**NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules**

**APPENDIX Q. PHYSICAL AND BIOLOGICAL CONTAINMENT FOR RECOMBINANT OR SYNTHETIC NUCLEIC ACID MOLECULE RESEARCH INVOLVING ANIMALS**

Appendix Q specifies containment and confinement practices for research involving whole animals, both those in which the animal's genome has been altered by stable introduction of recombinant or synthetic nucleic acid molecules, or DNA derived therefrom, into the germ-line (transgenic animals) and experiments involving viable recombinant or synthetic nucleic acid molecule-modified microorganisms tested on whole animals. The appendix applies to animal research activities with the following modifications:

Appendix Q shall supersede Appendix G (*Physical Containment*) when research animals are of a size or have growth requirements that preclude the use of containment for laboratory animals. Some animals may require other types of containment (see Appendix Q-III-D, *Footnotes and References for Appendix Q*). The animals covered in Appendix Q are those species normally categorized as animals including but not limited to cattle, swine, sheep, goats, horses, and poultry.

The Institutional Biosafety Committee shall include at least one scientist with expertise in animal containment principles when experiments utilizing Appendix Q require Institutional Biosafety Committee prior approval.

The institution shall establish and maintain a health surveillance program for personnel engaged in animal research involving viable recombinant or synthetic nucleic acid molecule-containing microorganisms that require Biosafety Level (BL) 3 or greater containment in the laboratory.

**Appendix Q-I. General Considerations**

**Appendix Q-I-A. Containment Levels**

The containment levels required for research involving recombinant or synthetic nucleic acid molecules associated with or in animals is based on classification of experiments in Section III, *Experiments Covered by the NIH Guidelines*. For the purpose of animal research, four levels of containment are established. These are referred to as BL1-Animals (N), BL2-N, BL3-N, and BL4-N and are described in the following appendices of Appendix Q. The descriptions include: (i) standard practices for physical and biological containment, and (ii) animal facilities.

**Appendix Q-I-B. Disposal of Animals (BL1-N through BL4-N)**

**Appendix Q-I-B-1.** When an animal covered by Appendix Q containing recombinant or synthetic nucleic acid molecules or a recombinant or synthetic nucleic acid molecule-derived organism is euthanized or dies, the carcass shall be disposed of to avoid its use as food for human beings or animals unless food use is specifically authorized by an appropriate Federal agency.

**Appendix Q-I-B-2.** A permanent record shall be maintained of the experimental use and disposal of each animal or group of animals.

**Appendix Q-II. Physical and Biological Containment Levels**

**Appendix Q-II-A. Biosafety Level 1 - Animals (BL1-N)**

**Appendix Q-II-A-1. Standard Practices (BL1-N)**

**Appendix Q-II-A-1-a. Animal Facility Access (BL1-N)**

**Appendix Q-II-A-1-a-(1).** The containment area shall be locked.

**Appendix Q-II-A-1-a-(2).** Access to the containment area shall be limited or restricted when experimental animals are being held.

**Appendix Q-II-A-1-a-(3).** The containment area shall be patrolled or monitored at frequent intervals.

**Appendix Q-II-A-1-b. Other (BL1-N)**

**Appendix Q-II-A-1-b-(1).** All genetically engineered neonates shall be permanently marked within 72 hours after birth, if their size permits. If their size does not permit marking, their containers should be marked. In addition, transgenic animals should contain distinct and biochemically assayable DNA sequences that allow identification of transgenic animals from among non-transgenic animals.

**Appendix Q-II-A-1-b-(2)** A double barrier shall be provided to separate male and female animals unless reproductive studies are part of the experiment or other measures are taken to avoid reproductive transmission. Reproductive incapacitation may be used.

**Appendix Q-II-A-1-b-(3).** The containment area shall be in accordance with state and Federal laws and animal care requirements.

**Appendix Q-II-A-2. Animal Facilities (BL1-N)**

**Appendix Q-II-A-2-a.** Animals shall be confined to securely fenced areas or be in enclosed structures (animal rooms) to minimize the possibility of theft or unintentional release.

**Appendix Q-II-B. Biosafety Level 2 - Animals (BL2-N)** (See Appendix Q-III-A, *Footnotes and References for Appendix Q*)

**Appendix Q-II-B-1. Standard Practices (BL2-N)**

**Appendix Q-II-B-1-a. Animal Facility Access (BL2-N)**

**Appendix Q-II-B-1-a-(1).** The containment area shall be locked.

**Appendix Q-II-B-1-a-(2).** The containment area shall be patrolled or monitored at frequent intervals.

**Appendix Q-II-B-1-a-(3).** The containment building shall be controlled and have a locking access.

**Appendix Q-II-B-1-a-(4).** The Animal Facility Director shall establish policies and procedures whereby only persons who have been advised of the potential hazard and who meet any specific entry requirements (e.g., vaccination) may enter the laboratory or animal rooms.

**Appendix Q-II-B-1-a-(5).** Animals of the same or different species, which are not involved in the work being performed, shall not be permitted in the animal area.

**Appendix Q-II-B-1-b. Decontamination and Inactivation (BL2-N)**

**Appendix Q-II-B-1-b-(1).** Contaminated materials that are decontaminated at a site away from the laboratory shall be placed in a closed durable leak-proof container prior to removal from the laboratory.

**Appendix Q-II-B-1-b-(2).** Needles and syringes shall be promptly placed in a puncture-resistant container and decontaminated, preferably by autoclaving, before discard or reuse.

**Appendix Q-II-B-1-c. Signs (BL2-N)**

**Appendix Q-II-B-1-c-(1).** When the animal research requires special provisions for entry (e.g., vaccination), a warning sign incorporating the universal biosafety symbol shall be posted on all access doors to the animal work area. The sign shall indicate: (i) the agent, (ii) the animal species, (iii) the name and telephone number of the Animal Facility Director or other responsible individual, and (iv) any special requirements for entering the laboratory.

**Appendix Q-II-B-1-d. Protective Clothing (BL2-N)**

**Appendix Q-II-B-1-d-(1).** Laboratory coats, gowns, smocks, or uniforms shall be worn while in the animal area or attached laboratory. Before entering non-laboratory areas (e.g., cafeteria, library, administrative offices), protective clothing shall be removed and kept in the work entrance area.

**Appendix Q-II-B-1-d-(2).** Special care shall be taken to avoid skin contamination with microorganisms containing recombinant or synthetic nucleic acid molecules. Impervious and/or protective gloves shall be worn when handling experimental animals and when skin contact with an infectious agent is unavoidable.

**Appendix Q-II-B-1-e. Records (BL2-N)**

**Appendix Q-II-B-1-e-(1).** Any incident involving spills and accidents that result in environmental release or exposures of animals or laboratory workers to organisms containing recombinant or synthetic nucleic acid molecules shall be reported immediately to the Animal Facility Director, Institutional Biosafety Committee, NIH

OSP, and other appropriate authorities (if applicable). Reports to the NIH OSP shall be sent to the Office of Science Policy, National Institutes of Health, preferably by e-mail to: NIHGuidelines@od.nih.gov; additional contact information is also available here and on the [OSP website](http://www.osp.od.nih.gov/) (www.osp.od.nih.gov). Medical evaluation, surveillance, and treatment shall be provided as appropriate and written records maintained. If necessary, the area shall be appropriately decontaminated.

**Appendix Q-II-B-1-e-(2).** When appropriate and giving consideration to the agent handled, baseline serum samples shall be collected and stored for animal care and other at-risk personnel. Additional serum specimens may be collected periodically depending on the agent handled and the function of the animal facility.

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**Appendix Q-II-B-1-f. Transfer of Materials (BL2-N)**

**Appendix Q-II-B-1-f-(1).** Biological materials removed from the animal containment area in a viable or intact state shall be transferred to a non-breakable sealed primary container and then enclosed in a non-breakable sealed secondary container. All containers, primary and secondary, shall be disinfected before removal from the animal facility. Advance approval for transfer of material shall be obtained from the Animal Facility Director. Packages containing viable agents may only be opened in a facility having an equivalent or higher level of physical containment unless the agent is biologically inactivated or incapable of reproduction.

**Appendix Q-II-B-1-g. Other (BL2-N)**

**Appendix Q-II-B-1-g-(1).** All genetically engineered neonates shall be permanently marked within 72 hours after birth, if their size permits. If their size does not permit marking, their containers should be marked. In addition, transgenic animals should contain distinct and biochemically assayable DNA sequences that allow identification of transgenic animals from among non-transgenic animals.

**Appendix Q-II-B-1-g-(2).** Needles and syringes shall be used only for parenteral injection and aspiration of fluids from laboratory animals and diaphragm bottles. Only needle-locking syringes or disposable syringeneedle units (i.e., needle is integral to the syringe) shall be used for the injection or aspiration of fluids containing organisms that contain recombinant or synthetic nucleic acid molecules. Extreme caution shall be used when handling needles and syringes to avoid autoinoculation and the generation of aerosols during use and disposal. Following use, needles shall not be bent, sheared, replaced in the needle sheath or guard, or removed from the syringe. Needles and syringes shall be promptly placed in a puncture-resistant container and decontaminated, preferably by autoclaving, before discard or reuse.

**Appendix Q-II-B-1-g-(3).** Appropriate steps should be taken to prevent horizontal transmission or exposure of laboratory personnel. If the agent used as a vector is known to be transmitted by a particular route (e.g., arthropods), special attention should be given to preventing spread by that route. In the absence of specific knowledge of a particular route of transmission, all potential means of horizontal transmission (e.g., arthropods, contaminated bedding, or animal waste, etc.) should be prevented.

**Appendix Q-II-B-1-g-(4).** Eating, drinking, smoking, and applying cosmetics shall not be permitted in the work area.

**Appendix Q-II-B-1-g-(5).** Individuals who handle materials and animals containing recombinant or synthetic nucleic acid molecules shall be required to wash their hands before exiting the containment area.

**Appendix Q-II-B-1-g-(6).** A double barrier shall be provided to separate male and female animals unless reproductive studies are part of the experiment or other measures are taken to avoid reproductive transmission. Reproductive incapacitation may be used.

**Appendix Q-II-B-1-g-(7).** The containment area shall be in accordance with state and Federal laws and animal care requirements.

**Appendix Q-II-B-1-g-(8).** A biosafety manual shall be prepared or adopted. Personnel shall be advised of special hazards and required to read and follow instructions on practices and procedures.

**Appendix Q-II-B-2. Animal Facilities (BL2-N)**

**Appendix Q-II-B-2-a.** Animals shall be contained within an enclosed structure (animal room or equivalent) to minimize the possibility of theft or unintentional release and to avoid arthropod access. The special provision to avoid the entry or escape of arthropods from the animal areas may be waived if the agent in use is not known to be transmitted by arthropods.

**Appendix Q-II-B-2-b.** Surfaces shall be impervious to water and resistant to acids, alkalis, organic solvents, and moderate heat.

**Appendix Q-II-B-2-c.** The animal containment area shall be designed so that it can be easily cleaned.

**Appendix Q-II-B-2-d.** Windows that open shall be fitted with fly screens.

**Appendix Q-II-B-2-e.** An autoclave shall be available for decontamination of laboratory wastes.

**Appendix Q-II-B-2-f.** If arthropods are used in the experiment or the agent under study can be transmitted by an arthropod, interior work areas shall be appropriately screened (52 mesh). All perimeter joints and openings shall be sealed and additional arthropod control mechanisms used to minimize arthropod entry and propagation, including appropriate screening of access doors or the equivalent.

**Appendix Q-II-C. Biosafety Level 3 - Animals (BL3-N)** (See Appendix Q-III-B, *Footnotes and References for Appendix Q*)

**Appendix Q-II-C-1. Standard Practices (BL3-N)**

**Appendix Q-II-C-1-a. Animal Facility Access (BL3-N)**

**Appendix Q-II-C-1-a-(1).** The containment area shall be locked.

**Appendix Q-II-C-1-a-(2).** The containment area shall be patrolled or monitored at frequent intervals.

**Appendix Q-II-C-1-a-(3).** The containment building shall be controlled and have a locking access.

**Appendix Q-II-C-1-a-(4).** The Animal Facility Director shall establish policies and procedures whereby only persons who have been advised of the potential hazard and who meet any specific entry requirements (e.g., vaccination) shall enter the laboratory or animal rooms.

**Appendix Q-II-C-1-a-(5).** Animal room doors, gates, or other closures shall be kept closed when experiments are in progress.

**Appendix Q-II-C-1-b. Decontamination and Inactivation (BL3-N)**

**Appendix Q-II-C-1-b-(1).** The work surfaces of containment equipment shall be decontaminated when work with organisms containing recombinant or synthetic nucleic acid molecules is finished. Where feasible, plasticbacked paper toweling shall be used on nonporous work surfaces to facilitate clean-up.

**Appendix Q-II-C-1-b-(2).** All animals shall be euthanized at the end of their experimental usefulness and the carcasses decontaminated before disposal in an approved manner.

**Appendix Q-II-C-1-b-(3).** Needles and syringes shall be promptly placed in a puncture-resistant container and decontaminated, preferably by autoclaving, before discard or reuse.

**Appendix Q-II-C-1-b-(4).** Special safety testing, decontamination procedures, and Institutional Biosafety Committee approval shall be required to transfer agents or tissue/organ specimens from a BL3-N animal facility to a facility with a lower containment classification.

**Appendix Q-II-C-1-b-(5).** Liquid effluent from containment equipment, sinks, biological safety cabinets, animal rooms, primary barriers, floor drains, and sterilizers shall be decontaminated by heat treatment before being released into the sanitary system. The procedure used for heat decontamination of liquid wastes shall be monitored with a recording thermometer. The effectiveness of the heat decontamination process system shall be revalidated at minimum on a yearly basis with an indicator organism. More frequent validation, based on the amount of use or other safety factors, shall be left to the discretion of the IBC.

**Appendix Q-II-C-1-c. Signs (BL3-N)**

**Appendix Q-II-C-1-c-(1).** When the animal research requires special provisions for entry (e.g., vaccination), a warning sign incorporating the universal biosafety symbol shall be posted on all access doors to the animal work area. The sign shall indicate: (i) the agent, (ii) the animal species, (iii) the name and telephone number of the Animal Facility Director or other responsible individual, and (iv) any special requirements for entering the laboratory.

**Appendix Q-II-C-1-d. Protective Clothing (BL3-N)**

**Appendix Q-II-C-1-d-(1).** Full protective clothing that protects the individual (e.g., scrub suits, coveralls, uniforms) shall be worn in the animal area. Clothing shall not be worn outside the animal containment area and shall be decontaminated before laundering or disposal. Personnel shall be required to shower before exiting the BL3-N area and wearing of personal clothing.

**Appendix Q-II-C-1-d-(2).** Special care shall be taken to avoid skin contamination with microorganisms containing recombinant or synthetic nucleic acid molecules. Impervious and/or protective gloves shall be worn when handling experimental animals and when skin contact with an infectious agent is unavoidable.

**Appendix Q-II-C-1-d-(3).** Appropriate respiratory protection shall be worn in rooms containing experimental animals.

**Appendix Q-II-C-1-e. Records (BL3-N)**

**Appendix Q-II-C-1-e-(1).** Documents regarding experimental animal use and disposal shall be maintained in a permanent record book.

**Appendix Q-II-C-1-e-(2).** Any incident involving spills and accidents that result in environmental release or exposure of animals or laboratory workers to organisms containing recombinant or synthetic nucleic acid molecules shall be reported immediately to the Biological Safety Office, Animal Facility Director, Institutional Biosafety Committee, NIH OSP, and other appropriate authorities (if applicable). Reports to the NIH OSP shall be sent to the Office of Science Policy, National Institutes of Health, preferably by e-mail to:

NIHGuidelines@od.nih.gov; additional contact information is also available here and on th[e OSP website](http://www.osp.od.nih.gov/) (www.osp.od.nih.gov). Medical evaluation, surveillance, and treatment shall be provided as appropriate and written records maintained. If necessary, the area shall be appropriately decontaminated.

**Appendix Q-II-C-1-e-(3).** When appropriate and giving consideration to the agent handled, baseline serum samples shall be collected and stored for animal care and other at-risk personnel. Additional serum specimens may be collected periodically depending on the agent handled or the function of the facility.

**Appendix Q-II-C-1-f. Transfer of Materials (BL3-N)**

**Appendix Q-II-C-1-f-(1).** Biological materials removed from the animal containment laboratory in a viable or intact state shall be transferred to a non-breakable sealed primary container and then enclosed in a nonbreakable sealed secondary container. All containers, primary and secondary, shall be disinfected before removal from the animal facility. Advance approval for transfer of material shall be obtained from the Animal Facility Director. Packages containing viable agents may be opened only in a facility having an equivalent or higher level of physical containment unless the agent is biologically inactivated or incapable of reproduction.

**Appendix Q-II-C-1-f-(2).** Special safety testing, decontamination procedures, and Institutional Biosafety Committee approval shall be required to transfer agents or tissue/organ specimens from a BL3-N animal facility to a facility with a lower containment classification.

**Appendix Q-II-C-1-g. Other (BL3-N)**

**Appendix Q-II-C-1-g-(1).** All genetically engineered neonates shall be permanently marked within 72 hours after birth, if their size permits. If their size does not permit marking, their containers should be marked. In addition, transgenic animals should contain distinct and biochemically assayable DNA sequences that allow identification of transgenic animals from among non-transgenic animals.

**Appendix Q-II-C-1-g-(2).** Appropriate steps should be taken to prevent horizontal transmission or exposure of laboratory personnel. If the agent used as the vector is known to be transmitted by a particular route (e.g., arthropods), special attention should be given to preventing spread by that route. In the absence of specific knowledge of a particular route of transmission, all potential means of horizontal transmission (e.g., arthropods, contaminated bedding, or animal waste) should be prevented.

**Appendix Q-II-C-1-g-(3).** Eating, drinking, smoking, and applying cosmetics shall not be permitted in the work area.

**Appendix Q-II-C-1-g-(4).** Individuals who handle materials and animals containing recombinant or synthetic nucleic acid molecules shall be required to wash their hands before exiting the containment area.

**Appendix Q-II-C-1-g-(5).** Experiments involving other organisms that require containment levels lower than BL3-N may be conducted in the same area concurrently with experiments requiring BL3-N containment provided that they are conducted in accordance with BL3-N practices.

**Appendix Q-II-C-1-g-(6).** Animal holding areas shall be cleaned at least once a day and decontaminated immediately following any spill of viable materials.

**Appendix Q-II-C-1-g-(7).** All procedures shall be performed carefully to minimize the creation of aerosols.

**Appendix Q-II-C-1-g-(8).** A double barrier shall be provided to separate male and female animals unless reproductive studies are part of the experiment or other measures are taken to avoid reproductive transmission. Reproductive incapacitation may be used.

**Appendix Q-II-C-1-g-(9).** The containment area shall be in accordance with state and Federal laws and animal care requirements.

**Appendix Q-II-C-1-g-(10).** All animals shall be euthanized at the end of their experimental usefulness and the carcasses decontaminated before disposal in an approved manner.

**Appendix Q-II-C-1-g-(11).** Personnel shall be required to shower before exiting the BL3-N area and wearing personal clothing.

**Appendix Q-II-C-1-g-(12).** Animals of the same or different species, which are not involved in the work being performed, shall not be permitted in the animal area.

**Appendix Q-II-C-1-g-(13).** Needles and syringes shall be used only for parenteral injection and aspiration of fluids from laboratory animals and diaphragm bottles. Only needle-locking syringes or disposable syringeneedle units (i.e., needle is integral to the syringe) shall be used for the injection or aspiration of fluids containing organisms that contain recombinant or synthetic nucleic acid molecules. Extreme caution shall be used when handling needles and syringes to avoid autoinoculation and the generation of aerosols during use and disposal. Following use, needles shall not be bent, sheared, replaced in the needle sheath or guard or removed from the syringe. The needles and syringes shall be promptly placed in a puncture-resistant container and decontaminated, preferably by autoclaving, before discard or reuse.

**Appendix Q-II-C-1-g-(14).** A biosafety manual shall be prepared or adopted. Personnel shall be advised of special hazards and required to read and follow instructions on practices and procedures.

**Appendix Q-II-C-2. Animal Facilities (BL3-N)**

**Appendix Q-II-C-2-a.** Animals shall be contained within an enclosed structure (animal room or equivalent) to minimize the possibility of theft or unintentional release and avoid arthropod access. The special provision to avoid the entry or escape of arthropods from the animal areas may be waived if the agent in use is not known to be transmitted by arthropods.

**Appendix Q-II-C-2-b.** The interior walls, floors, and ceilings shall be impervious to water and resistant to acids, alkalis, organic solvents, and moderate heat, to facilitate cleaning. Penetrations in these structures and surfaces (e.g., plumbing and utilities) shall be sealed.

**Appendix Q-II-C-2-c.** Windows in the animal facility shall be closed, sealed, and breakage resistant (e.g., double-pane tempered glass or equivalent). The need to maintain negative pressure should be considered when constructing or renovating the animal facility.

**Appendix Q-II-C-2-d.** An autoclave, incinerator, or other effective means to decontaminate animals and waste shall be available, preferably within the containment area. If feasible, a double-door autoclave is preferred and should be positioned to allow removal of material from the containment area.

**Appendix Q-II-C-2-e.** If arthropods are used in the experiment or the agent under study can be transmitted by an arthropod, the interior work area shall be appropriately screened (52 mesh). All perimeter joints and openings shall be sealed, and additional arthropod control mechanisms used to minimize arthropod entry and propagation, including appropriate screening, or the equivalent of access doors.

**Appendix Q-II-C-2-f.** Access doors to the containment area shall be self-closing.

**Appendix Q-II-C-2-g.** The animal area shall be separated from all other areas. Passage through two sets of doors shall be the basic requirement for entry into the animal area from access corridors or other contiguous areas. The animal containment area shall be physically separated from access corridors and other laboratories or areas by a double-door clothes change room, equipped with integral showers and airlock.

**Appendix Q-II-C-2-h.** Liquid effluent from containment equipment, sinks, biological safety cabinets, animal rooms, primary barriers, floor drains, and sterilizers shall be decontaminated by heat treatment before being released into the sanitary system. The procedure used for heat decontamination of liquid wastes shall be monitored with a recording thermometer. The effectiveness of the heat decontamination process system shall be revalidated at minimum on a yearly basis with an indicator organism. More frequent validation, based on the amount of use or other safety factors, shall be left to the discretion of the IBC.

**Appendix Q-II-C-2-i.** An exhaust air ventilation system shall be provided. This system shall create directional airflow that draws air into the animal room through the entry area. The building exhaust, or the exhaust from primary containment units, may be used for this purpose if the exhaust air is discharged to the outside and shall be dispersed away from occupied areas and air intakes. Personnel shall verify that the direction of the airflow (into the animal room) is proper.

**Appendix Q-II-C-2-j.** If the agent is transmitted by aerosol, then the exhaust air shall pass through a high efficiency particulate air/HEPA filter.

**Appendix Q-II-C-2-k.** Vacuum lines shall be protected with high efficiency particulate air/HEPA filters and liquid disinfectant traps.

**Appendix Q-II-C-2-l.** In lieu of open housing in the special animal room, animals held in a BL3-N area may be housed in partial-containment caging systems (e.g., Horsfall units or gnotobiotic systems, or other special containment primary barriers). Prudent judgment must be exercised to implement this ventilation system (e.g., animal species) and its discharge location.

**Appendix Q-II-C-2-m.** Each animal area shall contain a foot, elbow, or automatically operated sink for hand washing. The sink shall be located near the exit door.

**Appendix Q-II-C-2-n.** Restraining devices for animals may be required to avoid damage to the integrity of the animal containment facility.

**Appendix Q-II-D. Biosafety Level 4 - Animals (BL4-N)** (See Appendix Q-III-C, *Footnotes and References for Appendix Q*)

**Appendix Q-II-D-1. Standard Practices (BL4-N)**

**Appendix Q-II-D-1-a. Animal Facility Access (BL4-N)**

**Appendix Q-II-D-1-a-(1).** Individuals under 16 years of age shall not be permitted to enter the animal area.

**Appendix Q-II-D-1-a-(2).** The containment area shall be locked.

**Appendix Q-II-D-1-a-(3).** The containment area shall be patrolled or monitored at frequent intervals.

**Appendix Q-II-D-1-a-(4).** The containment building shall be controlled and have a locking access.

**Appendix Q-II-D-1-a-(5).** The Animal Facility Director shall establish policies and procedures whereby only persons who have been advised of the potential hazard and who meet any specific entry requirements (e.g., vaccination) may enter the laboratory or animal room.

**Appendix Q-II-D-1-a-(6).** Individuals shall enter and exit the animal facility only through the clothing change and shower rooms.

**Appendix Q-II-D-1-a-(7).** Personnel shall use the airlocks to enter or exit the laboratory only in an emergency.

**Appendix Q-II-D-1-a-(8).** Animal room doors, gates, and other closures shall be kept closed when experiments are in progress.

**Appendix Q-II-D-1-b. Decontamination and Inactivation (BL4-N)**

**Appendix Q-II-D-1-b-(1).** All contaminated liquid or solid wastes shall be decontaminated before disposal.

**Appendix Q-II-D-1-b-(2).** The work surfaces and containment equipment shall be decontaminated when work with organisms containing recombinant or synthetic nucleic acid molecules is finished. Where feasible, plasticbacked paper toweling shall be used on nonporous work surfaces to facilitate clean-up.

**Appendix Q-II-D-1-b-(3).** All wastes from animal rooms and laboratories shall be appropriately decontaminated before disposal in an approved manner.

**Appendix Q-II-D-1-b-(4).** No materials, except for biological materials that are to remain in a viable or intact state, shall be removed from the maximum containment laboratory unless they have been autoclaved or decontaminated. Equipment or material that might be damaged by high temperatures or steam shall be decontaminated by gaseous or vapor methods in an airlock or chamber designed for this purpose.

**Appendix Q-II-D-1-b-(5).** When ventilated suits are required, the animal personnel shower entrance/exit area shall be equipped with a chemical disinfectant shower to decontaminate the surface of the suit before exiting the area. A neutralization or water dilution device shall be integral with the chemical disinfectant discharge piping before entering the heat sterilization system. Entry to this area shall be through an airlock fitted with airtight doors.

**Appendix Q-II-D-1-b-(6).** Needles and syringes shall be promptly placed in a puncture-resistant container and decontaminated, preferably by autoclaving, before discard or reuse.

**Appendix Q-II-D-1-b-(7).** Supplies and materials needed in the animal facility shall be brought in by way of the double-door autoclave, fumigation chamber, or airlock that shall be appropriately decontaminated between each use.

**Appendix Q-II-D-1-b-(8).** An autoclave, incinerator, or other effective means to decontaminate animals and wastes shall be available, preferably within the containment area. If feasible, a double-door autoclave is preferred and should be positioned to allow removal of material from the containment area.

**Appendix Q-II-D-1-b-(9).** Liquid effluent from containment equipment, sinks, biological safety cabinets, animal rooms, primary barriers, floor drains, and sterilizers shall be decontaminated by heat treatment before being released into the sanitary system. If required by design, regulation, local ordinance or policy, liquid wastes from shower rooms and toilets shall be decontaminated with chemical disinfectants or heat by methods demonstrated to be effective. The procedure used for heat decontamination of liquid wastes shall be monitored with a recording thermometer. The effectiveness of the heat decontamination process system shall be revalidated at minimum on a yearly basis with an indicator organism. More frequent validation, based on the amount of use or other safety factors, shall be left to the discretion of the IBC. If required by design, regulation, local ordinance or policy, liquid wastes from the shower shall be chemically decontaminated using an Environmental Protection Agency-approved germicide. The efficacy of the chemical treatment process shall be validated with an indicator organism. Chemical disinfectants shall be neutralized or diluted before release into general effluent waste systems.

**Appendix Q-II-D-1-c. Signs (BL4-N)**

**Appendix Q-II-D-1-c-(1).** When the animal research requires special provisions for entry (e.g., vaccination), a warning sign incorporating the universal biosafety symbol shall be posted on all access doors to the animal work area. The sign shall indicate: (i) the agent, (ii) the animal species, (iii) the name and telephone number of the Animal Facility Director, or other responsible individual, and (iv) any special requirements for entering the laboratory.

**Appendix Q-II-D-1-d. Protective Clothing (BL4-N)**

**Appendix Q-II-D-1-d-(1).** Individuals shall enter and exit the animal facility only through the clothing change and shower rooms. Street clothing shall be removed and kept in the outer clothing change room. Complete laboratory clothing (may be disposable), including undergarments, pants, shirts, jump suits, and shoes shall be provided for all personnel entering the animal facility. When exiting the BL4-N area and before proceeding into the shower area, personnel shall remove their laboratory clothing in the inner change room. All laboratory clothing shall be autoclaved before laundering. Personnel shall shower each time they exit the animal facility.

**Appendix Q-II-D-1-d-(2).** A ventilated head-hood or a one-piece positive pressure suit, which is ventilated by a life-support system, shall be worn by all personnel entering rooms that contain experimental animals when appropriate. When ventilated suits are required, the animal personnel shower entrance/exit area shall be equipped with a chemical disinfectant shower to decontaminate the surface of the suit before exiting the area. A neutralization or water dilution device shall be integral with the chemical disinfectant discharge piping before entering the heat sterilization system. Entry to this area shall be through an airlock fitted with airtight doors.

**Appendix Q-II-D-1-d-(3).** Appropriate respiratory protection shall be worn in rooms containing experimental animals.

**Appendix Q-II-D-1-e. Records (BL4-N)**

**Appendix Q-II-D-1-e-(1).** Documents regarding experimental animal use and disposal shall be maintained in a permanent record book.

**Appendix Q-II-D-1-e-(2).** A system shall be established for: (i) reporting laboratory accidents and exposures that are a result of overt exposures to organisms containing recombinant or synthetic nucleic acid molecules, (ii) employee absenteeism, and (iii) medical surveillance of potential laboratory-associated illnesses. Permanent records shall be prepared and maintained. Any incident involving spills and accidents that results in environmental release or exposures of animals or laboratory workers to organisms containing recombinant or synthetic nucleic acid molecules shall be reported immediately to the Biological Safety Officer, Animal Facility Director, Institutional Biosafety Committee, NIH OSP, and other appropriate authorities (if applicable). Reports to the NIH OSP shall be sent to the Office of Science Policy, National Institutes of Health, preferably by e-mail to: NIHGuidelines@od.nih.gov; additional contact information is also available here and on th[e OSP website](http://www.osp.od.nih.gov/)

(www.osp.od.nih.gov). Medical evaluation, surveillance, and treatment shall be provided as appropriate and written records maintained. If necessary, the area shall be appropriately decontaminated.

**Appendix Q-II-D-1-e-(3).** When appropriate and giving consideration to the agents handled, baseline serum samples shall be collected and stored for animal care and other at-risk personnel. Additional serum specimens may be collected periodically depending on the agents handled or the function of the facility.

**Appendix Q-II-D-1-e-(4).** A permanent record book indicating the date and time of each entry and exit shall be signed by all personnel.

**Appendix Q-II-D-1-f. Transfer of Materials (BL4-N)**

**Appendix Q-II-D-1-f-(1).** No materials, except for biological materials that are to remain in a viable or intact state, shall be removed from the maximum containment laboratory unless they have been autoclaved or decontaminated. Equipment or material that might be damaged by high temperatures or steam shall be decontaminated by gaseous or vapor methods in an airlock or chamber designed for this purpose.

**Appendix Q-II-D-1-f-(2).** Biological materials removed from the animal maximum containment laboratory in a viable or intact state shall be transferred to a non-breakable sealed primary container and then enclosed in a non-breakable sealed secondary container that shall be removed from the animal facility through a disinfectant dunk tank, fumigation chamber, or an airlock designed for this purpose. Advance approval for transfer of material shall be obtained from the Animal Facility Director. Such packages containing viable agents can only be opened in another BL4-N animal facility if the agent is biologically inactivated or incapable of reproduction. Special safety testing, decontamination procedures, and Institutional Biosafety Committee approval shall be required to transfer agents or tissue/organ specimens from a BL4-N animal facility to one with a lower containment classification.

**Appendix Q-II-D-1-f-(3).** Supplies and materials needed in the animal facility shall be brought in by way of the double-door autoclave, fumigation chamber, or airlock that shall be appropriately decontaminated between each use. After securing the outer doors, personnel within the animal facility retrieve the materials by opening the interior doors of the autoclave, fumigation chamber, or airlock. These doors shall be secured after materials are brought into the animal facility.

**Appendix Q-II-D-1-g. Other (BL4-N)**

**Appendix Q-II-D-1-g-(1).** All genetically engineered neonates shall be permanently marked within 72 hours after birth, if their size permits. If their size does not permit marking, their containers should be marked. In addition, transgenic animals should contain distinct and biochemically assayable DNA sequences that allow identification of transgenic animals from among non-transgenic animals.

**Appendix Q-II-D-1-g-(2).** Eating, drinking, smoking, and applying cosmetics shall not be permitted in the work area.

**Appendix Q-II-D-1-g-(3).** Individuals who handle materials and animals containing recombinant or synthetic nucleic acid molecules shall be required to wash their hands before exiting the containment area.

**Appendix Q-II-D-1-g-(4).** Experiments involving other organisms that require containment levels lower than BL4-N may be conducted in the same area concurrently with experiments requiring BL4-N containment provided that they are conducted in accordance with BL4-N practices.

**Appendix Q-II-D-1-g-(5).** Animal holding areas shall be cleaned at least once a day and decontaminated immediately following any spill of viable materials.

**Appendix Q-II-D-1-g-(6).** All procedures shall be performed carefully to minimize the creation of aerosols.

**Appendix Q-II-D-1-g-(7).** A double barrier shall be provided to separate male and female animals. Animal isolation barriers shall be sturdy and accessible for cleaning. Reproductive incapacitation may be used.

**Appendix Q-II-D-1-g-(8).** The containment area shall be in accordance with state and Federal laws and animal care requirements.

**Appendix Q-II-D-1-g-(9).** The life support system for the ventilated suit or head hood is equipped with alarms and emergency back-up air tanks. The exhaust air from the suit area shall be filtered by two sets of high efficiency particulate air/HEPA filters installed in series or incinerated. A duplicate filtration unit, exhaust fan, and an automatically starting emergency power source shall be provided. The air pressure within the suit shall be greater than that of any adjacent area. Emergency lighting and communication systems shall be provided. A double-door autoclave shall be provided for decontamination of waste materials to be removed from the suit area.

**Appendix Q-II-D-1-g-(10).** Needles and syringes shall be used only for parenteral injection and aspiration of fluids from laboratory animals and diaphragm bottles. Only needle-locking syringes or disposable syringeneedle units (i.e., needle is integral to the syringe) shall be used for the injection or aspiration of fluids containing organisms that contain recombinant or synthetic nucleic acid molecules. Extreme caution shall be used when handling needles and syringes to avoid autoinoculation and the generation of aerosols during use and disposal. Following use, needles shall not be bent, sheared, replaced in the needle sheath or guard, or removed from the syringe. The needles and syringes shall be promptly placed in a puncture-resistant container and decontaminated, preferably by autoclaving, before discard or reuse.

**Appendix Q-II-D-1-g-(11).** An essential adjunct to the 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 Q-II-D-1-g-(12).** A biosafety manual shall be prepared or adopted. Personnel shall be advised of special hazards and required to read and follow instructions on practices and procedures.

**Appendix Q-II-D-1-g-(13).** Vacuum lines shall be protected with high efficiency particulate air/HEPA filters and liquid disinfectant traps.

**Appendix Q-II-D-2. Animal Facilities (BL4-N)**

**Appendix Q-II-D-2-a.** Animals shall be contained within an enclosed structure (animal room or equivalent) to minimize the possibility of theft or unintentional release and avoid arthropod access.

**Appendix Q-II-D-2-b.** The interior walls, floors, and ceilings shall be impervious to water and resistant to acids, alkalis, organic solvents, and moderate heat, to facilitate cleaning. Penetrations in these structures and surfaces (e.g., plumbing and utilities) shall be sealed.

**Appendix Q-II-D-2-c.** Windows in the animal facility shall be closed, sealed, and breakage resistant (e.g., double-pane tempered glass or equivalent).

**Appendix Q-II-D-2-d.** An autoclave, incinerator, or other effective means to decontaminate animals and wastes shall be available, preferably within the containment area. If feasible, a double-door autoclave is preferred and should be positioned to allow removal of material from the containment area.

**Appendix Q-II-D-2-e.** Access doors to the containment area shall be self-closing.

**Appendix Q-II-D-2-f.** All perimeter joints and openings shall be sealed to form an arthropod-proof structure.

**Appendix Q-II-D-2-g.** The BL4-N laboratory provides a double barrier to prevent the release of recombinant or synthetic nucleic acid molecule containing microorganisms into the environment. Design of the animal facility shall be such that if the barrier of the inner facility is breached, the outer barrier will prevent release into the environment. The animal area shall be separated from all other areas. Passage through two sets of doors shall be the basic requirement for entry into the animal area from access corridors or other contiguous areas. Physical separation of the animal containment area from access corridors or other laboratories or activities shall be provided by a double-door clothes change room equipped with integral showers and airlock.

**Appendix Q-II-D-2-h.** A necropsy room shall be provided within the BL4-N containment area.

**Appendix Q-II-D-2-i.** Liquid effluent from containment equipment, sinks, biological safety cabinets, animal rooms, primary barriers, floor drains, and sterilizers shall be decontaminated by heat treatment before being released into the sanitary system. If required by design, regulation, local ordinance or policy, liquid wastes from shower rooms and toilets shall be decontaminated with chemical disinfectants or heat by methods demonstrated to be effective. The procedure used for heat decontamination of liquid wastes shall be monitored with a recording thermometer. The effectiveness of the heat decontamination process system shall be revalidated at minimum on a yearly basis with an indicator organism. More frequent validation, based on the amount of use or other safety factors, shall be left to the discretion of the IBC. If required by design, regulation, local ordinance or policy, liquid wastes from the shower shall be chemically decontaminated using an Environmental Protection Agency-approved germicide. The efficacy of the chemical treatment process shall be validated with an indicator organism. Chemical disinfectants shall be neutralized or diluted before release into general effluent waste systems.

**Appendix Q-II-D-2-j.** A ducted exhaust air ventilation system shall be provided that creates directional airflow that draws air into the laboratory through the entry area. The exhaust air, which is not recirculated to any other area of the building, shall be discharged to the outside and dispersed away from the occupied areas and air intakes. Personnel shall verify that the direction of the airflow (into the animal room) is proper.

**Appendix Q-II-D-2-k.** Exhaust air from BL4-N containment area shall be double high efficiency particulate air/HEPA filtered or treated by passing through a certified HEPA filter and an air incinerator before release to the atmosphere. Double HEPA filters shall be required for the supply air system in a BL4-N containment area.

**Appendix Q-II-D-2-l.** All high efficiency particulate air/HEPA filters' frames and housings shall be certified to have no detectable smoke [dioctyl phthalate] leaks when the exit face (direction of flow) of the filter is scanned above 0.01 percent when measured by a linear or logarithmic photometer. The instrument must demonstrate a threshold sensitivity of at least 1x10-3 micrograms per liter for 0.3 micrometer diameter dioctyl phthalate particles and a challenge concentration of 80-120 micrograms per liter. The air sampling rate should be at least 1 cfm (28.3 liters per minute).

**Appendix Q-II-D-2-m.** If an air incinerator is used in lieu of the second high efficiency particulate air/HEPA filter, it shall be biologically challenged to prove all viable test agents are sterilized. The biological challenge must be minimally 1x108 organisms per cubic foot of airflow through the incinerator. It is universally accepted if bacterial spores are used to challenge and verify that the equipment is capable of killing spores, then assurance is provided that all other known agents are inactivated by the parameters established to operate the equipment. Test spores meeting this criterion are *Bacillus* *subtilis* var. *niger* or *Bacillus* *stearothermophilus*. The operating temperature of the incinerator shall be continuously monitored and recorded during use.

**Appendix Q-II-D-2-n.** All equipment and floor drains shall be equipped with deep traps (minimally 5 inches). Floor drains shall be fitted with isolation plugs or fitted with automatic water fill devices.

**Appendix Q-II-D-2-o.** Each animal area shall contain a foot, elbow, or automatically operated sink for hand washing. The sink shall be located near the exit door.

**Appendix Q-II-D-2-p.** Restraining devices for animals may be required to avoid damage to the integrity of the containment animal facility.

**Appendix Q-II-D-2-q.** The supply water distribution system shall be fitted with a back-flow preventer or break tank.

**Appendix Q-II-D-2-r.** All utilities, liquid and gas services, shall be protected with devices that avoid back-flow.

**Appendix Q-II-D-2-s.** Sewer and other atmospheric ventilation lines shall be equipped minimally with a single high efficiency particulate/HEPA filter. Condensate drains from these type housings shall be appropriately connected to a contaminated or sanitary drain system. The drain position in the housing dictates the appropriate system to be used.

**Appendix Q-III. Footnotes and References for Appendix Q**

**Appendix Q-III-A.** If a recombinant or synthetic nucleic acid molecule is derived from a Class 2 organism requiring BL2 containment, personnel shall be required to have specific training in handling pathogenic agents and directed by knowledgeable scientists.

**Appendix Q-III-B.** Personnel who handle pathogenic and potentially lethal agents shall be required to have specific training and be supervised by knowledgeable scientists who are experienced in working with these agents. BL3-N containment also minimizes escape of recombinant or synthetic nucleic acid moleculecontaining organisms from exhaust air or waste material from the containment area.

**Appendix Q-III-C.** Risk Group 4 and restricted microorganisms (see Appendix B, *Classification of Human Etiologic Agents on the Basis of Hazard*, and Sections V-G and V-L, *Footnotes and References of Sections I through IV*) pose a high level of individual risk for acquiring life-threatening diseases to personnel and/or animals. To import animal or plant pathogens, special approval must be obtained from [U.S. Department of Agriculture, Animal and Plant Health Inspection Service (APHIS),](http://www.aphis.usda.gov/) Veterinary Services, National Center for Import-Export, Products Program, 4700 River Road, Unit 40, Riverdale, MD 20737. Phone: (301) 734-8499; Fax: (301) 734-8226.

Laboratory staff shall be required to have specific and thorough training in handling extremely hazardous infectious agents, primary and secondary containment, standard and special practices, and laboratory design characteristics. The laboratory staff shall be supervised by knowledgeable scientists who are trained and experienced in working with these agents and in the special containment facilities.

Within work areas of the animal facility, all activities shall be confined to the specially equipped animal rooms or support areas. The maximum animal containment area and support areas shall have special engineering and design features to prevent the dissemination of microorganisms into the environment via exhaust air or waste disposal.

**Appendix Q-III-D.**  Other research with non-laboratory animals, which may not appropriately be conducted under conditions described in Appendix Q, may be conducted safely by applying practices routinely used for controlled culture of these biota. In aquatic systems, for example, BL1 equivalent conditions could be met by utilizing growth tanks that provide adequate physical means to avoid the escape of the aquatic species, its gametes, and introduced exogenous genetic material. A mechanism shall be provided to ensure that neither the organisms nor their gametes can escape into the supply or discharge system of the rearing container (e.g., tank, aquarium, etc.) Acceptable barriers include appropriate filtration, irradiation, heat treatment, chemical treatment, etc. Moreover, the top of the rearing container shall be covered to avoid escape of the organism and its gametes. In the event of tank rupture, leakage, or overflow, the construction of the room containing these tanks should prevent the organisms and gametes from entering the building's drains before the organism and its gametes have been inactivated.

Other types of non-laboratory animals (e.g., nematodes, arthropods, and certain forms of smaller animals) may be accommodated by using the appropriate BL1 through BL4 or BL1-P through BL4-P containment practices and procedures as specified in Appendices G and P.