

Chapter 3 Laboratory Containment Levels for Biological Research Involving Microorganisms or Materials Potentially Harboring Microorganisms

HAZARD LEVELS

STANDARD MICROBIOLOGICAL PRACTICES

SPECIAL PRACTICES

CONTAINMENT EQUIPMENT

LABORATORY FACILITIES

OTHER REQUIREMENTS

	BL-1	BL-2	BL-3 Practice in BL-2 Facilities	BL-3
A. HAZARD LEVELS				
Degree of risk	Low Risk: <i>E. coli</i> K-12	Low to Moderate: Influenza viruses, Mycobacterium sp., Herpes viruses	Moderate to High: specific experiments w/BSL-3 agents which the IBC authorize in BSL-2 facilities	High (serious or potentially lethal consequences): <i>M. tuberculosis</i> , HTLV, HIV, <i>Brucella</i> sp.
B. STANDARD MICROBIOLOGICAL PRACTICES				
1. Public access while experiments are in process.	Not recommended	Access to the lab is limited when BSL-2 work is being conducted.	Restricted	Not permitted
2. Daily contamination	Daily & upon spills	Daily & upon spills	Daily, upon completion of work with biohazardous materials & spills.	Daily, upon completion of work with biohazardous materials & spills.
3. Biohazardous waste decontamination	Before disposal	Before disposal	Before disposal (See note 1).	Before removal from building.
4. Pipetting	Mechanical device	Mechanical device	Mechanical device	Mechanical device
5. Eating, drinking, application of cosmetics or contact lenses.	Permitted only in designated areas.	Not permitted in the laboratory.	Not permitted at any time.	Not permitted at any time.
			Required	Required

6. Handwashing facilities	Required	Required	(foot/elbow/electronic operation recommended)	(foot/elbow/electronic operation recommended)
7. Aerosol minimization procedures.	Required	Required	Required	Required
8. Laboratory coats and closed toed shoes.	Front button lab coats and closed toed shoes are recommended.	Front button lab coats are recommended and closed toed shoes are required.	Wrap around disposable clothing required for all workers with potential exposure to biohazardous materials. Closed toed shoes with shoe covers are required.	Wrap around disposable clothing required for all workers with potential exposure to biohazardous materials. Closed toed shoes with shoe covers are required.
C. SPECIAL PRACTICES				
1. Autoclave	Not Required	Must be available.	Must be available (See note 1).	Required, preferably in laboratory.
2. Insect/rodent control program	Required	Required	Required	Required
3. Transport of biohazardous waste material for processing (decontamination) away from lab.	Double orange bags in durable, closed, specially marked, leak-proof containers.	Double red bags in durable, closed, specially marked, leak-proof containers.	Double red bags in durable, closed, specially marked, leak-proof containers.	Biohazardous waste must be deactivated in double red bags in the laboratory and then transported in durable, closed, specially marked, leak-proof containers.
D. CONTAINMENT EQUIPMENT				
1. Biological Safety Cabinet (BSC)	Not required	Required for all aerosol generating processes.	Required for all work with biohazardous agents.	Required for all work with biohazardous agents.
2. Other physical containment	Equipment must be decontaminated immediately after use.	Physical containment devices are used when procedures with a high potential for creating aerosols are being conducted with biohazardous materials. (See note 2) If high concentrations or large volumes of biohazardous materials are used, some types of	Physical containment devices, such as centrifuge safety cups, sealed centrifuge rotors and containment caging for animals are used for all activities with biohazardous materials that pose a	Physical containment devices, such as centrifuge safety cups, sealed centrifuge rotors and containment caging for animals are used for all activities with biohazardous materials that pose a

		material may be centrifuged in the open laboratory if sealed heads or centrifuge safety cups are used, and if the containers are opened only in a biological safety cabinet. Equipment must be decontaminated immediately after use.	threat of aerosol exposure. (See note 3). Equipment must be decontaminated immediately after use.	threat of aerosol exposure. (See note 3). Equipment must be decontaminated immediately after use.
3. Freezers and refrigerators	No biohazard sign required.	Biohazard sign must be posted and containers must be clearly labeled.	Biohazard sign must be posted and containers must be clearly labeled.	Biohazard sign must be posted and containers must be clearly labeled.
4. BSC Certification	Certified annually	Certified annually	Certified annually	Certified annually
5. HEPA-filtered vacuum lines	Recommended	Required	Required	Required
6. BSC work surface decontamination	Daily & following spills.	Required after each use.	Required after each use.	Required after each use.
7. Personal Protective Equipment (PPE) when working within primary containment (e.g., BSC)	Required - gloves should be worn when handling infected animals and when skin contact with biohazardous materials is unavoidable.	Required - appropriate combinations of special protective clothing, gloves, etc., are used for all activities with biohazardous materials. (See note 4).	Required - appropriate combinations of special protective clothing, gloves, face masks or dust-mist respirators, are used for all activities with biohazardous materials. (See note 4)	Required - appropriate combinations of special protective clothing plus NIOSH N95 respirators or better must be worn in rooms containing infected animals.
8. Personal Protective Equipment (PPE) when working outside of primary containment.	Required - gloves should be worn when handling infected animals and when skin contact with biohazardous materials is unavoidable.	Required - appropriate combinations of special protective clothing plus NIOSH N95 respirators or better must be worn in rooms containing infected animals.	Required - appropriate combinations of special protective clothing plus NIOSH N95 respirators or better must be worn in rooms containing infected animals.	Required - appropriate combinations of special protective clothing plus NIOSH N95 respirators or better must be worn in rooms containing infected animals.
E. LABORATORY FACILITIES				

1. Ventilation	Negative pressure; no recirculation of air to other areas of the building.	Negative pressure; no recirculation of air to other areas of the building.	Air flows from low hazard to higher hazard areas; no recirculation of air is permitted.	Negative pressure; no recirculation of air to other areas of the building.
2. Posted biohazardous material/biosafety level signs	Not required	Required on lab doors in areas where BSL-2 materials are stored and where work is done.	Required on lab doors in areas where BSL-3 materials are stored and where work is done.	Required on lab doors in areas where BSL-3 materials are stored and where work is done.
3. Bench top work	Permitted	Permitted only for low-risk procedures.	Not permitted for biohazardous materials.	Not permitted for biohazardous materials.
4. Operable windows	Permitted with fly screens.	Not permitted	Not permitted	Not permitted
5. Laboratory separated from the general public.	No	Yes. Doors must be closed when work is being done with biohazardous materials.	Yes. Doors must be closed when work is being done with biohazardous materials.	Yes. Doors must be closed at all times.
F. OTHER REQUIREMENTS				
1. Technical training	Required with documentation.	Required with documentation.	Required with documentation.	Required with documentation.
2. Medical Surveillance (baseline serology)	Required when appropriate.	Required when appropriate.	Required when appropriate.	Required when appropriate.
3. Spill/accidents	Report immediately to lab director; medical evaluation, surveillance and treatment are provided as appropriate; maintain written records.	Report immediately to lab director; medical evaluation, surveillance and treatment are provided as appropriate; maintain written records.	Report immediately to lab director; medical evaluation, surveillance and treatment are provided as appropriate; maintain written records.	Report immediately to lab director; medical evaluation, surveillance and treatment are provided as appropriate; maintain written records.
4. Biosafety manual	Manual prepared or adopted; personnel required to be familiar with policies & procedures.	Manual prepared or adopted; personnel required to be familiar with policies & procedures.	Manual prepared or adopted; personnel required to be familiar with policies & procedures.	Manual prepared or adopted; personnel required to be familiar with policies & procedures.
	Check with the building monitor to obtain a copy	Check with the building monitor to obtain a copy	Check with the	Check with the

5. Evacuation procedures	of the evacuation procedures for the building. Evacuation procedures should be posted near laboratory exit door.	of the evacuation procedures for the building. Evacuation procedures should be posted near laboratory exit door.	building monitor to obtain a copy of the evacuation procedures for the building. Evacuation procedures should be posted near laboratory exit door.	building monitor to obtain a copy of the evacuation procedures for the building. Evacuation procedures should be posted near laboratory exit door.
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Note 1: Biohazardous waste must be placed in a marked, closed, leak-proof containers and under direct control of the responsible lab worker(s) until it is autoclaved. See Chapter 12 for detailed information on handling and disposing of various laboratory wastes properly.

Note 2: These procedures include centrifuging, grinding, blending, vigorous shaking or mixing, sonic disruption, opening containers of biohazardous materials whose internal pressures may be different from ambient pressures, inoculating animals intra-nasally, and harvesting infected tissues from animals or eggs.

Note 3: These procedures include manipulation of cultures and of clinical or environmental material that may be a source of aerosols containing biohazardous materials; the aerosol challenge of experimental animals; harvesting of tissues from infected animals and embryonic eggs; and necropsies of infected animals.

Note 4: Required with aerosol generating equipment; manipulation of high concentrations or large volumes of biohazardous materials; activity involving all clinical specimens; body fluids and tissues from humans or from infected animals or eggs; human cell culture; necropsies of infected animals.