hands away from needles
- Use mechanical methods for needle removal
- Never bend, recap or manipulate sharps by hand.
- Dispose into sharps container
- Collect reusable sharps in puncture-proof, leak-proof containers





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.

