

NIH Guidelines for Research Involving Recombinant DNA Molecules (April 2002)

APPENDIX G. PHYSICAL CONTAINMENT

Appendix G specifies physical containment for standard laboratory experiments and defines Biosafety Level 1 through Biosafety Level 4. For large-scale (over 10 liters) research or production, Appendix K (*Physical Containment for Large Scale Uses of Organisms Containing Recombinant DNA Molecules*) supersedes Appendix G. Appendix K defines Good Large Scale Practice through Biosafety Level 3 - Large Scale. For certain work with plants, Appendix P (*Physical and Biological Containment for Recombinant DNA Research Involving Plants*) supersedes Appendix G. Appendix P defines Biosafety Levels 1 through 4 - Plants. For certain work with animals, Appendix Q (*Physical and Biological Containment for Recombinant DNA Research Involving Animals*) supersedes Appendix G. Appendix Q defines Biosafety Levels 1 through 4 - Animals.

Appendix G-I. Standard Practices and Training

The first principle of containment is strict adherence to good microbiological practices (see [Appendices G-III-A through G-III-J](#), *Footnotes and References of Appendix G*). Consequently, all personnel directly or indirectly involved in experiments using recombinant DNA shall receive adequate instruction (see [Sections IV-B-1-h](#), *Responsibilities of the Institution--General Information*, and [IV-B-7-d](#), *Responsibilities of the Principal Investigator Prior to Initiating Research*). At a minimum, these instructions include training in aseptic techniques and in the biology of the organisms used in the experiments so that the potential biohazards can be understood and appreciated. Any research group working with agents that are known or potential biohazards shall have an emergency plan that describes the procedures to be followed if an accident contaminates personnel or the environment. The Principal Investigator shall ensure that everyone in the laboratory is familiar with both the potential hazards of the work and the emergency plan (see [Sections IV-B-7-d](#), *Responsibilities of the Principal Investigator Prior to Initiating Research* and [IV-B-7-e](#), *Responsibilities of the Principal Investigator During the Conduct of the Research*). If a research group is working with a known pathogen for which there is an effective vaccine, the vaccine should be made available to all workers. Serological monitoring, when clearly appropriate, will be provided (see [Section IV-B-1-f](#), *Responsibilities of the Institution--General Information*). The *Laboratory Safety Monograph* (see [Appendix G-III-O](#), *Footnotes and References of Appendix G*) and *Biosafety in Microbiological and Biomedical Laboratories* (see [Appendix G-III-B](#), *Footnotes and References of Appendix G*) describe practices, equipment, and facilities in detail.

Appendix G-II. Physical Containment Levels

The objective of physical containment is to confine organisms containing recombinant DNA molecules and to reduce the potential for exposure of the laboratory worker, persons outside of the laboratory, and the environment to organisms containing recombinant DNA molecules. Physical containment is achieved through the use of laboratory practices, containment equipment, and special laboratory design. Emphasis is placed on primary means of physical containment which are provided by laboratory practices and containment equipment.

Special laboratory design provides a secondary means of protection against the accidental release of organisms outside the laboratory or to the environment. Special laboratory design is used primarily in facilities in which experiments of moderate to high potential hazard are performed.

Combinations of laboratory practices, containment equipment, and special laboratory design can be made to achieve different levels of physical containment. Four levels of physical containment, which are designated as BL1, BL2, BL3, and BL4 are described. It should be emphasized that the descriptions and assignments of physical containment detailed below are based on existing approaches to containment of pathogenic organisms (see [Appendix G-III-B](#), *Footnotes and References of Appendix G*). The National Cancer Institute describes three levels for research on oncogenic viruses which roughly correspond to our BL2, BL3, and BL4 levels (see [Appendix G-III-C](#), *Footnotes and References of Appendix G*).

It is recognized that several different combinations of laboratory practices, containment equipment, and special laboratory design may be appropriate for containment of specific research activities. The *NIH Guidelines*, therefore, allow alternative selections of primary containment equipment within facilities that have been designed to provide BL3 and BL4 levels of physical containment. The selection of alternative methods of primary containment is dependent, however, on the level of biological containment provided by the host-vector system used in the experiment. Consideration will be given to other combinations which achieve an equivalent level of containment (see [Sections IV-C-1-b-\(1\)](#), *Major Actions* and [IV-C-1-b-\(2\)](#), *Minor Actions*).

Appendix G-II-A. Biosafety Level 1 (BL1) (See [Appendix G-III-M](#), *Footnotes and References of Appendix G*)

Appendix G-II-A-1. Standard Microbiological Practices (BL1)

Appendix G-II-A-1-a.

Access to the laboratory is limited or restricted at the discretion of the Principal Investigator when experiments are in progress.

Appendix G-II-A-1-b.

Work surfaces are decontaminated once a day and after any spill of viable material.

Appendix G-II-A-1-c.

All contaminated liquid or solid wastes are decontaminated before disposal.

Appendix G-II-A-1-d.

Mechanical pipetting devices are used; mouth pipetting is prohibited.

Appendix G-II-A-1-e.

Eating, drinking, smoking, and applying cosmetics are not permitted in the work area. Food may be stored in cabinets or refrigerators designated and used for this purpose only.

Appendix G-II-A-1-f.

Persons wash their hands: (i) after they handle materials involving organisms containing recombinant DNA molecules and animals, and (ii) before exiting the laboratory.

Appendix G-II-A-1-g.

All procedures are performed carefully to minimize the creation of aerosols.

Appendix G-II-A-1-h.

In the interest of good personal hygiene, facilities (e.g., hand washing sink, shower, changing room) and protective clothing (e.g., uniforms, laboratory coats) shall be provided that are appropriate for the risk of exposure to viable organisms containing recombinant DNA molecules.

Appendix G-II-A-2. Special Practices (BL1)

Appendix G-II-A-2-a.

Contaminated materials that are to be decontaminated at a site away from the laboratory are placed in a durable leak-proof container which is closed before being removed from the laboratory.

Appendix G-II-A-2-b.

An insect and rodent control program is in effect.

Appendix G-II-A-3. Containment Equipment (BL1)

Appendix G-II-A-3-a.

Special containment equipment is generally not required for manipulations of agents assigned to BL1.

Appendix G-II-A-4. Laboratory Facilities (BL1)

Appendix G-II-A-4-a.

The laboratory is designed so that it can be easily cleaned.

Appendix G-II-A-4-b.

Bench tops are impervious to water and resistant to acids, alkalis, organic solvents, and moderate heat.

Appendix G-II-A-4-c.

Laboratory furniture is sturdy. Spaces between benches, cabinets, and equipment are accessible for cleaning.

Appendix G-II-A-4-d.

Each laboratory contains a sink for hand washing.

Appendix G-II-A-4-e.

If the laboratory has windows that open, they are fitted with fly screens.

Appendix G-II-B. Biosafety Level 2 (BL2) (See [Appendix G-III-N](#), *Footnotes and References of Appendix G*)

Appendix G-II-B-1. Standard Microbiological Practices (BL2)

Appendix G-II-B-1-a.

Access to the laboratory is limited or restricted by the Principal Investigator when work with organisms containing recombinant DNA molecules is in progress.

Appendix G-II-B-1-b.

Work surfaces are decontaminated at least once a day and after any spill of viable material.

Appendix G-II-B-1-c.

All contaminated liquid or solid wastes are decontaminated before disposal.

Appendix G-II-B-1-d.

Mechanical pipetting devices are used; mouth pipetting is prohibited.

Appendix G-II-B-1-e.

Eating, drinking, smoking, and applying cosmetics are not permitted in the work area. Food may be stored in cabinets or refrigerators designated and used for this purpose only.

Appendix G-II-B-1-f.

Persons wash their hands: (i) after handling materials involving organisms containing recombinant DNA molecules and animals, and (ii) when exiting the laboratory.

Appendix G-II-B-1-g.

All procedures are performed carefully to minimize the creation of aerosols.

Appendix G-II-B-1-h.

Experiments of lesser biohazard potential can be conducted concurrently in carefully demarcated areas of the same laboratory.

Appendix G-II-B-2. Special Practices (BL2)

Appendix G-II-B-2-a.

Contaminated materials that are to be decontaminated at a site away from the laboratory are placed in a durable leak-proof container which is closed before being removed from the laboratory.

Appendix G-II-B-2-b.

The Principal Investigator limits access to the laboratory. The Principal Investigator has the final responsibility for assessing each circumstance and determining who may enter or work in the laboratory.

Appendix G-II-B-2-c.

The Principal Investigator establishes policies and procedures whereby only persons who have been advised of the potential hazard and meet any specific entry requirements (e.g., immunization) may enter the laboratory or animal rooms.

Appendix G-II-B-2-d.

When the organisms containing recombinant DNA molecules in use in the laboratory require special provisions for entry (e.g., vaccination), a hazard warning sign incorporating the universal biosafety symbol is posted on the access door to the laboratory work area. The hazard warning sign identifies the agent, lists the name and telephone number of the Principal Investigator or other responsible person(s), and indicates the special requirement(s) for entering the laboratory.

Appendix G-II-B-2-e.

An insect and rodent control program is in effect.

Appendix G-II-B-2-f.

Laboratory coats, gowns, smocks, or uniforms are worn while in the laboratory. Before exiting the laboratory for non-laboratory areas (e.g., cafeteria, library, administrative offices), this protective clothing is removed and left in the laboratory or covered with a clean coat not used in the laboratory.

Appendix G-II-B-2-g.

Animals not involved in the work being performed are not permitted in the laboratory.

Appendix G-II-B-2-h.

Special care is taken to avoid skin contamination with organisms containing recombinant DNA molecules; gloves should be worn when handling experimental animals and when skin contact with the agent is unavoidable.

Appendix G-II-B-2-i.

All wastes from laboratories and animal rooms are appropriately decontaminated before disposal.

Appendix G-II-B-2-j.

Hypodermic needles and syringes are used only for parenteral injection and aspiration of fluids from laboratory animals and diaphragm bottles. Only needle-locking syringes or disposable syringe-needle units (i.e., needle is integral to the syringe) are used for the injection or aspiration of fluids containing organisms that contain recombinant DNA molecules. Extreme caution should be used when handling needles and syringes to avoid autoinoculation and the generation of aerosols during use and disposal. Needles should not be bent, sheared, replaced in the needle sheath or guard, or removed from the syringe following use. The needle and syringe should be promptly placed in a puncture-resistant container and decontaminated, preferably autoclaved, before discard or reuse.

Appendix G-II-B-2-k.

Spills and accidents which result in overt exposures to organisms containing recombinant DNA molecules are immediately reported to the Institutional Biosafety Committee and NIH/OBA. Reports to NIH/OBA shall be sent to the Office of Biotechnology Activities, National Institutes of Health, 6705 Rockledge Drive, Suite 750, MSC 7985, Bethesda, MD 20892-7985 (20817 for non-USPS mail), 301-496-9838, 301-496- 9839 (fax). Medical evaluation, surveillance, and treatment are provided as appropriate and written records are maintained.

Appendix G-II-B-2-l.

When appropriate, considering the agent(s) handled, baseline serum samples for laboratory and other at-risk personnel are collected and stored. Additional serum specimens may be collected periodically depending on the agents handled or the function of the facility.

Appendix G-II-B-2-m.

A biosafety manual is prepared or adopted. Personnel are advised of special hazards and are required to read and follow instructions on practices and procedures.

Appendix G-II-B-3. Containment Equipment (BL2)

Appendix G-II-B-3-a.

Biological safety cabinets (Class I or II) (see [Appendix G-III-L](#), *Footnotes and References of Appendix G*) or other appropriate personal protective or physical containment devices are used whenever:

Appendix G-II-B-3-a-(1).

Procedures with a high potential for creating aerosols are conducted (see [Appendix G-III-O](#), *Footnotes and References of Appendix G*). These may include centrifuging, grinding, blending,

vigorous shaking or mixing, sonic disruption, opening containers of materials whose internal pressures may be different from ambient pressures, intranasal inoculation of animals, and harvesting infected tissues from animals or eggs.

Appendix G-II-B-3-a-(2).

High concentrations or large volumes of organisms containing recombinant DNA molecules are used. Such materials may be centrifuged in the open laboratory if sealed beads or centrifuge safety cups are used and if they are opened only in a biological safety cabinet.

Appendix G-II-B-4. Laboratory Facilities (BL2)

Appendix G-II-B-4-a.

The laboratory is designed so that it can be easily cleaned.

Appendix G-II-B-4-b.

Bench tops are impervious to water and resistant to acids, alkalis, organic solvents, and moderate heat.

Appendix G-II-B-4-c.

Laboratory furniture is sturdy and spaces between benches, cabinets, and equipment are accessible for cleaning.

Appendix G-II-B-4-d.

Each laboratory contains a sink for hand washing.

Appendix G-II-B-4-e.

If the laboratory has windows that open, they are fitted with fly screens.

Appendix G-II-B-4-f.

An autoclave for decontaminating laboratory wastes is available.

Appendix G-II-C. Biosafety Level 3 (BL3) (See [Appendix G-III-P](#), *Footnotes and References of Appendix G*)

Appendix G-II-C-1. Standard Microbiological Practices (BL3)

Appendix G-II-C-1-a.

Work surfaces are decontaminated at least once a day and after any spill of viable material.

Appendix G-II-C-1-b.

All contaminated liquid or solid wastes are decontaminated before disposal.

Appendix G-II-C-1-c.

Mechanical pipetting devices are used; mouth pipetting is prohibited.

Appendix G-II-C-1-d.

Eating, drinking, smoking, storing food, and applying cosmetics are not permitted in the work area.

Appendix G-II-C-1-e.

Persons wash their hands: (i) after handling materials involving organisms containing recombinant DNA molecules, and handling animals, and (ii) when exiting the laboratory.

Appendix G-II-C-1-f.

All procedures are performed carefully to minimize the creation of aerosols.

Appendix G-II-C-1-g.

Persons under 16 years of age shall not enter the laboratory.

Appendix G-II-C-1-h.

If experiments involving other organisms which require lower levels of containment are to be conducted in the same laboratory concurrently with experiments requiring BL3 level physical containment, they shall be conducted in accordance with all BL3 level laboratory practices.

Appendix G-II-C-2. Special Practices (BL3)

Appendix G-II-C-2-a.

Laboratory doors are kept closed when experiments are in progress.

Appendix G-II-C-2-b.

Contaminated materials that are to be decontaminated at a site away from the laboratory are placed in a durable leak-proof container which is closed before being removed from the laboratory.

Appendix G-II-C-2-c.

The Principal Investigator controls access to the laboratory and restricts access to persons whose presence is required for program or support purposes. The Principal Investigator has the final responsibility for assessing each circumstance and determining who may enter or work in the laboratory.

Appendix G-II-C-2-d.

The Principal Investigator establishes policies and procedures whereby only persons who have been advised of the potential biohazard, who meet any specific entry requirements (e.g., immunization), and who comply with all entry and exit procedures entering the laboratory or animal rooms.

Appendix G-II-C-2-e.

When organisms containing recombinant DNA molecules or experimental animals are present in the laboratory or containment module, a hazard warning sign incorporating the universal biosafety symbol is posted on all laboratory and animal room access doors. The hazard warning sign identifies the agent, lists the name and telephone number of the Principal Investigator or other responsible person(s), and indicates any special requirements for entering the laboratory such as the need for immunizations, respirators, or other personal protective measures.

Appendix G-II-C-2-f.

All activities involving organisms containing recombinant DNA molecules are conducted in biological safety cabinets or other physical containment devices within the containment module. No work in open vessels is conducted on the open bench.

Appendix G-II-C-2-g.

The work surfaces of biological safety cabinets and other containment equipment are decontaminated when work with organisms containing recombinant DNA molecules is finished. Plastic-backed paper toweling used on non-perforated work surfaces within biological safety cabinets facilitates clean-up.

Appendix G-II-C-2-h.

An insect and rodent program is in effect.

Appendix G-II-C-2-i.

Laboratory clothing that protects street clothing (e.g., solid front or wrap-around gowns, scrub suits, coveralls) is worn in the laboratory. Laboratory clothing is not worn outside the laboratory, and it is decontaminated prior to laundering or disposal.

Appendix G-II-C-2-j.

Special care is taken to avoid skin contamination with contaminated materials; gloves should be worn when handling infected animals and when skin contact with infectious materials is unavoidable.

Appendix G-II-C-2-k.

Molded surgical masks or respirators are worn in rooms containing experimental animals.

Appendix G-II-C-2-l.

Animals and plants not related to the work being conducted are not permitted in the laboratory.

Appendix G-II-C-2-m.

Laboratory animals held in a BL3 area shall be housed in partial-containment caging systems, such as Horsfall units (see [Appendix G-III-K](#), *Footnotes and References of Appendix G*), open cages placed in ventilated enclosures, solid-wall and -bottom cages covered by filter bonnets or solid-wall and -bottom cages placed on holding racks equipped with ultraviolet in radiation lamps and reflectors.

Note: Conventional caging systems may be used provided that all personnel wear appropriate personal protective devices. These protective devices shall include at a minimum wrap-around gowns, head covers, gloves, shoe covers, and respirators. All personnel shall shower on exit from areas where these devices are required.

Appendix G-II-C-2-n.

All wastes from laboratories and animal rooms are appropriately decontaminated before disposal.

Appendix G-II-C-2-o.

Vacuum lines are protected with high efficiency particulate air/HEPA filters and liquid disinfectant traps.

Appendix G-II-C-2-p.

Hypodermic needles and syringes are used only for parenteral injection and aspiration of fluids from laboratory animals and diaphragm bottles. Only needle locking syringes or disposable syringe-needle units (i.e., needle is integral to the syringe) are used for the injection or aspiration of fluids containing organisms that contain recombinant DNA molecules. Extreme caution should be used when handling needles and syringes to avoid autoinoculation and the generation of aerosols during use and disposal. Needles should not be bent, sheared, replaced in the needle sheath or guard, or removed from the syringe following use. The needle and syringe should be promptly placed in a puncture-resistant container and decontaminated, preferably by autoclaving, before discard or reuse.

Appendix G-II-C-2-q.

Spills and accidents which result in overt or potential exposures to organisms containing recombinant DNA molecules are immediately reported to the Biological Safety Officer, Institutional Biosafety Committee, and NIH/OBA. Reports to NIH/OBA shall be sent to the Office of Biotechnology Activities, National Institutes of Health, 6705 Rockledge Drive, Suite 750, MSC 7985, Bethesda, MD 20892-7985 (20817 for non-USPS mail), 301-496-9838, 301-496-9839 (fax). Appropriate medical evaluation, surveillance, and treatment are provided and written records are maintained.

Appendix G-II-C-2-r.

Baseline serum samples for all laboratory and other at-risk personnel should be collected and stored. Additional serum specimens may be collected periodically depending on the agents handled or the function of the laboratory.

Appendix G-II-C-2-s.

A biosafety manual is prepared or adopted. Personnel are advised of special hazards and are required to read and follow the instructions on practices and procedures.

Appendix G-II-C-2-t. Alternative Selection of Containment Equipment (BL3)

Experimental procedures involving a host-vector system that provides a one-step higher level of biological containment than that specified may be conducted in the BL3 laboratory using containment equipment specified for the BL2 level of physical containment. Experimental procedures involving a host-vector system that provides a one-step lower level of biological containment than that specified may be conducted in the BL3 laboratory using containment equipment specified for the BL4 level of physical containment. Alternative combination of containment safeguards are shown in [Appendix G-Table 1, Possible Alternate Combinations of Physical and Biological Containment Safeguards](#).

Appendix G-II-C-3. Containment Equipment (BL3)

Appendix G-II-C-3-a.

Biological safety cabinets (Class I, II, or III) (see [Appendix G-III-L, Footnotes and References of Appendix G](#)) or other appropriate combinations of personal protective or physical containment devices (e.g., special protective clothing, masks, gloves, respirators, centrifuge safety cups, sealed centrifuge rotors, and containment caging for animals) are used for all activities with organisms containing recombinant DNA molecules which pose a threat of aerosol exposure. These include: manipulation of cultures and of those clinical or environmental materials which may be a source of aerosols; the aerosol challenge of experimental animals; the harvesting of infected tissues or fluids from experimental animals and embryonated eggs; and the necropsy of experimental animals.

Appendix G-II-C-4. Laboratory Facilities (BL3)

Appendix G-II-C-4-a.

The laboratory is separated from areas which are open to unrestricted traffic flow within the building. Passage through two sets of doors is the basic requirement for entry into the laboratory from access corridors or other contiguous areas. Physical separation of the high containment laboratory from access corridors or other laboratories or activities may be provided by a double-doored clothes change room (showers may be included), airlock, or other access facility which requires passage through two sets of doors before entering the laboratory.

Appendix G-II-C-4-b.

The interior surfaces of walls, floors, and ceilings are water resistant so that they can be easily cleaned. Penetrations in these surfaces are sealed or capable of being sealed to facilitate decontaminating the area.

Appendix G-II-C-4-c.

Bench tops are impervious to water and resistant to acids, alkalis, organic solvents, and moderate heat.

Appendix G-II-C-4-d.

Laboratory furniture is sturdy and spaces between benches, cabinets, and equipment are accessible for cleaning.

Appendix G-II-C-4-e.

Each laboratory contains a sink for hand washing. The sink is foot, elbow, or automatically operated and is located near the laboratory exit door.

Appendix G-II-C-4-f.

Windows in the laboratory are closed and sealed.

Appendix G-II-C-4-g.

Access doors to the laboratory or containment module are self-closing.

Appendix G-II-C-4-h.

An autoclave for decontaminating laboratory wastes is available preferably within the laboratory.

Appendix G-II-C-4-i.

A ducted exhaust air ventilation system is provided. This system creates directional airflow that draws air into the laboratory through the entry area. The exhaust air is not recirculated to any other area of the building, is discharged to the outside, and is dispersed away from the occupied areas and air intakes. Personnel shall verify that the direction of the airflow (into the laboratory) is proper. The exhaust air from the laboratory room may be discharged to the outside without being filtered or otherwise treated.

Appendix G-II-C-4-j.

The high efficiency particulate air/HEPA filtered exhaust air from Class I or Class II biological safety cabinets is discharged directly to the outside or through the building exhaust system. Exhaust air from Class I or II biological safety cabinets may be recirculated within the laboratory if the cabinet is tested and certified at least every twelve months. If the HEPA-filtered exhaust air from Class I or II biological safety cabinets is to be discharged to the outside through the building exhaust air system, it is connected to this system in a manner (e.g., thimble unit connection (see [Appendix G-III-L, Footnotes and References of Appendix G](#))) that avoids any interference with the air balance of the cabinets or building exhaust system.

Appendix G-II-D. Biosafety Level 4 (BL4)

Appendix G-II-D-1. Standard Microbiological Practices (BL4)

Appendix G-II-D-1-a.

Work surfaces are decontaminated at least once a day and immediately after any spill of viable material.

Appendix G-II-D-1-b.

Only mechanical pipetting devices are used.

Appendix G-II-D-1-c.

Eating, drinking, smoking, storing food, and applying cosmetics are not permitted in the laboratory.

Appendix G-II-D-1-d.

All procedures are performed carefully to minimize the creation of aerosols.

Appendix G-II-D-2. Special Practices (BL4)

Appendix G-II-D-2-a.

Biological materials to be removed from the Class III cabinets or from the maximum containment laboratory in a viable or intact state are transferred to a non-breakable, sealed primary container and then enclosed in a non-breakable, sealed secondary container which is removed from the facility through a disinfectant dunk tank, fumigation chamber, or an airlock designed for this purpose.

Appendix G-II-D-2-b.

No materials, except for biological materials that are to remain in a viable or intact state, are removed from the maximum containment laboratory unless they have been autoclaved or decontaminated before exiting the facility. Equipment or material which might be damaged by high temperatures or steam is decontaminated by gaseous or vapor methods in an airlock or chamber designed for this purpose.

Appendix G-II-D-2-c.

Only persons whose presence in the facility or individual laboratory rooms is required for program or support purposes are authorized to enter. The supervisor has the final responsibility for assessing each circumstance and determining who may enter or work in the laboratory. Access to the facility is limited by means of secure, locked doors; accessibility is managed by the Principal Investigator, Biological Safety Officer, or other person responsible for the physical security of the facility. Before entering, persons are advised of the potential biohazards and instructed as to appropriate safeguards for ensuring their safety.

Authorized persons comply with the instructions and all other applicable entry and exit procedures. A logbook signed by all personnel indicates the date and time of each entry and exit. Practical and effective protocols for emergency situations are established.

Appendix G-II-D-2-d.

Personnel enter and exit the facility only through the clothing change and shower rooms. Personnel shower each time they exit the facility. Personnel use the air locks to enter or exit the laboratory only in an emergency.

Appendix G-II-D-2-e.

Street clothing is removed in the outer clothing change room and kept there. Complete laboratory clothing (may be disposable), including undergarments, pants and shirts or jump suits, shoes, and gloves, is provided and used by all personnel entering the facility. Head covers are provided for personnel who do not wash their hair during the exit shower. When exiting the laboratory and before proceeding into the shower area, personnel remove their laboratory clothing and store it in a locker or hamper in the inner change room. Protective clothing shall be decontaminated prior to laundering or disposal.

Appendix G-II-D-2-f.

When materials that contain organisms containing recombinant DNA molecules or experimental animals are present in the laboratory or animal rooms, a hazard warning sign incorporating the universal biosafety symbol is posted on all access doors. The sign identifies the agent, lists the name of the Principal Investigator or other responsible person(s), and indicates any special requirements for entering the area (e.g., the need for immunizations or respirators).

Appendix G-II-D-2-g.

Supplies and materials needed in the facility are brought in by way of the double-doored autoclave, fumigation chamber, or airlock which is appropriately decontaminated between each use. After securing the outer doors, personnel within the facility retrieve the materials by opening the interior doors or the autoclave, fumigation chamber, or airlock. These doors are secured after materials are brought into the facility.

Appendix G-II-D-2-h.

An insect and rodent control program is in effect.

Appendix G-II-D-2-i.

Materials (e.g., plants, animals, and clothing) not related to the experiment being conducted are not permitted in the facility.

Appendix G-II-D-2-j.

Hypodermic needles and syringes are used only for parenteral injection and aspiration of fluids from laboratory animals and diaphragm bottles. Only needle-locking syringes or disposable syringe-needle units (i.e., needle is integral part of unit) are used for the injection or aspiration of fluids containing organisms that contain recombinant DNA molecules. Needles should not be bent, sheared, replaced in the needle sheath or guard, or removed from the syringe following use. The needle and syringe should be placed in a puncture-resistant container and decontaminated, preferably by autoclaving before discard or reuse. Whenever possible, cannulas are used instead of sharp needles (e.g., gavage).

Appendix G-II-D-2-k.

A system is set up for reporting laboratory accidents, exposures, employee absenteeism, and for the medical surveillance of potential laboratory-associated illnesses. Spills and accidents which result in overt exposures to organisms containing recombinant DNA molecules are immediately reported to the Biological Safety

Officer, Institutional Biosafety Committee, and NIH/OBA. Reports to the NIH/OBA shall be sent to the Office of Biotechnology Activities, National Institutes of Health, 6705 Rockledge Drive, Suite 750, MSC 7985, Bethesda, MD 20892-7985 (20817 for non-USPS mail), 301-496-9838, 301-496-9839 (fax). Written records are prepared and maintained. An essential adjunct to such a reporting-surveillance system is the availability of a facility for quarantine, isolation, and medical care of personnel with potential or known laboratory associated illnesses.

Appendix G-II-D-2-l.

Laboratory animals involved in experiments requiring BL4 level physical containment shall be housed either in cages contained in Class III cabinets or in partial containment caging systems, such as Horsfall units (see [Appendix G-III-K](#), *Footnotes and References of Appendix G*), open cages placed in ventilated enclosures, or solid-wall and -bottom cages placed on holding racks equipped with ultraviolet irradiation lamps and reflectors that are located in a specially designed area in which all personnel are required to wear one-piece positive pressure suits.

Appendix G-II-D-2-m. Alternative Selection of Containment Equipment (BL4)

Experimental procedures involving a host-vector system that provides a one-step higher level of biological containment than that specified may be conducted in the BL4 facility using containment equipment requirements specified for the BL3 level of physical containment. Alternative combinations of containment safeguards are shown in Appendix G-Table 1, *Possible Alternate Combinations of Physical and Biological Containment Safeguards*.

Appendix G-II-D-3. Containment Equipment (BL4)

Appendix G-II-D-3-a.

All procedures within the facility with agents assigned to Biosafety Level 4 are conducted in the Class III biological safety cabinet or in Class I or II biological safety cabinets used in conjunction with one-piece positive pressure personnel suits ventilated by a life-support system.

Appendix G-II-D-4. Laboratory Facilities (BL4)

Appendix G-II-D-4-a.

The maximum containment facility consists of either a separate building or a clearly demarcated and isolated zone within a building. Outer and inner change rooms separated by a shower are provided for personnel entering and exiting the facility. A double-doored autoclave, fumigation chamber, or ventilated airlock is provided for passage of those materials, supplies, or equipment which are not brought into the facility through the change room.

Appendix G-II-D-4-b.

Walls, floors, and ceilings of the facility are constructed to form a sealed internal shell which facilitates fumigation and is animal and insect proof. The internal surfaces of this shell are resistant to liquids and chemicals, thus facilitating cleaning and decontamination of the area. All penetrations in these structures and surfaces are sealed. Any drains in the floors contain traps filled with a chemical disinfectant of demonstrated efficacy against the target agent, and they are connected directly to the liquid waste decontamination system. Sewer and other ventilation lines contain high efficiency particulate air/HEPA filters.

Appendix G-II-D-4-c.

Internal facility appurtenances, such as light fixtures, air ducts, and utility pipes, are arranged to minimize the horizontal surface area on which dust can settle.

Appendix G-II-D-4-d.

Bench tops have seamless surfaces which are impervious to water and resistant to acids, alkalis, organic solvents, and moderate heat.

Appendix G-II-D-4-e.

Laboratory furniture is simple and of sturdy construction; and spaces between benches, cabinets, and equipment are accessible for cleaning.

Appendix G-II-D-4-f.

A foot, elbow, or automatically operated hand washing sink is provided near the door of each laboratory room in the facility.

Appendix G-II-D-4-g.

If there is a central vacuum system, it does not serve areas outside the facility. In-line high efficiency particulate air/HEPA filters are placed as near as practicable to each use point or service cock. Filters are installed to permit in-place decontamination and replacement. Other liquid and gas services to the facility are protected by devices that prevent back-flow.

Appendix G-II-D-4-h.

If water fountains are provided, they are foot operated and are located in the facility corridors outside the laboratory. The water service to the fountain is not connected to the back-flow protected distribution system supplying water to the laboratory areas.

Appendix G-II-D-4-i.

Access doors to the laboratory are self-closing and locking.

Appendix G-II-D-4-j.

Any windows are breakage resistant.

Appendix G-II-D-4-k. A double-doored autoclave is provided for decontaminating materials passing out of the facility. The autoclave door which opens to the area external to the facility is sealed to the outer wall and automatically controlled so that the outside door can only be opened after the autoclave "sterilization" cycle has been completed.

Appendix G-II-D-4-l.

A pass-through dunk tank, fumigation chamber, or an equivalent decontamination method is provided so that materials and equipment that cannot be decontaminated in the autoclave can be safely removed from the facility.

Appendix G-II-D-4-m.

Liquid effluent from laboratory sinks, biological safety cabinets, floors, and autoclave chambers are decontaminated by heat treatment before being released from the maximum containment facility. Liquid wastes from shower rooms and toilets may be decontaminated with chemical disinfectants or

by heat in the liquid waste decontamination system. The procedure used for heat decontamination of liquid wastes is evaluated mechanically and biologically by using a recording thermometer and an indicator microorganism with a defined heat susceptibility pattern. If liquid wastes from the shower room are decontaminated with chemical disinfectants, the chemical used is of demonstrated efficacy against the target or indicator microorganisms.

Appendix G-II-D-4-n.

An individual supply and exhaust air ventilation system is provided. The system maintains pressure differentials and directional airflow as required to assure flows inward from areas outside of the facility toward areas of highest potential risk within the facility. Manometers are used to sense pressure differentials between adjacent areas maintained at different pressure levels. If a system malfunctions, the manometers sound an alarm. The supply and exhaust airflow is interlocked to assure inward (or zero) airflow at all times.

Appendix G-II-D-4-o.

The exhaust air from the facility is filtered through high efficiency particulate air/HEPA filters and discharged to the outside so that it is dispersed away from occupied buildings and air intakes. Within the facility, the filters are located as near the laboratories as practicable in order to reduce the length of potentially contaminated air ducts. The filter chambers are designed to allow *in situ* decontamination before filters are removed and to facilitate certification testing after they are replaced. Coarse filters and HEPA filters are provided to treat air supplied to the facility in order to increase the lifetime of the exhaust HEPA filters and to protect the supply air system should air pressures become unbalanced in the laboratory.

Appendix G-II-D-4-p.

The treated exhaust air from Class I and II biological safety cabinets may be discharged into the laboratory room environment or the outside through the facility air exhaust system. If exhaust air from Class I or II biological safety cabinets is discharged into the laboratory the cabinets are tested and certified at six-month intervals. *The exhaust air from Class III biological safety cabinets is discharged, without recirculation through two sets of high efficiency particulate air/HEPA filters in series, via the facility exhaust air system.* If the treated exhaust air from any of these cabinets is discharged to the outside through the facility exhaust air system, it is connected to this system in a manner (e.g., thimble unit connection (see [Appendix G-III-L, Footnotes and References of Appendix G](#))) that avoids any interference with the air balance of the cabinets or the facility exhaust air system.

Appendix G-II-D-4-q.

A specially designed suit area may be provided in the facility. Personnel who enter this area shall wear a one-piece positive pressure suit that is ventilated by a life-support system. The life-support system includes alarms and emergency backup breathing air tanks. Entry to this area is through an airlock fitted with airtight doors. A chemical shower is provided to decontaminate the surface of the suit before the worker exits the area. The exhaust air from the suit area is filtered by two sets of high efficiency particulate air/HEPA filters installed in series. A duplicate filtration unit, exhaust fan, and an automatically starting emergency power source are provided. The air pressure within the suit area is greater than that of any adjacent area. Emergency lighting and communication systems are provided. All penetrations into the internal shell of the suit are sealed. A double-doored autoclave is provided for decontaminating waste materials to be removed from the suit areas.

Appendix G - Table 1. Possible Alternate Combinations Of Physical And Biological Containment

Classification of Physical & Biological Containment	Alternate Physical Containment			Alternate Biological Containment
	Laboratory Facilities	Laboratory Practices	Laboratory Equipment	
BL3/HV2	BL3 BL3	BL3 BL3	BL3 BL4	HV2 HV1
BL3/HV1	BL3 BL3	BL3 BL3	BL3 BL2	HV1 HV2
BL4/HV1	BL4 BL4	BL4 BL4	BL4 BL3	HV1 HV2

BL - Biosafety Level
HV - Host-Vector System

Appendix G-III. Footnotes and References of Appendix G

Appendix G-III-A. *Biosafety in Microbiological and Biomedical Laboratories*, 4th edition, May, 1999, DHHS, Public Health Service, [Centers for Disease Control and Prevention](#), Atlanta, Georgia, and National Institutes of Health, Bethesda, Maryland.

Appendix G-III-B. *Biosafety in Microbiological and Biomedical Laboratories*, 3rd edition, May 1993, U.S. DHHS, Public Health Service, Centers for Disease Control and Prevention, Atlanta, Georgia, and NIH, Bethesda, Maryland.

Appendix G-III-C. *National Cancer Institute Safety Standards for Research Involving Oncogenic Viruses*, U.S. Department of Health, Education, and Welfare Publication No. (NIH) 75-790, October 1974.

Appendix G-III-D. *National Institutes of Health Biohazards Safety Guide*, U.S. Department of Health, Education, and Welfare, Public Health Service, NIH, U.S. Government Printing Office, Stock No. 1740-00383, 1974.

Appendix G-III-E. A. Hellman, M. N. Oxman, and R. Pollack (eds.), *Biohazards in Biological Research*, Cold Spring Harbor Laboratory 1973.

Appendix G-III-F. N. V. Steere (ed.), *Handbook of Laboratory Safety*, 2nd edition, The Chemical Rubber Co., Cleveland, Ohio, 1971.

Appendix G-III-G. Bodily, J. L., "General Administration of the Laboratory," H. L. Bodily, E. L. Updyke, and J. O. Mason (eds.), *Diagnostic Procedures for Bacterial, Mycotic, and Parasitic Infections*, American Public Health Association, New York, 1970, pp. 11-28.

Appendix G-III-H. Darlow, H. M. (1969). "Safety in the Microbiological Laboratory," in J. R. Norris and D. W. Robbins (eds.), *Methods in Microbiology*, Academic Press, Inc., New York, pp. 169-204.

Appendix G-III-I. *The Prevention of Laboratory Acquired Infection*, C. H. Collins, E. G. Hartley, and R. Pilsworth, Public Health Laboratory Service, Monograph Series No. 6, 1974.

Appendix G-III-J. Chatigny, M. A., "Protection Against Infection in the Microbiological Laboratory: Devices and Procedures," in W. W. Umbreit (ed.), *Advances in Applied Microbiology*, Academic Press, New York, New York, 1961, 3:131-192.

Appendix G-III-K.

Horsfall, F. L. Jr., and J. H. Baner, *Individual Isolation of Infected Animals in a Single Room*, J. Bact., 1940, 40, 569-580.

Appendix G-III-L.

Biological safety cabinets referred to in this section are classified as Class I, Class II, or Class III cabinets. A Class I is a ventilated cabinet for personnel protection having an inward flow of air away from the operator. The exhaust air from this cabinet is filtered through a high efficiency particulate air/HEPA filter. This cabinet is used in three operational modes: (i) with a full-width open front, (ii) with an installed front closure panel (having four 6-inch diameter openings) without gloves, and (iii) with an installed front closure panel equipped with arm-length rubber gloves. The face velocity of the inward flow of air through the full-width open front is 75 feet per minute or greater. A Class II cabinet is a ventilated cabinet for personnel and product protection having an open front with inward air flow for personnel protection, and HEPA filtered mass recirculated air flow for product protection. The cabinet exhaust air is filtered through a HEPA filter. The face velocity of the inward flow of air through the full-width open front is 75 feet per minute or greater. Design and performance specifications for Class II cabinets have been adopted by the National Sanitation Foundation, Ann Arbor, Michigan. A Class III cabinet is a closed-front ventilated cabinet of gas tight construction which provides the highest level of personnel protection of all biosafety safety cabinets. The interior of the cabinet is protected from contaminants exterior to the cabinet. The cabinet is fitted with arm-length rubber gloves and is operated under a negative pressure of at least 0.5 inches water gauge. All supply air is filtered through HEPA filters. Exhaust air is filtered through two HEPA filters or one HEPA filter and incinerator before being discharged to the outside environment. National Sanitation Foundation Standard 49. 1976. Class II (Laminar Flow) Biohazard Cabinetry, Ann Arbor, Michigan.

Appendix G-III-M.

Biosafety Level 1 is suitable for work involving agents of unknown or minimal potential hazard to laboratory personnel and the environment. The laboratory is not separated from the general traffic patterns in the building. Work is generally conducted on open bench tops. Special containment equipment is not required or generally used. Laboratory personnel have specific training in the procedures conducted in the laboratory and are supervised by a scientist with general training in microbiology or a related science (see [Appendix G-III-B](#), *Footnotes and References of Appendix G*).

Appendix G-III-N.

Biosafety Level 2 is similar to Level 1 and is suitable for work involving agents of moderate potential hazard to personnel and the environment. It differs in that: (1) laboratory personnel have specific training in handling pathogenic agents and are directed by competent scientists; (2) access to the laboratory is limited when work is being conducted; and (3) certain procedures in which infectious aerosols are created are conducted in biological safety cabinets or other physical containment equipment (see [Appendix G-III-B](#), *Footnotes and References of Appendix G*).

Appendix G-III-O.

Office of Research Safety, National Cancer Institute, and the Special Committee of Safety and Health Experts, *Laboratory Safety Monograph: A Supplement to the NIH Guidelines for Recombinant DNA Research*, NIH, Bethesda, Maryland 1978.

Appendix G-III-P.

Biosafety Level 3 is applicable to clinical, diagnostic, teaching, research, or production facilities in which work is conducted with indigenous or exotic agents which may cause serious or potentially lethal disease as a result of exposure by the inhalation route. Laboratory personnel have specific training in handling pathogenic and potentially lethal agents and are supervised by competent scientists who are experienced in working with these agents. All procedures involving the manipulation of infectious material are conducted within biological safety cabinets or other physical containment devices or by personnel wearing appropriate personal protective clothing and devices. The laboratory has special engineering and design features. It is recognized, however, that many existing facilities may not have all the facility safeguards recommended for BL3 (e.g., access zone, sealed penetrations, and directional airflow, etc.). In these circumstances, acceptable safety may be achieved for routine or repetitive operations (e.g., diagnostic procedures involving the propagation of an agent for identification, typing, and susceptibility testing) in laboratories where facility features satisfy BL2 recommendations provided the recommended "Standard Microbiological Practices," "Special Practices," and "Containment Equipment" for BL3 are rigorously followed. The decision to implement this modification of BL3 recommendations should be made only by the Principal Investigator.