The 1, 2, 3's of Biosafety Levels

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adapted from the CDC/NIH 3rd edition of *Biosafety in Microbiological and Biomedical Laboratories* [1]

Slides - 6/page - b/w - Adobe Acrobat Format (.pdf)

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Introduction

From the earliest days of microbiological research, laboratorians have recognized that acquiring infections from the agents they manipulated was a recognized occupational hazard (slide 1). The most commonly-acquired lab infections were caused by bacterial agents; as microbiologists learned to culture animal viruses, they also found ways to become infected with these agents (slide 2). From the literature reviews of Sulkin and Pike, we also learned that a significant number of these infections were fatal and that most infections were of unknown origin. Exposure to infectious aerosols were implicated in about eighty per cent of the reported infections (slide 3).

Guidelines evolved as a means of protecting microbiological workers based on these data and an understanding of the risks associated with various manipulations of many agents transmissible by different routes. These guidelines work from the premise that safe work sites result from a combination of engineering controls, management policies, work practices and procedures (slide 4), and, occasionally, medical interventions. The different biosafety levels developed for microbiological and biomedical laboratories provide increasing levels of personnel and environmental protection (slide 5).

There is a definite hierarchy of administrative controls that need to be in effect. Upper level management must set the general tone that safety is a high priority at their institute. Though this is often expressed in

broad policy statements, it must be supported by resource allocation decisions: financial, personnel staffing, training, a safety performance reward structure, etc. For each biosafety level there are also specific supervisory qualifications as assurance that the laboratorians are provided appropriate role models and knowledgeable mentors (slide 6). Crucial to safe working conditions are the various types of specialized equipment available to serve as primary barriers between the microorganism and the laboratorian. These range from simple gloves and other personnel protective equipment to simple (sealed centrifuge heads) or complex (biosafety cabinets) containment devices (slide 7).

Biosafety Level 1

BSL-1 is appropriate for working with microorganisms that are not known to cause disease in healthy human humans (slide 8). This is the type of laboratory found in municipal water-testing laboratories, in high schools, and in some community colleges teaching introductory microbiology classes, where the agents are not considered hazardous (slide 9). The lay-out of a typical BSL-1 laboratory is shown in slide 10.

There is a door that can be closed to keep visitors out of the lab while work with the agents is in progress. Hazard warning signs may be posted on the door indicating any hazards that may be present, including radioactive materials, lazar lights, high noise emitting equipment, or toxic chemicals. There is a hand-washing sink available, preferably near the door. Waste materials are segregated according to hazard type, and there is an appropriate chemical decon tray for collecting contaminated implements. Work is done

on the open bench, and plastic-backed absorbent pads can be placed on the work surface to collect splatter or droplets associated with the work. The bench tops should be impervious to acid and all furniture should be sturdy (slide 11). If there are openable windows in the lab, they should be fitted with screens.

The lab should be constructed in such a manner that it can be easily cleaned and decontaminated (slide 12). At BSL-1 there is no specific recommendation that the laboratory be isolated from other parts of the building (slide 13). Although there is no specific biological safety reason for having more than six air changes per hour in a BSL-1 laboratory, it may be necessary if there are volatile or toxic chemicals in use. In general, inward directional airflow is the ideal.

At BSL-1, standard microbiological practices include the use of mechanical pipetting devices (slide 14), having a prohibition on eating, drinking and smoking in the lab, and requiring hand washing by all persons when they finish their work or when exiting the laboratory (slide 15, 16). Persons working in the lab should wear a lab coat (slide 17) to protect their street clothes. It is a recommended practice to wear gloves while manipulating the agents. Additional protective equipment may include working behind a splatter shield or wearing eye or face protection (slide 18). At BSL-1, no special precautions are needed (slide 19).

Hand washing is one of the most important procedures that can be used by laboratorians to prevent removal of unwanted microbiological agents, radioactive materials, or chemicals from the laboratory environment (slide 20). Use of liquid soap is generally preferable to bar soap; twenty seconds of vigorous lathering will remove most of these materials very

effectively. After drying your hands with a paper towel, you can use the towel to turn off the faucets and thus prevent recontaminating your hands.

The scientist who provides overall supervision to a BSL-1 laboratory needs to have general training in microbiology or a related science (slide 21). The supervisor is responsible for establishing the general lab safety procedures and for ensuring that each laboratorian is properly educated in these procedures. Lab personnel, on the other hand, need to accept such training and follow the proscribed protocols.

Biosafety Level 2

The facility, the containment devices, the administrative controls, and the practices and procedures that constitute BSL-2 are designed to maximize safe working conditions for laboratorians working with agents of moderate risk to personnel and the environment (slide 22). The agents manipulated at BSL-2 are often ones to which the workers have had exposure to in the community, often as children, and to which they have already experienced an immune response. Unlike the guidelines for BSL-1, there are a number of immunizations recommended before working with specific agents (slide 23). Most notable is Hepatitis B virus immunization which is recommended by the Occupational Safety and Health Administration for persons, including laboratorians, at high risk of exposure to blood and blood products [2]. These agents are generally transmissible following ingestion, exposure of mucous membranes, or intradermal exposure. Eating, drinking and smoking are prohibited in BSL-2 laboratories, and extreme precautions are taken while handling needles and other sharp instruments (slide 24).

The basic lay-out of a BSL-2 laboratory is depicted in slide 25. Access to the laboratory is restricted by the supervisor, who establishes the biosafety level, the need for specified personal protective equipment, the need for training., or other appropriate requirements. The door to the laboratory is kept closed to minimize unnecessary access by casual visitors, vendors, or persons not needing to be in the laboratory. There is no requirement for directional inward air flow in a BSL-2 laboratory, except as may be required for chemical odor control; however, many BSL-2 laboratories opt for this feature.

Some work may be done on the open bench by persons wearing appropriate protective clothing or gear. Any work that may produce splatters or aerosols of infectious materials should be done inside a biological safety cabinet (BSC) or other containment device, such as aerosol-containing centrifuge cups. Waste materials need to be segregated into chemical, radioactive, bio-hazardous, or general waste streams. Infectious waste should be decontaminated (by treating with chemical disinfectants or by steam autoclaving).

As the biosafety level increases, all those microbiological practices and procedures delineated for the lower level(s) are carried forward to the next higher level. Thus, the standard microbiological practices found at BSL-1 are still in effect at BSL-2 (slide 26), with emphasis on wearing gloves, using mechanical pipetting devices, and attention to handling sharps. In any situation, do not break or bend needles; in most situations it is prudent to use single-use needles and syringes. Do not recap needles. Needles and syringes, butterfly needles and associated tubing, and similar devices should be discarded intact

into a puncture- and leak-proof container (slide 27, 28). Other sharps items (such as broken glass, slide 29) should not be handled by hand. Consider substituting plastic ware for glass laboratory items (slide 30).

Specific policies and procedures regarding access to the BSL-2 laboratory should be developed and posted (slide 31). On the one hand, it is prudent to allow entry to repair technicians or engineers only if they are very familiar with the activities of the lab or are escorted by a laboratorian who is. On the other hand, it needs to be emphasized that the posting of a BSL-2 biohazard sign on the door does not mean that the agent is everywhere in the room; rather, the agent is normally confined to the BSC, an incubator or refrigerator or freezer. It is prudent to schedule entry by non-laboratorians to times when there is no active work with the agent being conducted.

A leak proof box, preferably equipped with a gasket seal lid, should be used for transport of infectious materials from one location to another (slide 32). This is particularly important when moving samples from patient care areas to laboratories, or from an off-site collection center to the lab.

Storing a base-line serum sample may be required prior to working in certain laboratories (slide 33). This sample can be used at to compare with future serum samples to determine any changes in immunological response to the agents used in the laboratory. Alternatively, a base-line serum sample may be drawn at the time of a possible exposure, then compared to a future sample for possible rising antibody titer. In any event, written employee informed consent must be obtained before obtaining the sample and for

subsequent testing. Informed consent is also needed for any immunizations that are offered.

Other special practices include: decontaminating work surfaces after completing the work with the infectious materials, keeping non-research animals out of the laboratory, and reporting all spills and accidents (slide 34). An incident log book is a useful means for recording events that have gone wrong; it is important to document these events, not for punitive action, but to be able to better understand what happened with an eye to preventing similar events in the future.

At BSL-2, all work that might create aerosols of infectious materials should be done in containment (slide 35). The most common device is the biological safety cabinet, and the most common cabinet in use is referred to as a Class II, type A BSC, shown in cross-sectional diagram in slide 36. Room air is drawn in at the face opening and is immediately drawn through the front grille (A) and under the work surface. The air is then blown (F) through the rear air plenum (E) to the top of the cabinet where it is divided into two chambers (D). Thirty percent of the air is exhausted out of the cabinet (C) through a high efficiency particulate air (HEPA) filter into the laboratory room. The remaining seventy per cent of the air (B) is directed through another HEPA filter down onto the work surface in a laminar flow directional air pattern. The typical HEPA filter removes 99.97% of all particles that are 0.3 micron or larger in size, which means that all microbial agents will be trapped in the filter. The air returned to the laboratory and delivered to the work surface is virtually sterile, which means that an open flame (Bunsen burner) is not needed within the BSC.

Before materials are introduced into the BSC, they should be wiped with 70% alcohol to remove any external contaminants. Experience has shown that clean materials should be kept to one side of the work surface, dirty items on the other (slide 37). Management of workflow within the BSC is crucial to preventing cross-contamination. Rapid air movement outside the cabinet (caused by co-workers walking past, air supply vents directed across the face of the BSC, etc.) will interrupt the rather fragile air curtain, which may cause air-borne contaminants in the cabinet to be drawn into the lap of the worker. The chair should be adjusted so that the lower portion of the sash is even with the worker's armpits.

Any paper or plastic materials introduced into the BSC should not be allowed to interfere with air flow through the front or rear grilles (slide 38). The downward airflow from the supply filter "splits" about one third of the way into the cabinet; in the front third, air moves to the front grille, with the remainder of the air flowing to the rear. This means that aerosol-generating activities should be performed towards the rear of the cabinet to provide further worker protection.

Infectious waste materials should be chemically disinfected or, preferably, decontaminated in a steam autoclave (slide 39). Infectious waste materials to be removed from a BSC should be placed in a pan or tray that can be covered during transport to the autoclave, or placed in a biohazard autoclave bag. By placing an inch or two of water in the bag before sealing it for transport, steam will be generated within the bag during the autoclave cycle.

The supervisor of a BSL-2 laboratory should be a

competent scientist who has a technical understanding of the risks associated with the microbiological agents in use (slide 40). The supervisor limits access to those persons who have received the appropriate immunizations and establishes the personal protective standards for the laboratory; he/she is also responsible for developing the lab's biological safety manual. Laboratory personnel should be aware of the potential hazards associated with the work and be proficient in the specified practices and procedures.

Biosafety Level 3

BSL-3 is suitable for work with infectious agents which may cause serious or potentially lethal diseases as a result of exposure by the inhalation route (slide 41). BSL-3 laboratories should be located away from high-traffic areas. Examples of agents that should be manipulated at BSL-3 are *M. tuberculosis* (research activities), St. Louis encephalitis virus, and *Coxiella burnetii* (slide 42).

There are some specific secondary barriers needed at BSL-3 (slide 43), that tend to set these laboratories apart from BSL-2. At CDC the current main BSL-3 laboratories are located in a unique high containment building (slide 44) that also houses the BSL-4 laboratory (slide 45). A typical BSL-3 laboratory lay out is shown in slide 46. These laboratories are characterized by having a double-door entry (shown here as an ante-room; other configurations are also used). Because the agents manipulated at BSL-3 are transmissible by the aerosol route, particular attention is given to air movement in these labs. Air moves from areas of lesser contamination to areas of higher contamination, such as from the corridor into the laboratory. Air movement is also single pass; exhaust

air is not recirculated to other rooms. Exhaust air does not have to be HEPA filtered, unless local conditions are such that reentrainment into building air supply systems is unavoidable.

All work that may create aerosols or splatter is done inside a biological safety cabinet (slide 47). Wall, ceiling and floor penetrations are sealed to keep aerosols in and to keep gaseous decontaminants in. The floor is monolithic, and there are continuous cove moldings that extend at least 4" up the wall. Acoustic tiles are not used in BSL-3 laboratories; ceilings should be waterproof for ease of cleaning Centrifuge tubes are placed into containment cups or heads in the BSC (slide 48), transferred to the centrifuge, spun, then returned to the BSC to be unloaded. In some laboratories the centrifuges themselves are enclosed in a vented area to minimize possible aerosol exposures created in the event of a centrifuge failure. Vacuum lines are protected with HEPA filters so that maintenance personnel are not exposed to infectious aerosols (slide 49).

Standard microbiological practices are the same as for BSL-1 and BSL-2 laboratories (slide 50). Class II type A biological safety cabinets [3] are suitable in BSL-3 laboratories. Sometimes Class II type B3 cabinets are installed, requiring thimble connection to the building exhaust systems (slide 51). Depending on the nature of the work being done in the BSL-3 laboratory, additional personnel protective devices may be worn, such as respirators (slide 52). When pulmonary protection is required, the laboratorians need to have appropriate medical evaluations and be trained in proper fit testing and care of their respirators.

Supervisors of BSL-3 laboratories should be

competent scientists experienced in working with the agents (slide 53). They establish criteria for entry into the laboratory, restrict access, develop appropriate practices and procedures, and train the laboratorians. They are also responsible for developing the laboratory safety manual. The lab personnel must rigorously follow the established guidelines, demonstrate proficiency in performing their various procedures, and receive appropriate training (slide 54). They must participate in specified medical surveillance programs, and report all incidents that constitute potential exposures.

Summary

The guidelines presented in the CDC/NIH publication *Biosafety in Microbiological and Biomedical Laboratories* present standard and special practices, safety equipment recommendations, and performance standards for the facilities that, taken together, should be considered as optimal for most laboratory situations (slide 55). There may be instances where unique needs, unknown hazards associated with unknown pathogens, or other contributing requirements will cause supervisors or biosafety professionals to seek higher biosafety requirements. These can be established after appropriate risk assessments have been conducted.

References

1. Richmond, JY and RW McKinney, 1993: Biosafety in Microbiological and Biomedical Laboratories. US Department of Health and Human Services, CDC/NIH, 3rd Edition. US Government Printing Office, Washington, DC.

2. US Department of Labor, Occupational Safety and Health Administration, 1991. Occupational Exposures to Bloodborne Pathogens, Final Rule. Fed. Register 56:64175-64182.

3. Richmond, JY and RW McKinney, 1995: Primary Containment for Biohazards: Selection, Installation and Use of Biological Safety Cabinets. US Department of Health and Human Services, CDC/NIH.. US Government Printing Office, Washington, DC.

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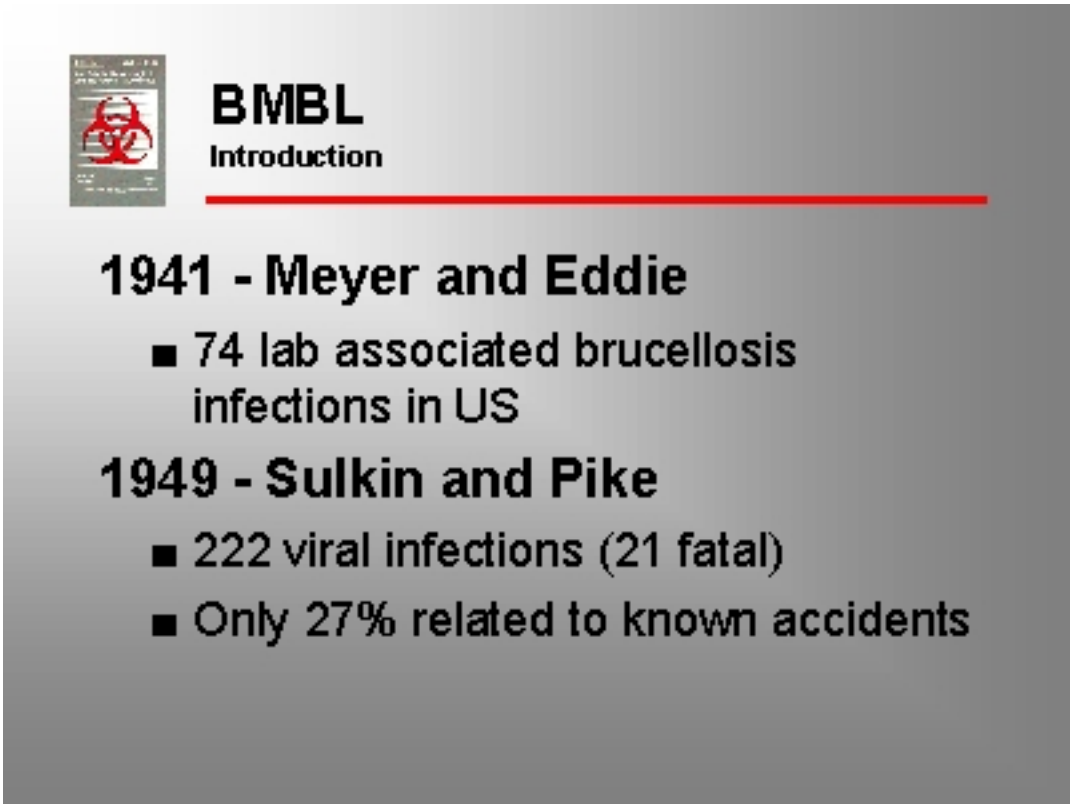

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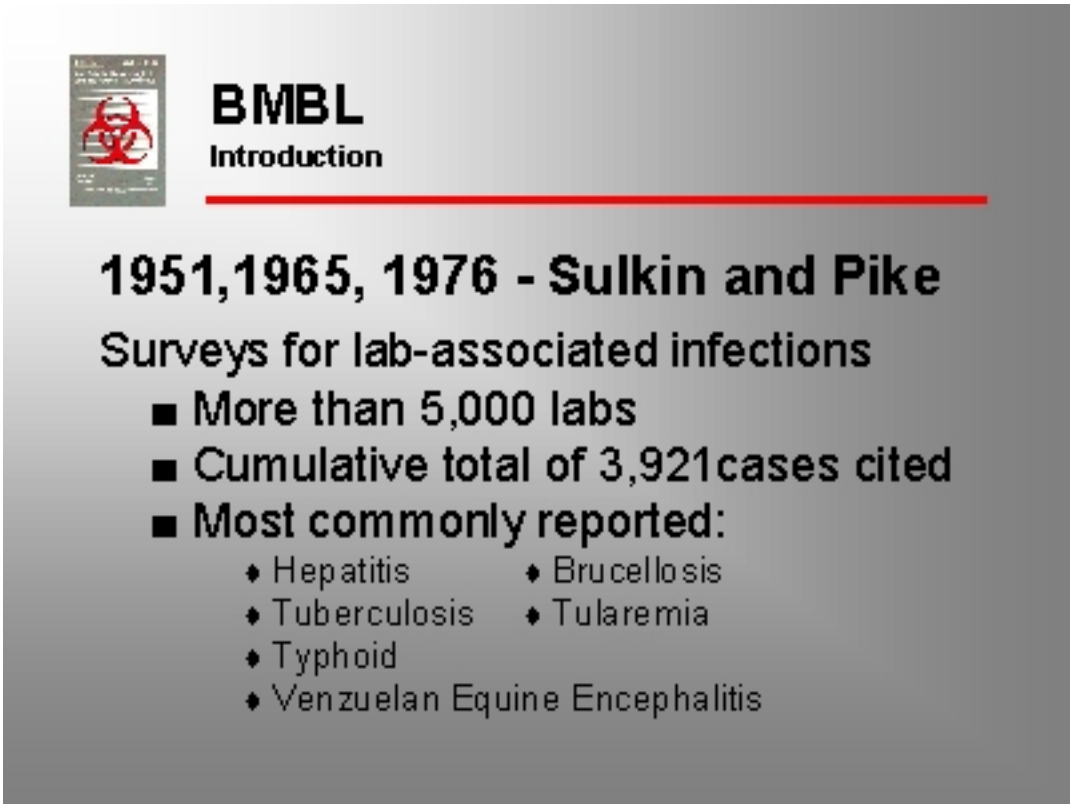

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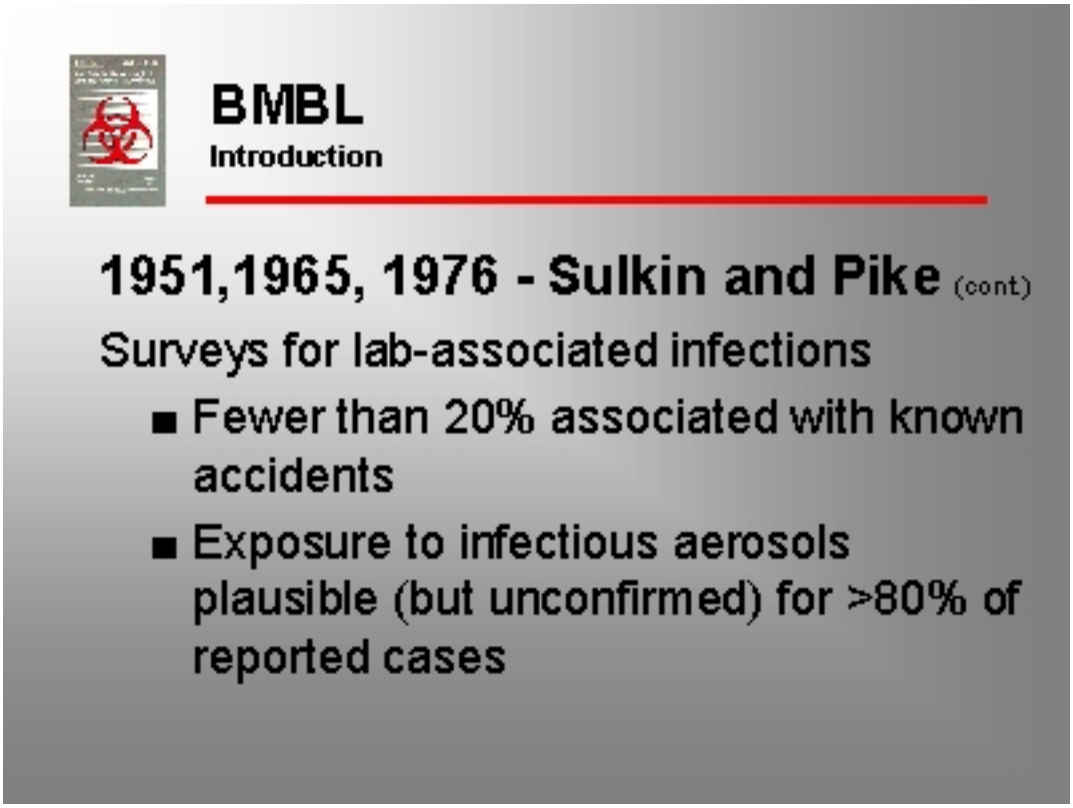

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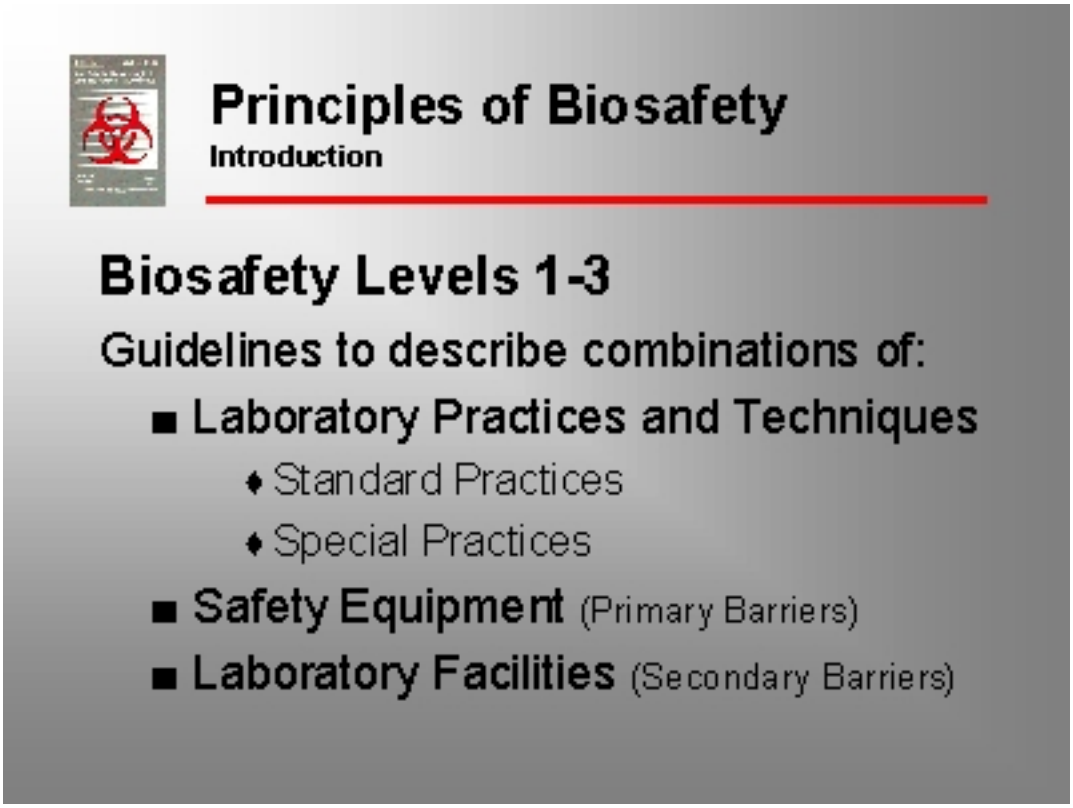

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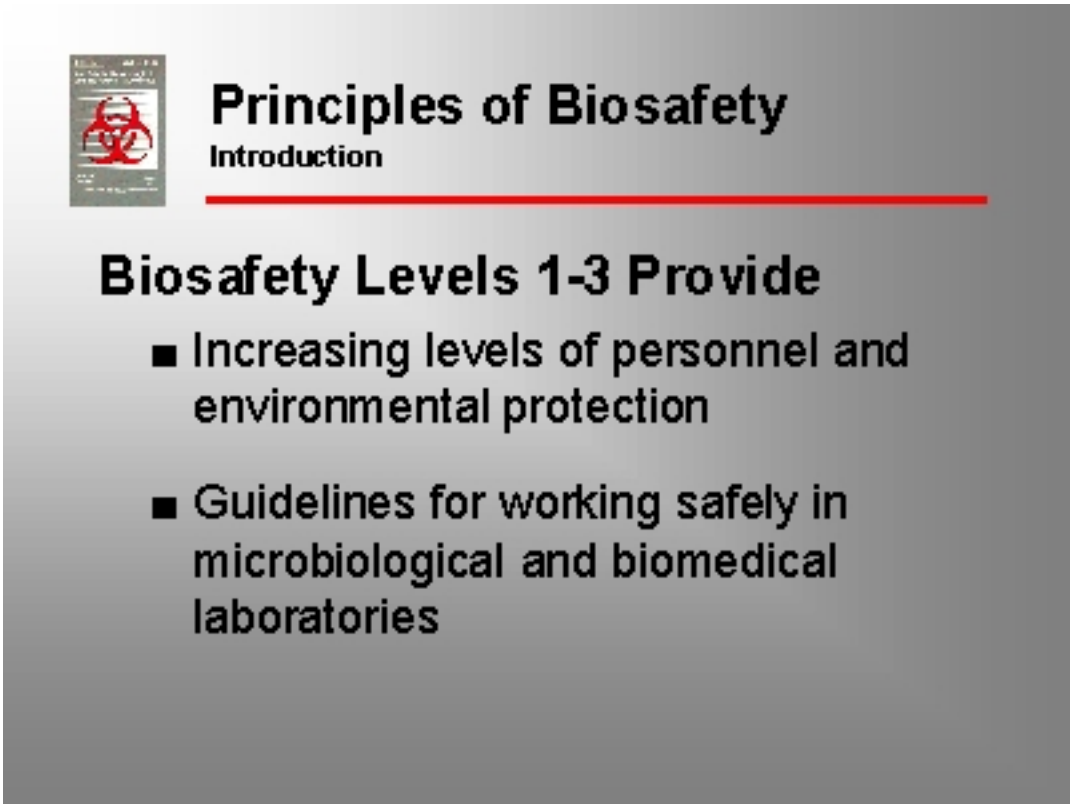

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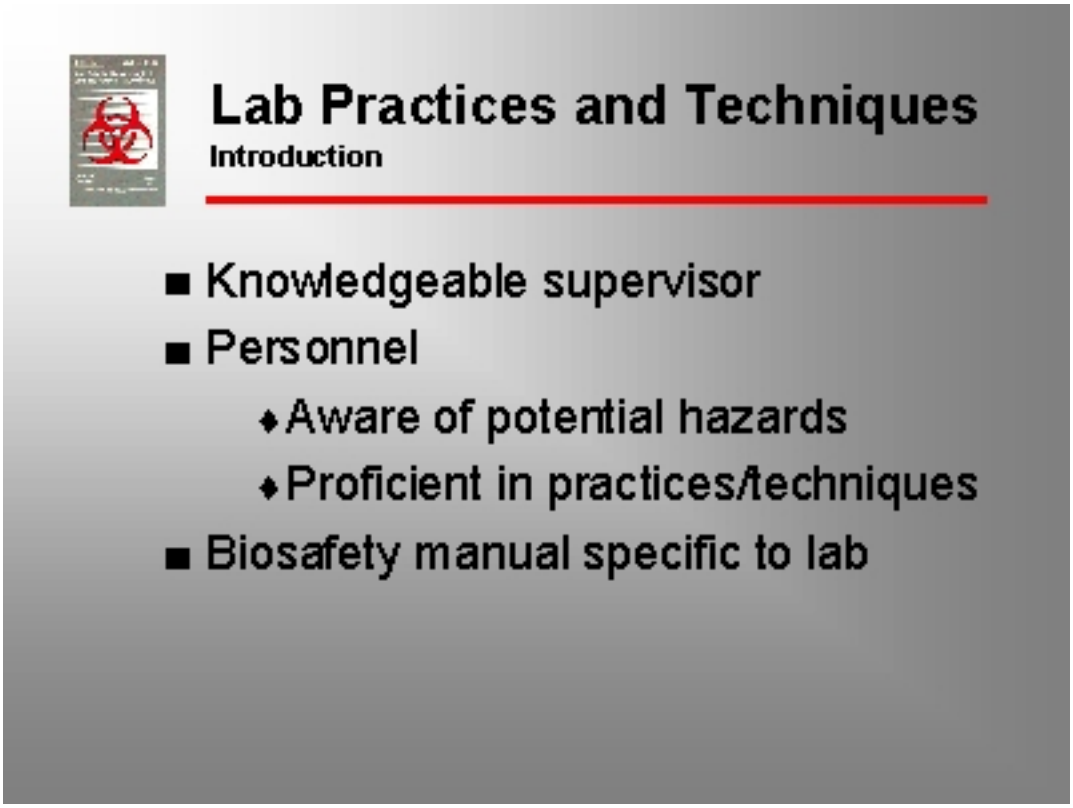

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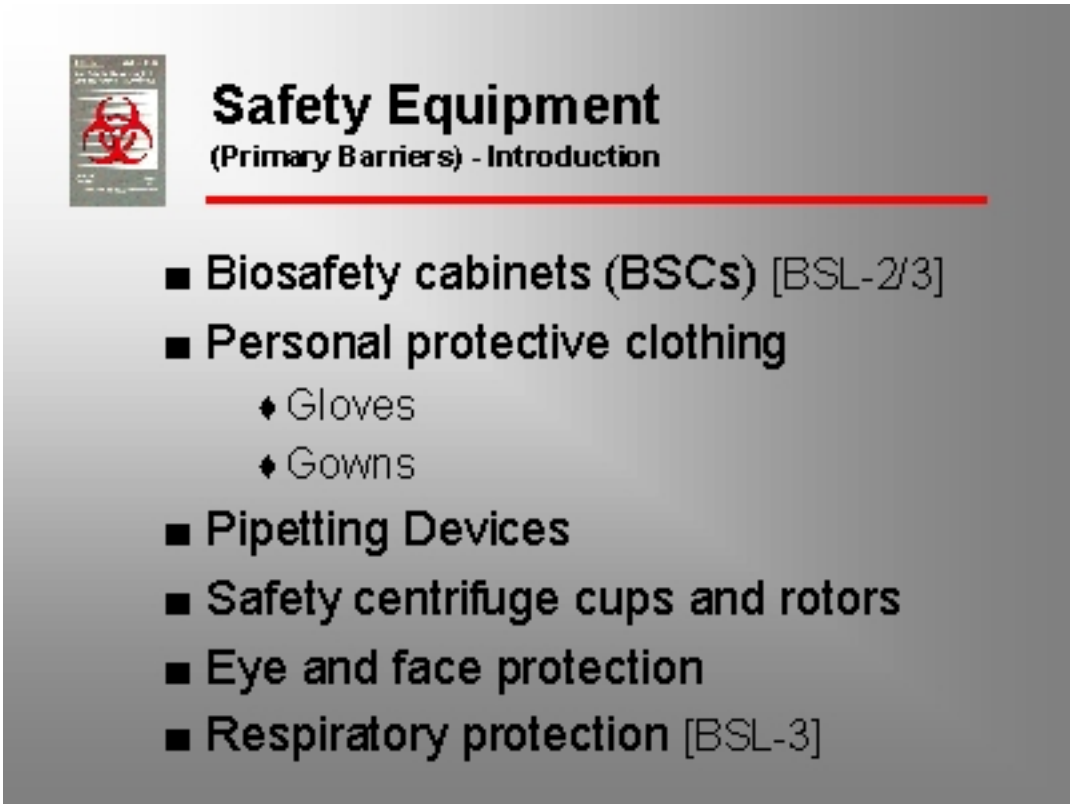

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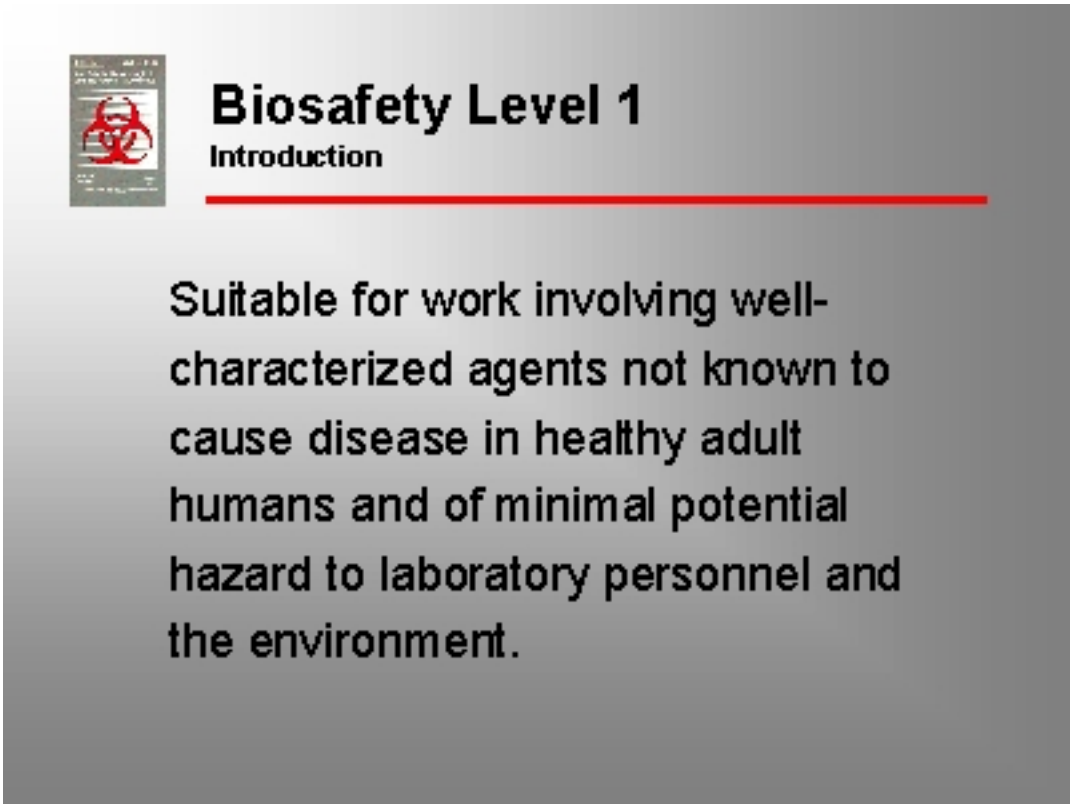

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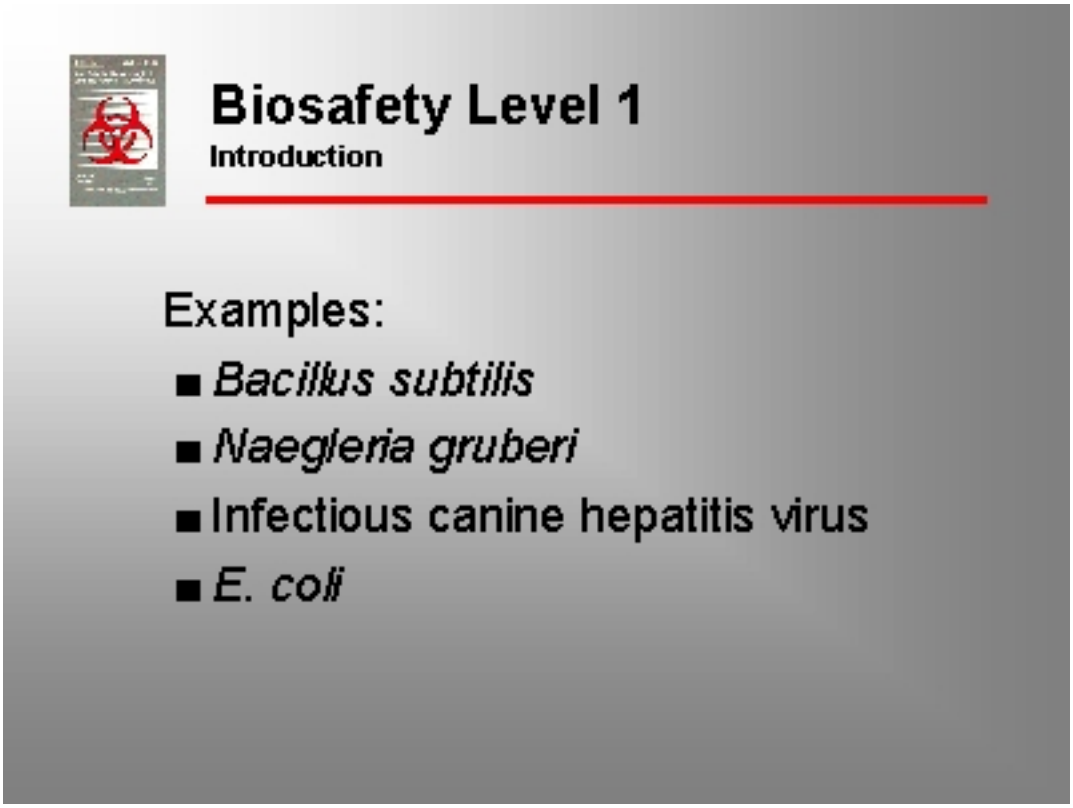

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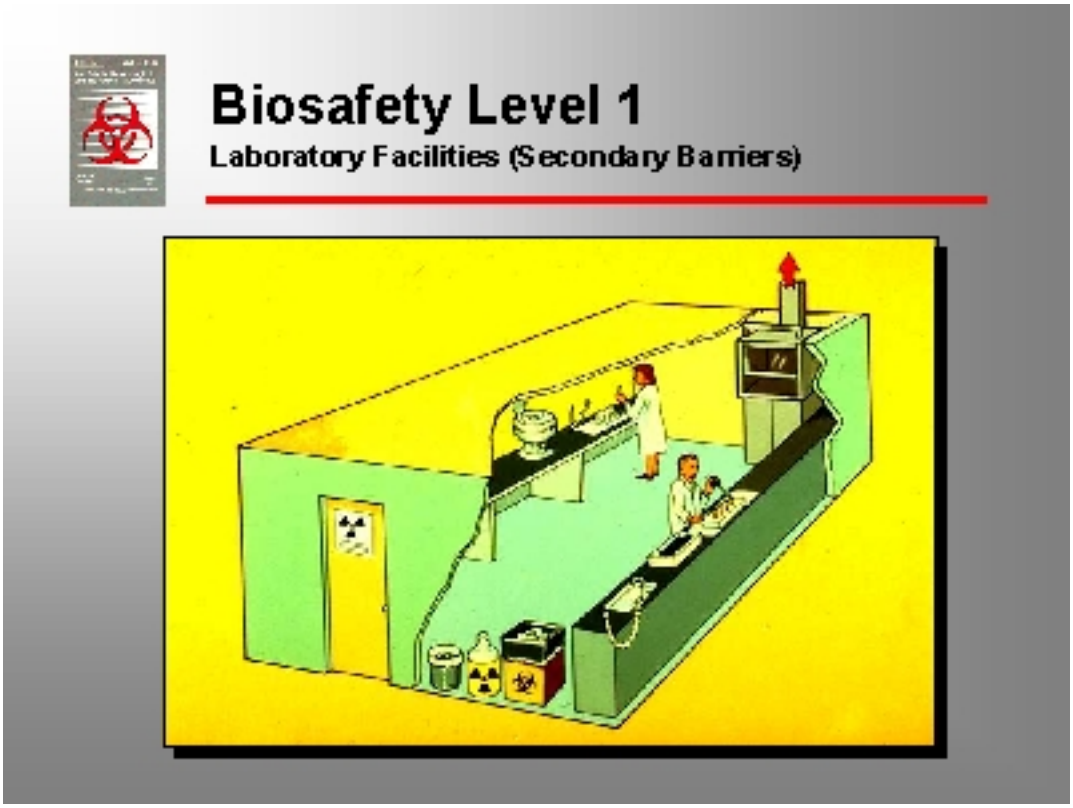

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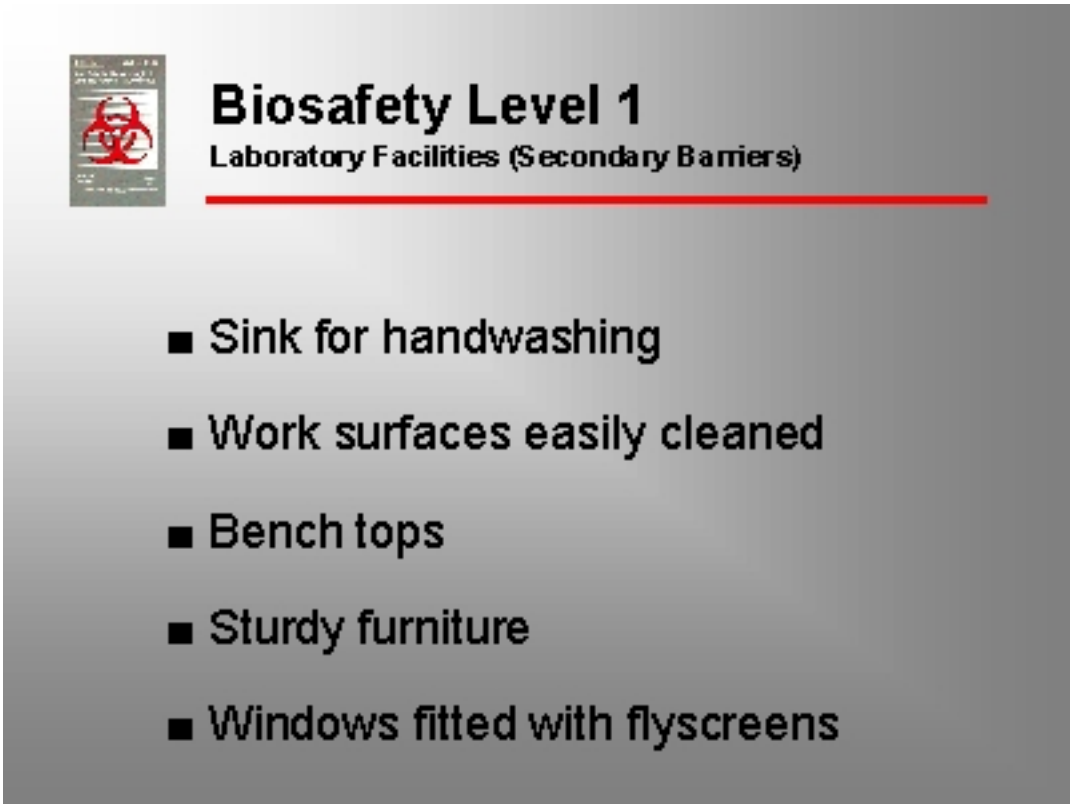

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Facility Design

(Secondary Barriers) - Introduction

Easily cleaned and decontaminated

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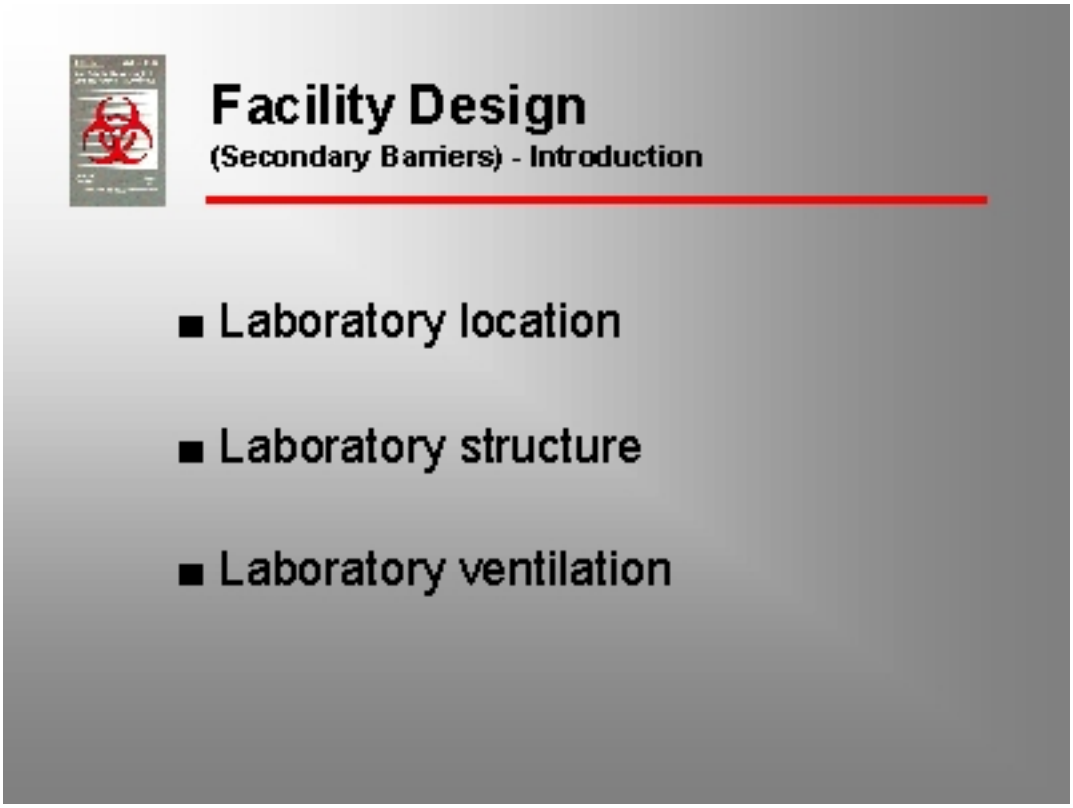

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Biosafety Level 1

Standard Microbiological Practices

Use mechanical pipetting devices

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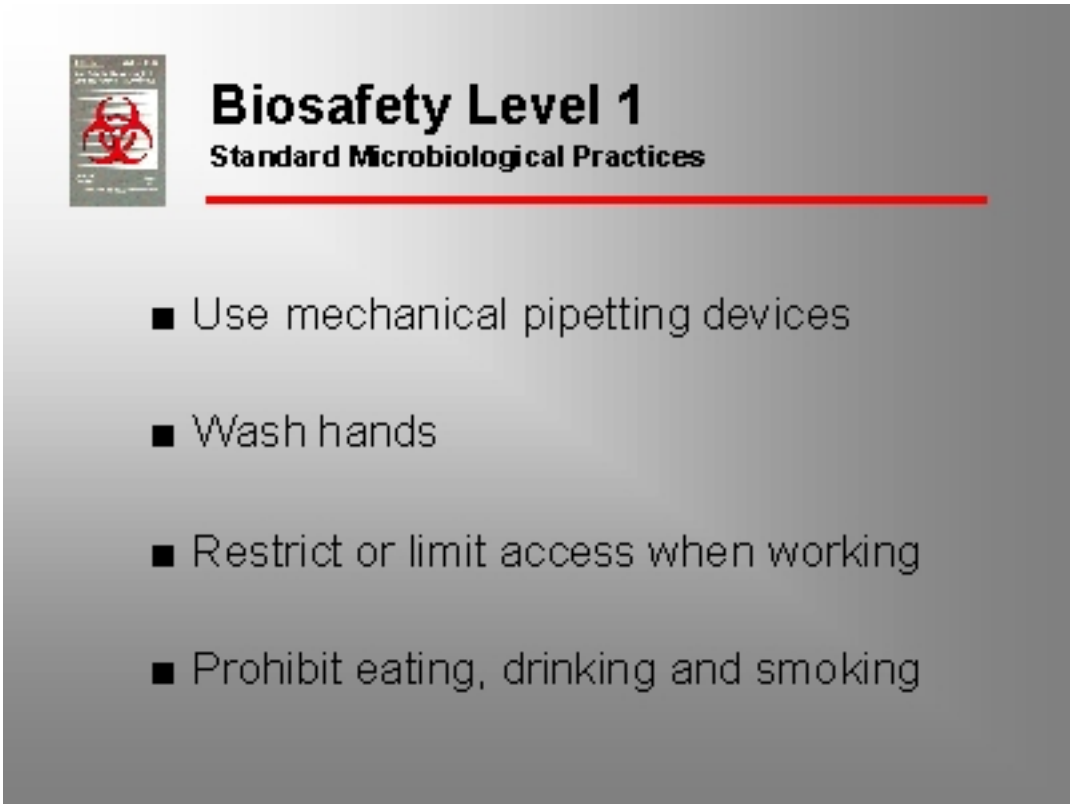

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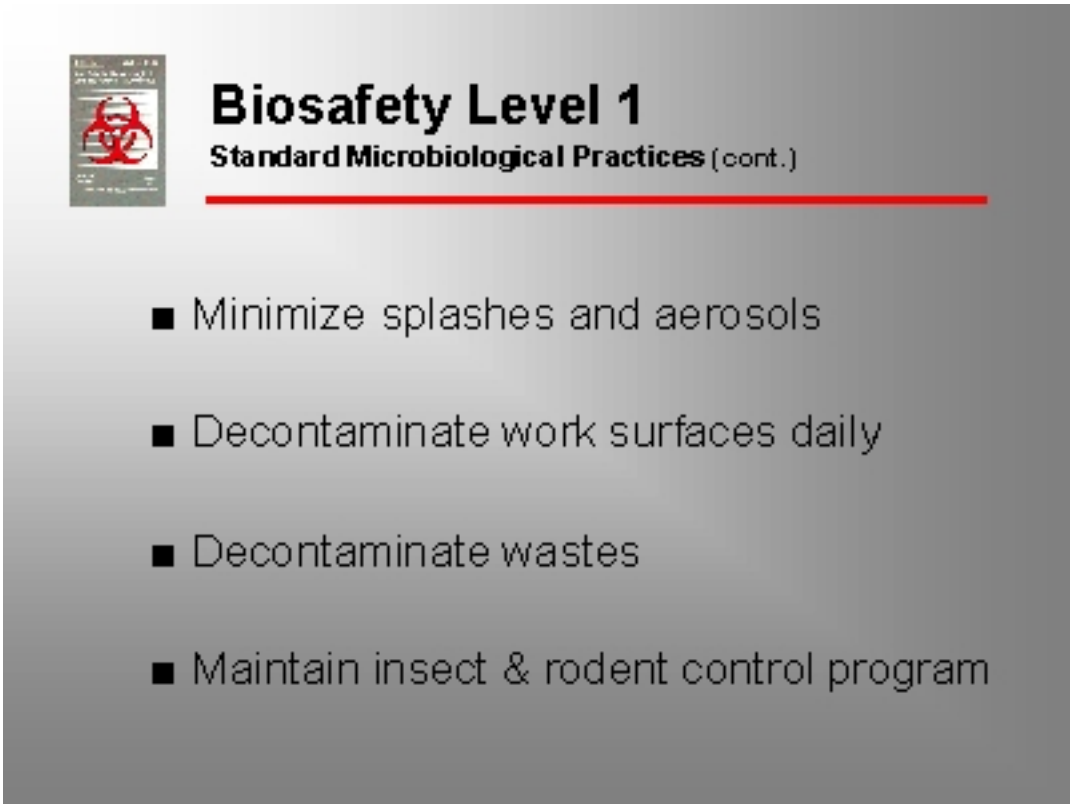

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Biosafety Level 1

Safety Equipment (Primary Barriers)

Protective clothing

- Lab coat
- Gloves

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Biosafety Level 1

Safety Equipment (Primary Barriers)

Additionally, PPE may be needed

- Face protection
- Eye protection

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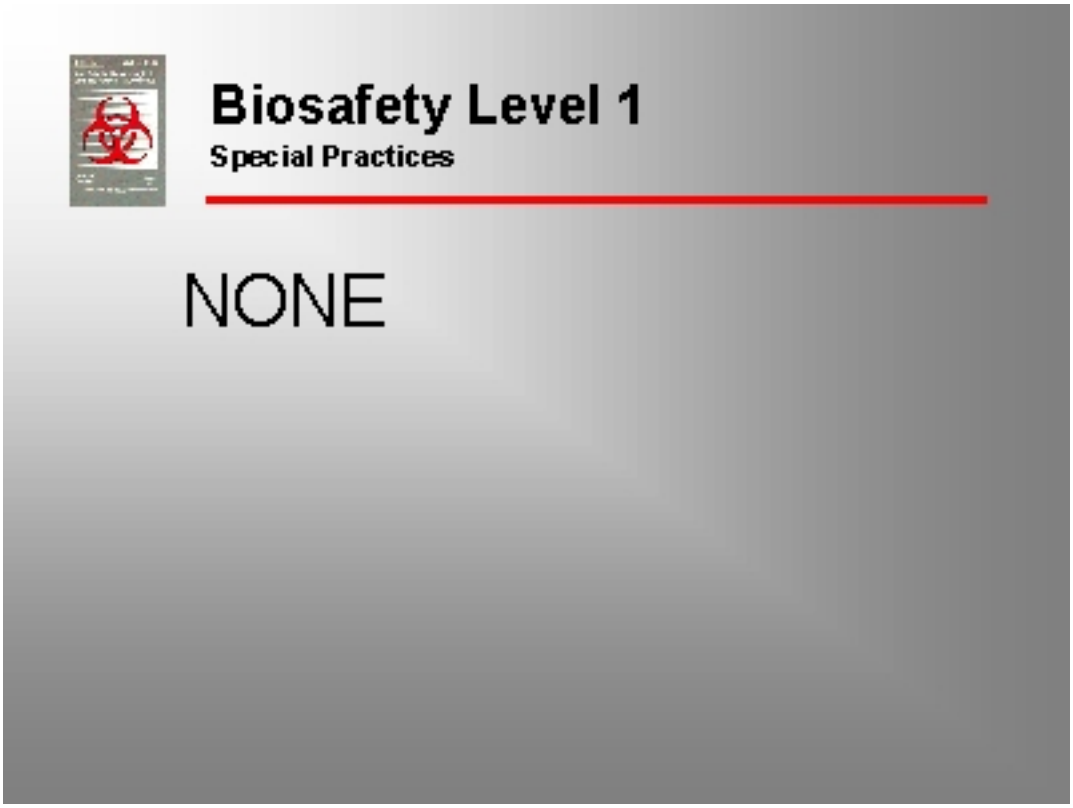

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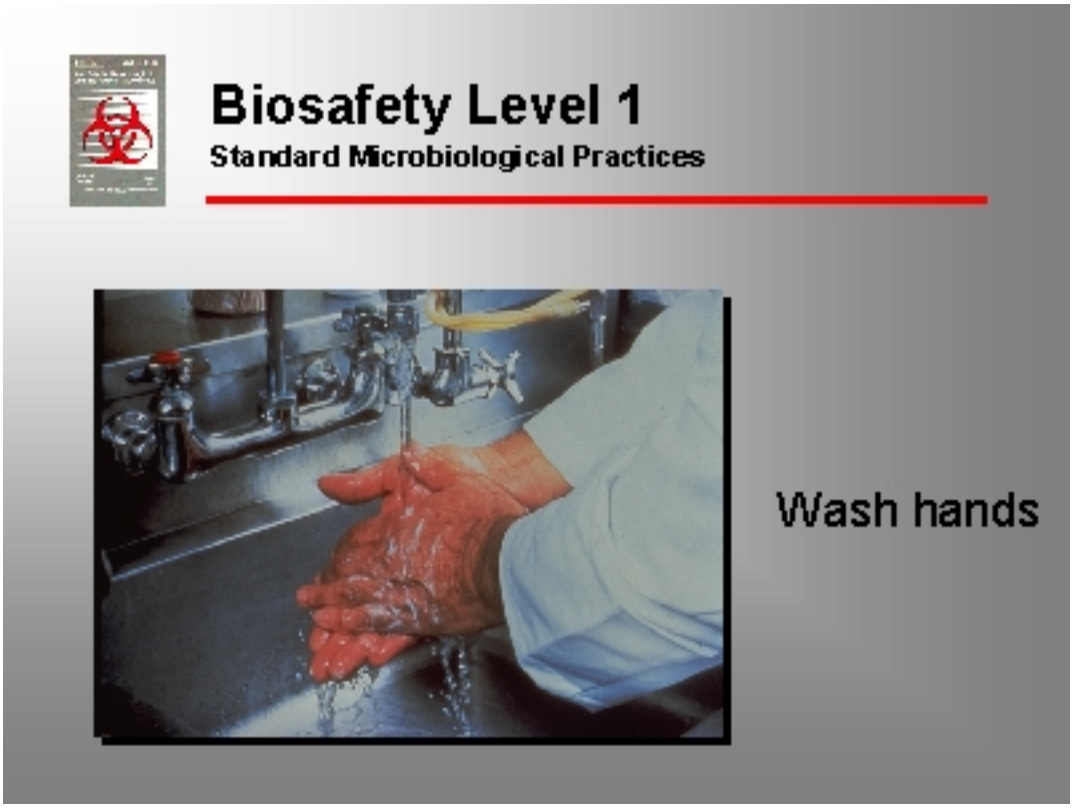

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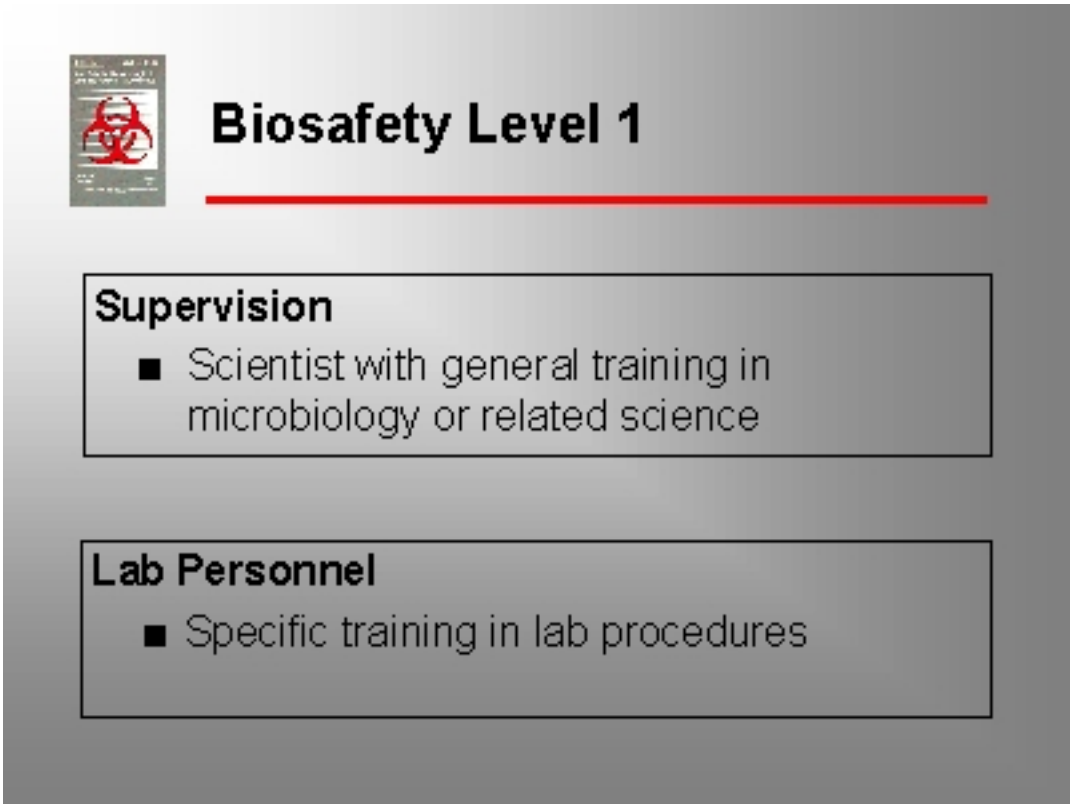

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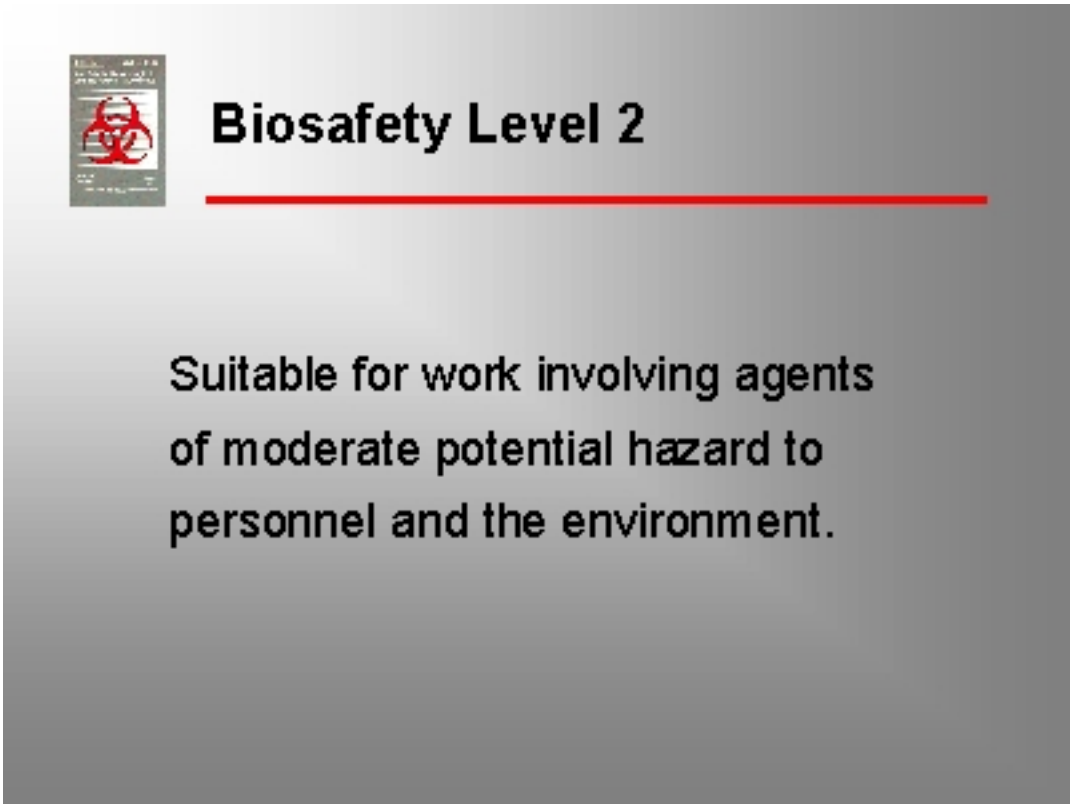
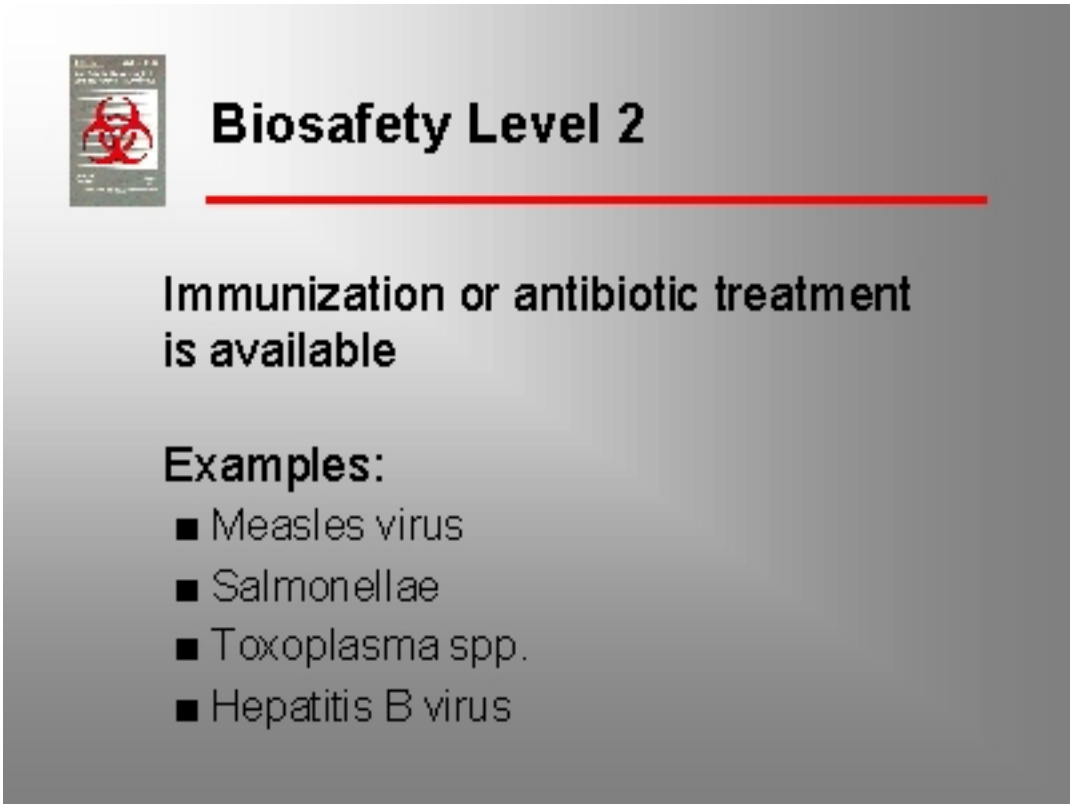
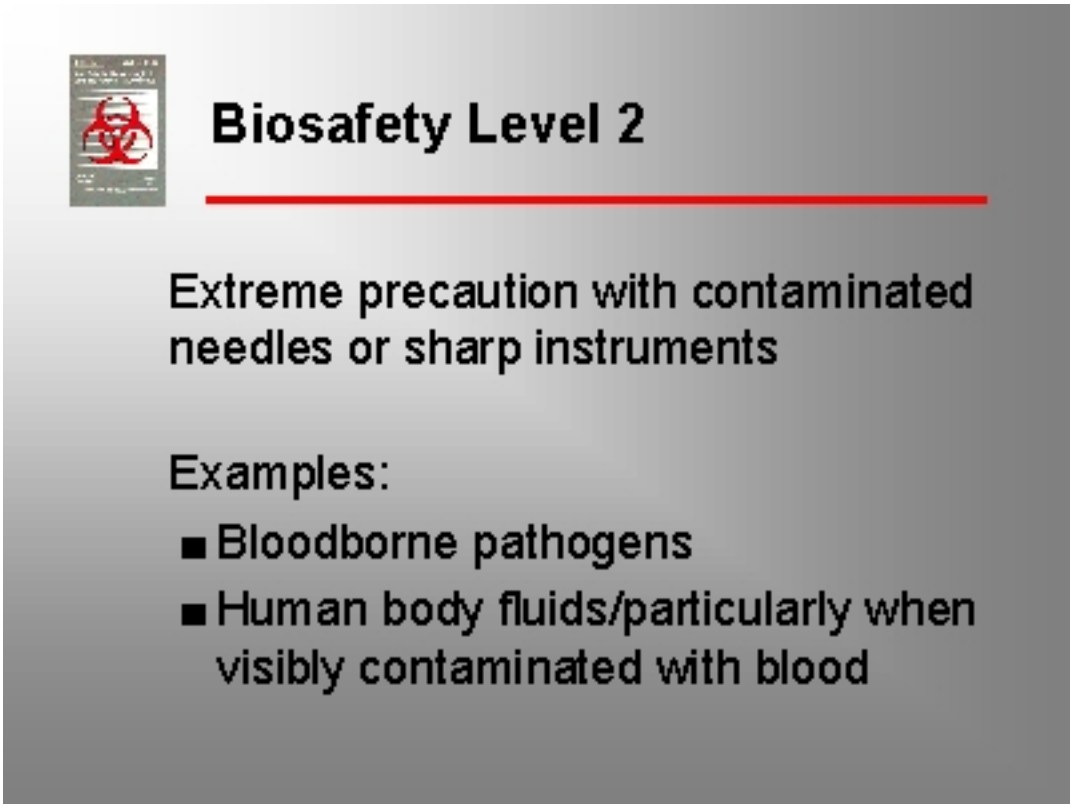

Biosafety Level 2
Suitable for work involving agents of moderate potential hazard to personnel and the environment.


Biosafety Level 2
Immunization or antibiotic treatment is available
Examples:
■ Measles virus
■ Salmonellae
■ Toxoplasma spp.
■ Hepatitis B virus



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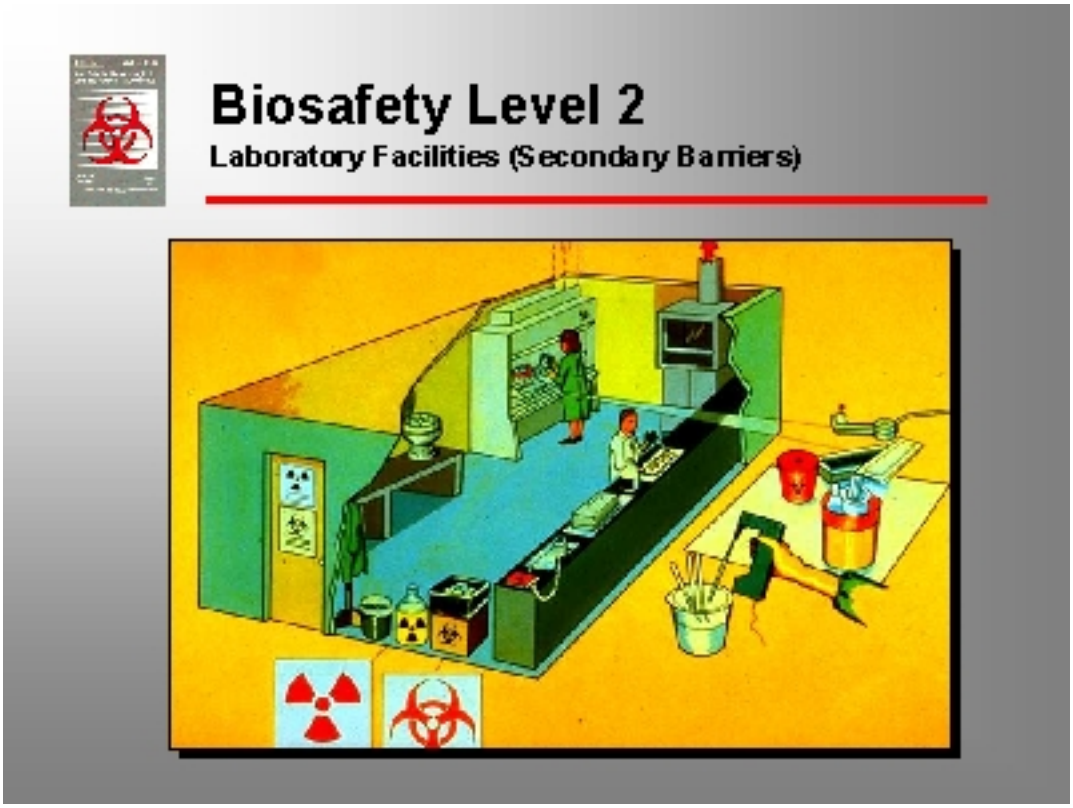

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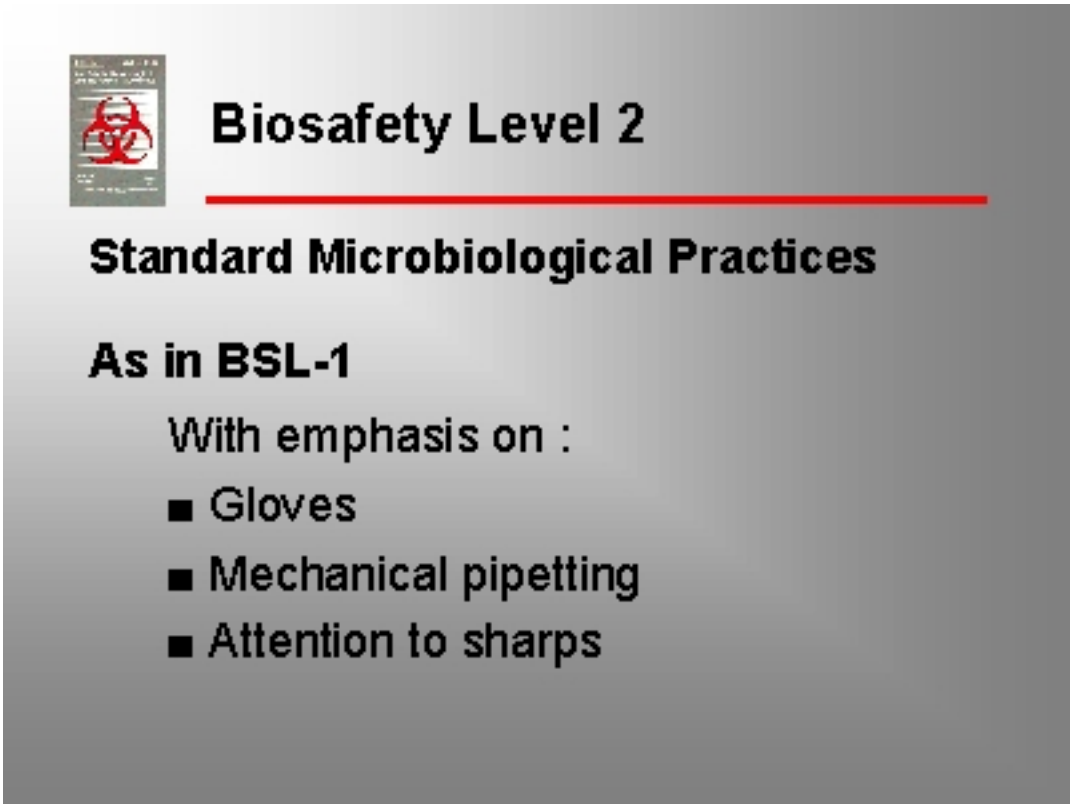
Biosafety Level 2
Standard Microbiological Practices
As in BSL-1
With emphasis on :
■ Gloves
■ Mechanical pipetting
■ Attention to sharps

Biosafety Level 2

Special Practices

Needles & Sharps Precautions

DON'T

Break, bend, resheath or reuse syringes or needles

DO

Use sharps containers

Biosafety Level 2

Special Practices (cont.)

Needles & Sharps Precautions

So someone won't be injured later

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Biosafety Level 2

Special Practices (cont.)

Needles & Sharps Precautions

DON'T

Touch broken glass with hands

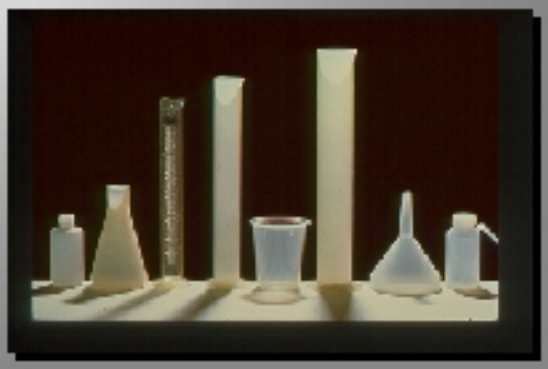
Biosafety Level 2

Special Practices (cont.)

Needles & Sharps Precautions

DO

Use plasticware



Biosafety Level 2

Special Practices (cont.)

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

Biosafety Level 2

Special Practices (cont.)

- Use leak-proof transport containers

Biosafety Level 2

Special Practices (cont.)

- Immunizations
- Baseline serum samples

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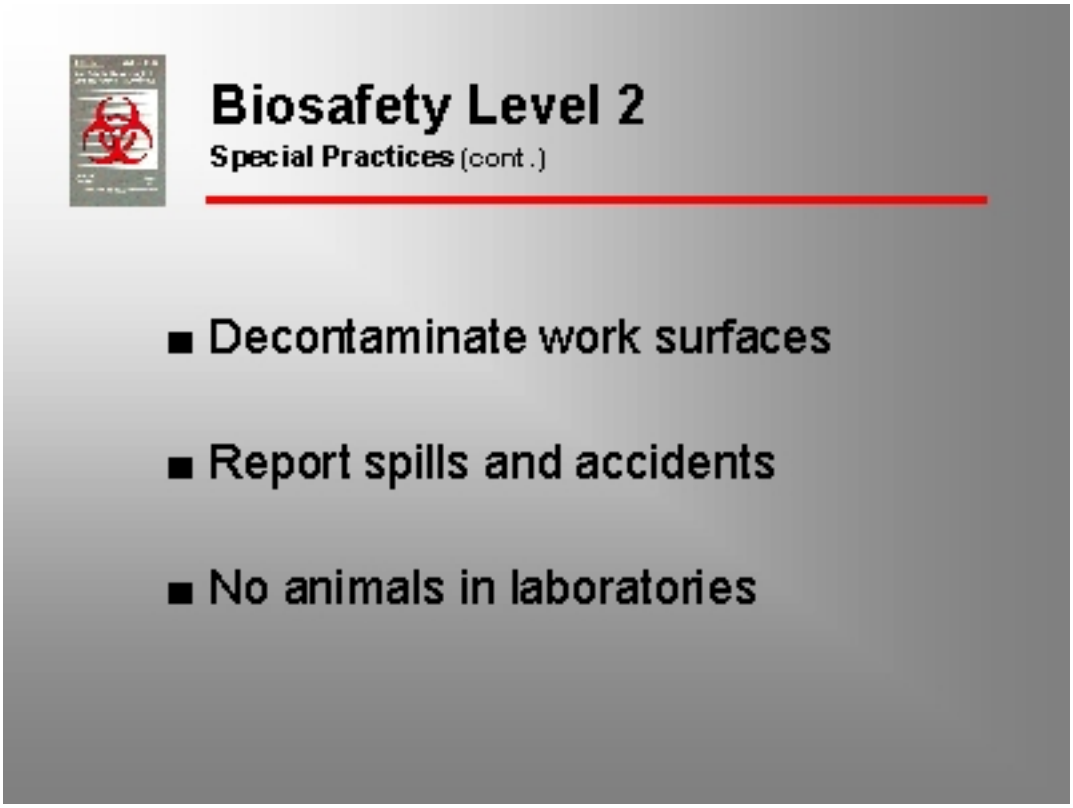

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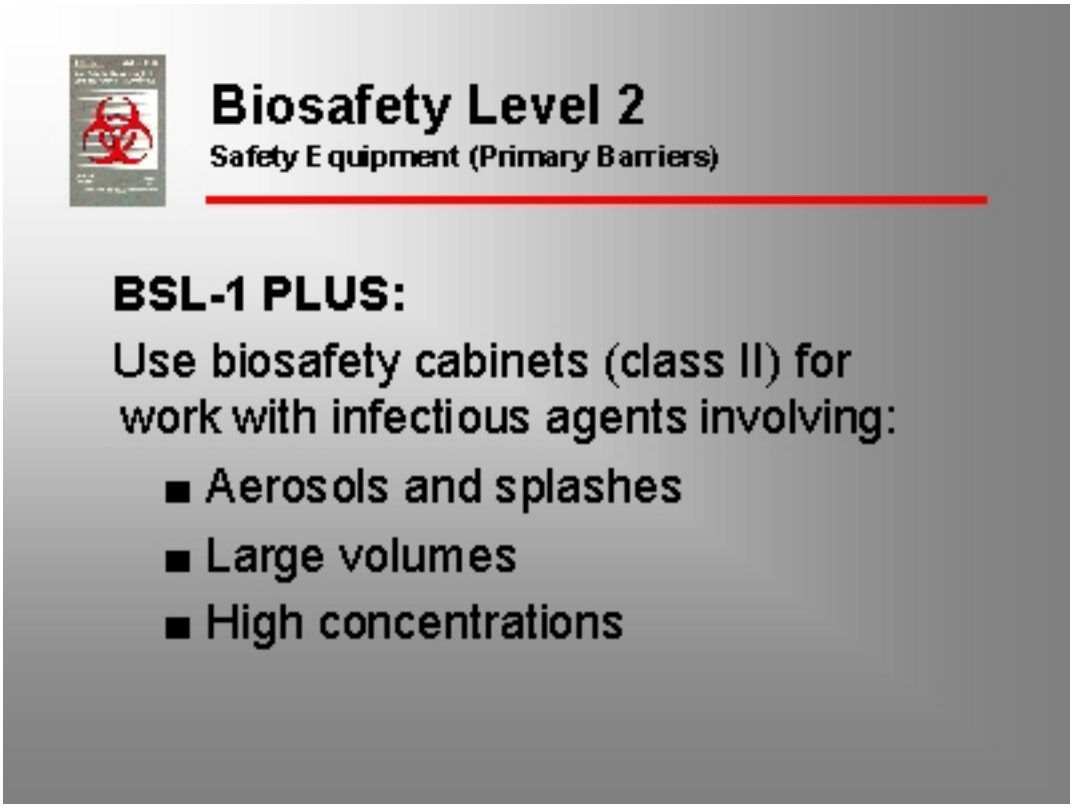
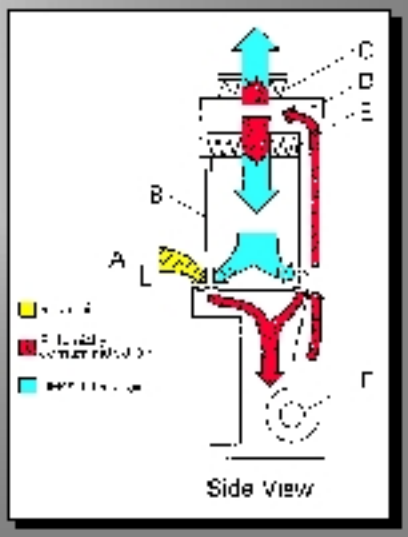

Biosafety Level 2
Safety Equipment (Primary Barriers)
BSL-1 PLUS:
Use biosafety cabinets (class II) for work with infectious agents involving:
■ Aerosols and splashes
■ Large volumes
■ High concentrations

Biosafety Level 2

Safety Equipment (Primary Barriers)

Class II Biosafety Cabinets

- Airflow

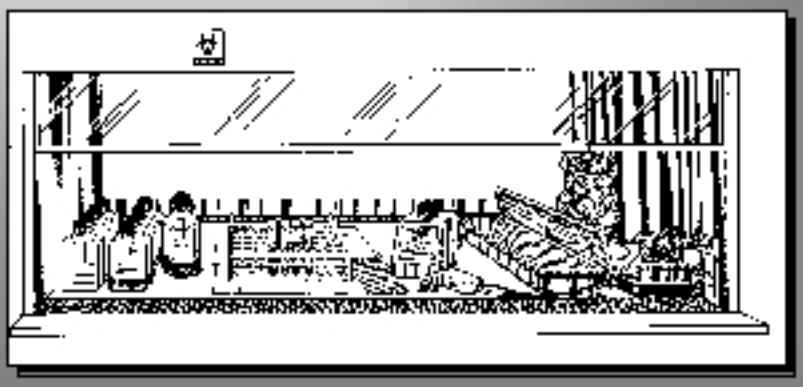


Biosafety Level 2

Safety Equipment (Primary Barriers)

Class II Biosafety Cabinets

- Layout of equipment



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Biosafety Level 2

Safety Equipment (Primary Barriers)

Class II Biosafety Cabinets

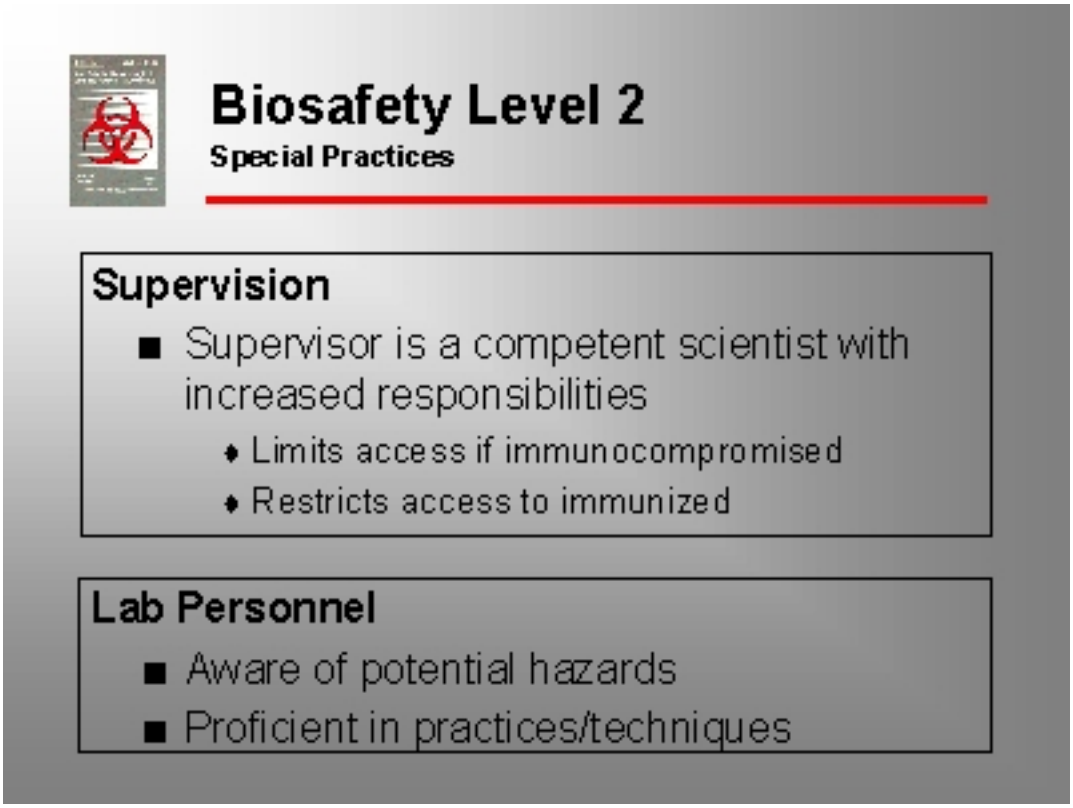
- Technique

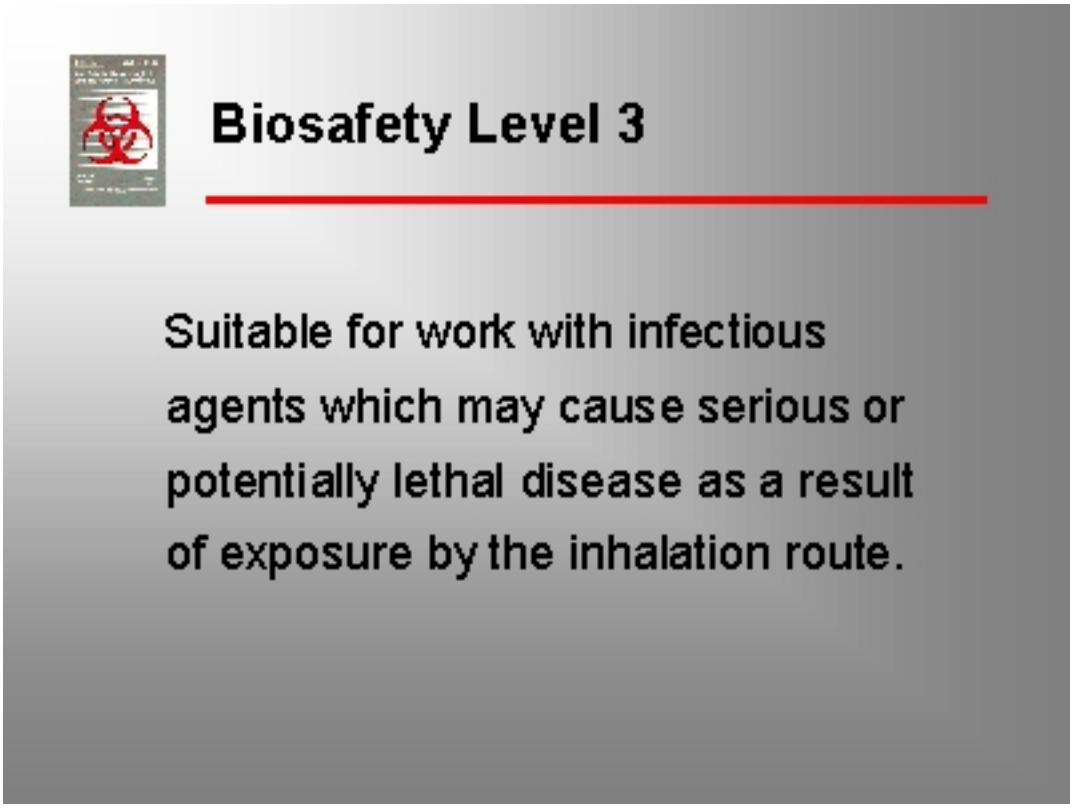
Biosafety Level 2

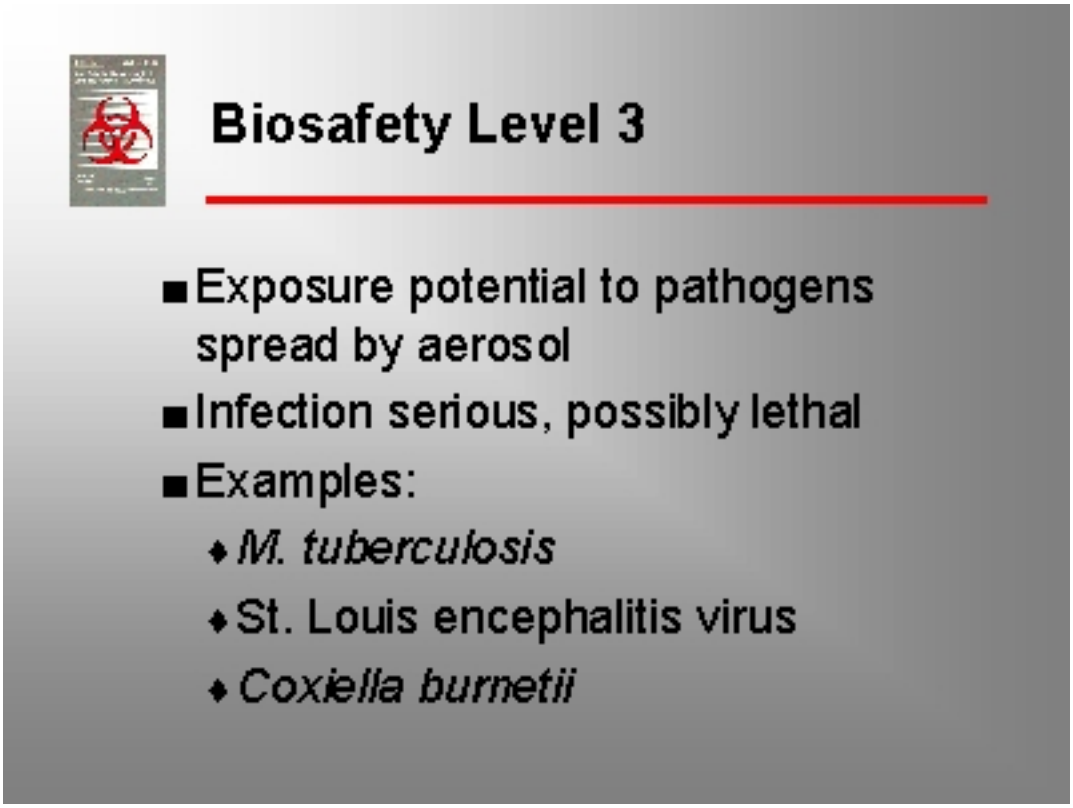
Laboratory Facilities (Secondary Barriers)

BSL-1 Facilities PLUS:

- Autoclave available
- Eyewash station available


Biosafety Level 2
Special Practices
Supervision
Supervisor is a competent scientist with increased responsibilities
Limits access if immunocompromised
Restricts access to immunized
Lab Personnel
Aware of potential hazards
Proficient in practices/techniques


Biosafety Level 3
Suitable for work with infectious agents which may cause serious or potentially lethal disease as a result of exposure by the inhalation route.



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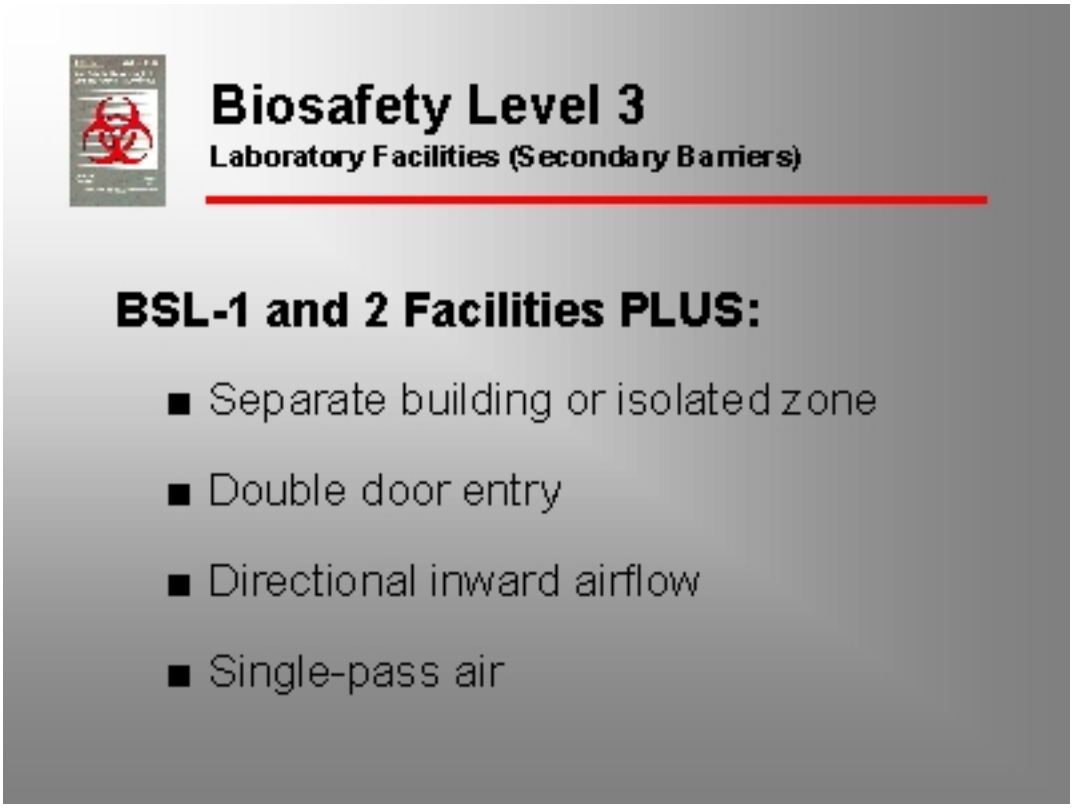

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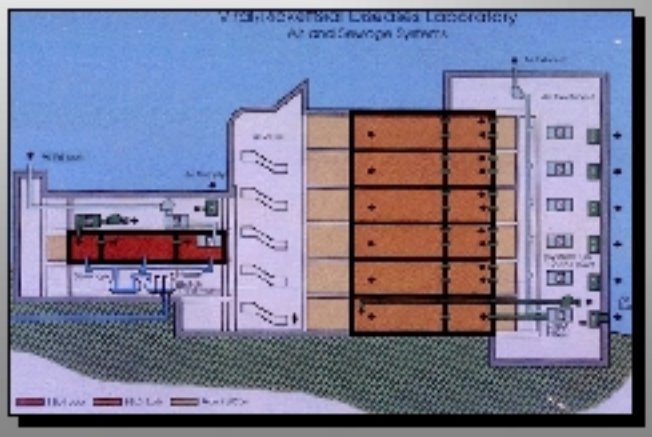
Facility Design

(Tertiary Barriers)

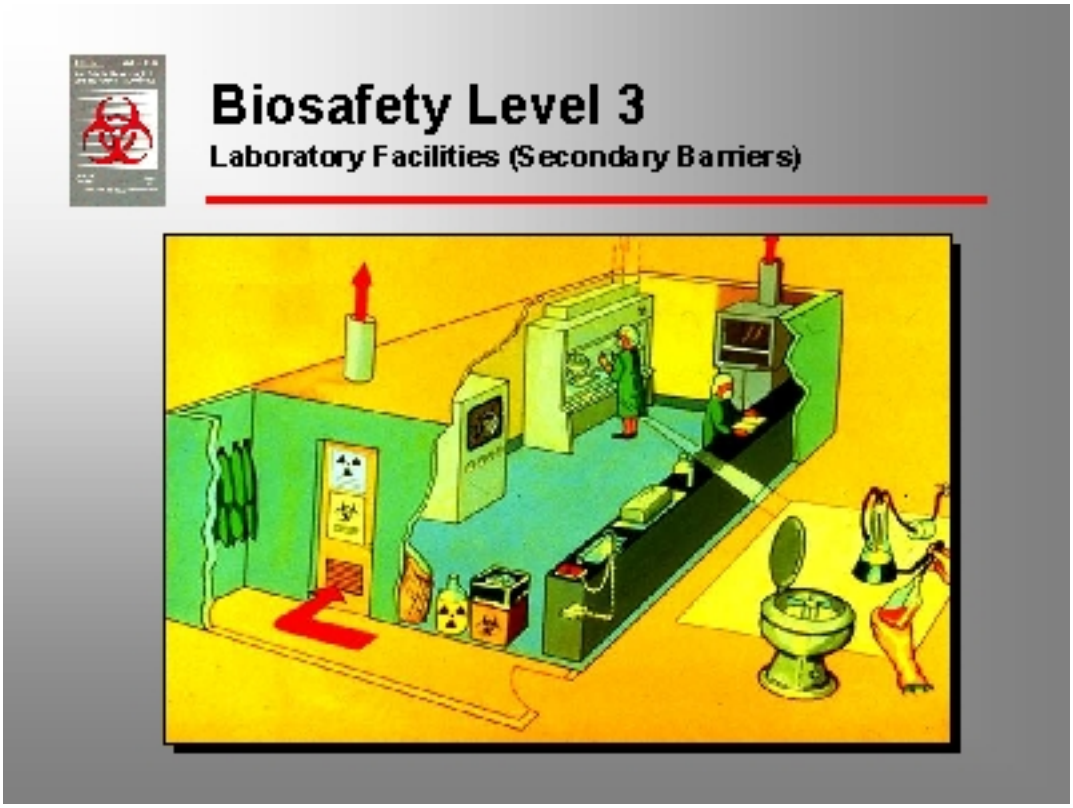
Location of CDC's MCL

Facility Design

(Tertiary Barriers)



Lab structure
Lab ventilation



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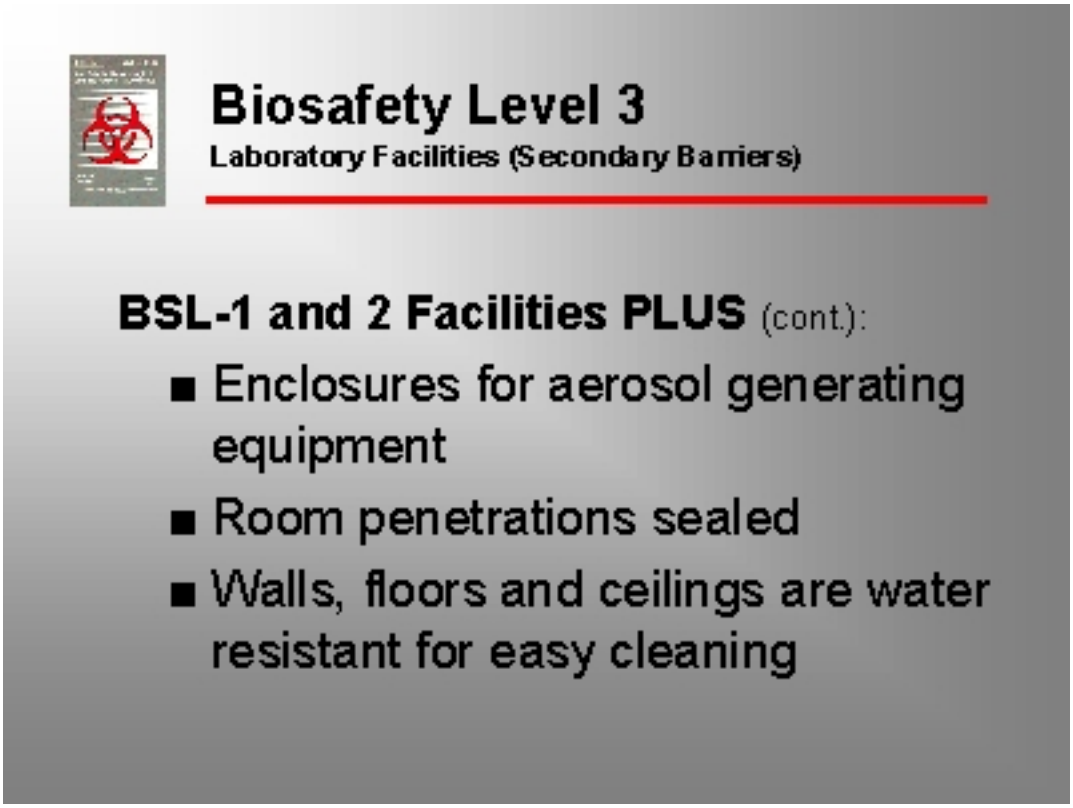

Biosafety Level 3
Laboratory Facilities (Secondary Barriers)
BSL-1 and 2 Facilities PLUS (cont.):
■ Enclosures for aerosol generating equipment
■ Room penetrations sealed
■ Walls, floors and ceilings are water resistant for easy cleaning

Biosafety Level 3

Special Practices

BSL-2 Special Practices PLUS:

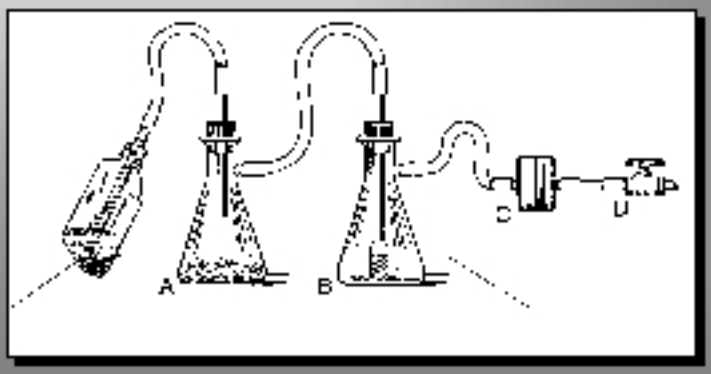
- Work in certified BSC
- Use bioaerosol-containing equipment
- Decontaminate spills promptly

Biosafety Level 3

Laboratory Facilities (Secondary Barriers)

BSL-1 and 2 Facilities PLUS:

- Vacuum lines protected



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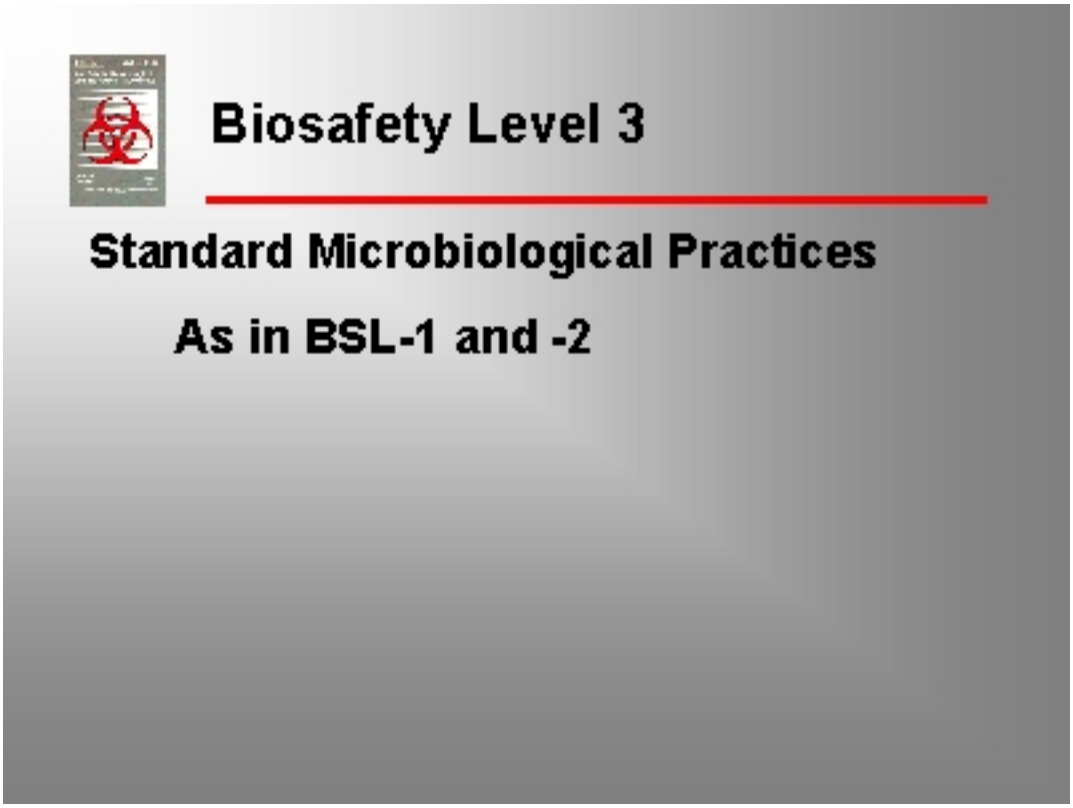

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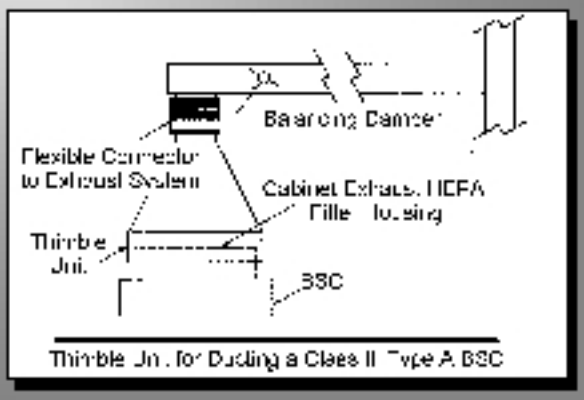
Biosafety Level 3

Safety Equipment (Primary Barriers)

BSL-1 and 2 Safety Equipment PLUS:

- BSC class II or III to manipulate infectious material



Thimble Jnt. for Ducting a Class II Type A BSC

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Biosafety Level 3

Safety Equipment (Primary Barriers)

BSL-1 and 2 Safety Equipment PLUS:

- Respiratory protection may be indicated

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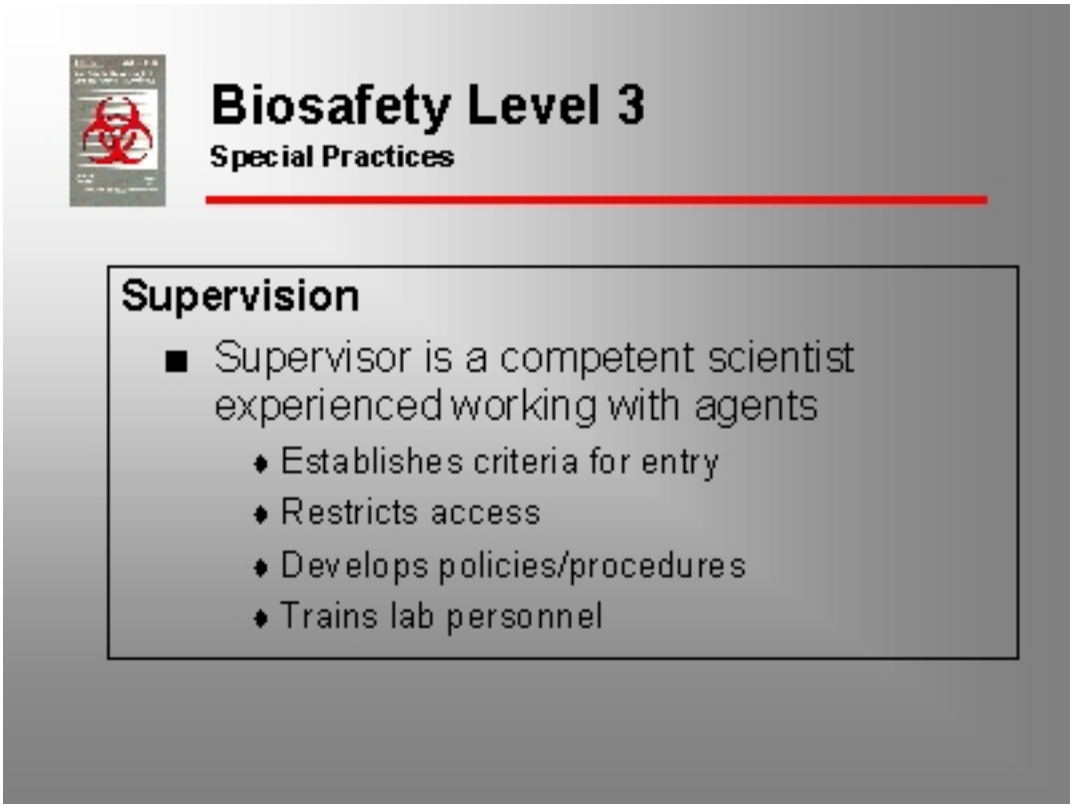
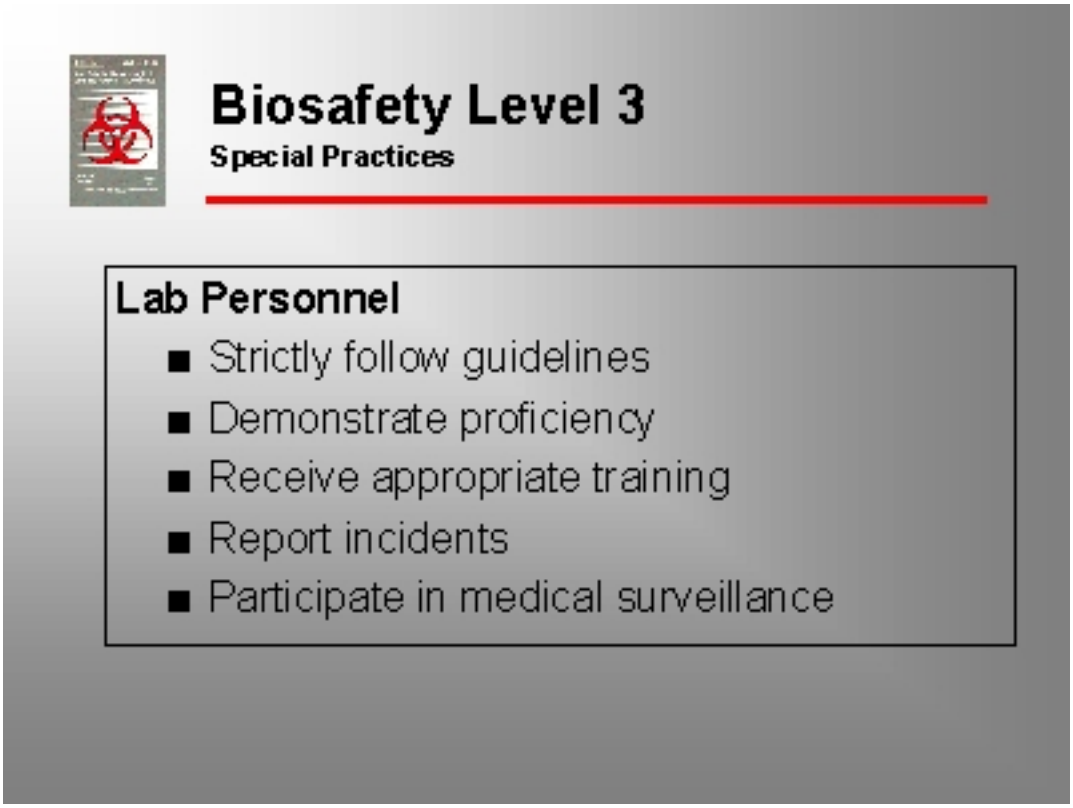

Biosafety Level 3
Special Practices
Supervision
Supervisor is a competent scientist experienced working with agents
Establishes criteria for entry
Restricts access
Develops policies/procedures
Trains lab personnel



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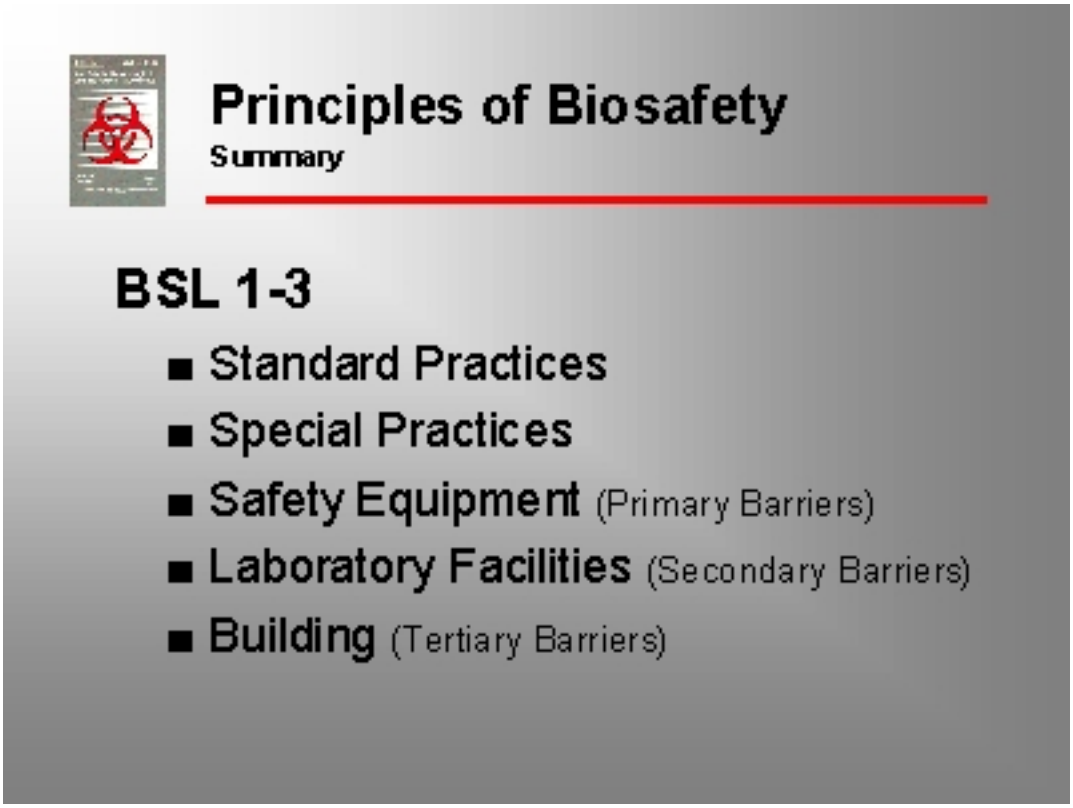

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5. Principles of Biosafety - Introduction Text
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41. Biosafety Level 3 Text

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44. Facility Design - (Tertiary Barriers) Text

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48. Biosafety Level 3 - Special Practices Text

49. Biosafety Level 3 - Laboratory Facilities (Secondary Barriers) Text

50. Biosafety Level 3 Text

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53. Biosafety Level 3 - Special Practices Text

54. Biosafety Level 3 - Special Practices Text

55. Principles of Biosafety - Summary Text

Office of Health and Safety, Centers for Disease Control and Prevention,
1600 Clifton Road N.E., Mail Stop F05 Atlanta, Georgia 30333, USA
Last Modified: 2/2/98

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