

## Inspection Checklist for NIH BL4-N Laboratories (7 CFR 331; 9 CFR 121; 42 CFR 73; NIH Guidelines)

Entity Name:  
 Inspection Date:  
 Street Address:  
 City, State, Zip:  
 RO:  
 ARO(s):

Lead Inspector:  
 Other Inspectors:

Building/Room(s):

PI(s):

HHS Agents:

Overlap Agents:

USDA Agents:

*When information is entered in this form, the form is to be considered Sensitive Select Agent Information.*

Entity Name:		Inspection Date:			
Reference	Statement	Yes	No	N/A	Comments
<b>NIH BL4-N (rDNA) REQUIREMENTS</b>					
<b>CFR: Section 12(a)</b>	An individual or entity required to register under this part must develop and implement a written biosafety (biocontainment) plan that is commensurate with the risk of the select agent or toxin, given its intended use.				
<b>CFR: Section 12(a)</b>	The biosafety (biocontainment) plan must contain sufficient information and documentation to describe the biosafety and containment procedures for the select agent or toxin, including any animals (including arthropods) or plants intentionally or accidentally exposed to or infected with a select agent.				
<b>CFR: Section 12(b)</b>	The biosafety and containment procedures must be sufficient to contain the select agent or toxin (e.g., physical structure and features of the entity, and operational and procedural safeguards).				
<b>CFR: Section 12 (c)(1)</b>	In developing a biosafety plan, an individual or entity should consider: The CDC/NIH publication, Biosafety in Microbiological and Biomedical Laboratories. This document is available on the National Select Agent Registry website at <a href="http://www.selectagents.gov/">http://www.selectagents.gov/</a> .				
<b>CFR: Section 12 (c)(3)</b>	In developing a biosafety plan, an individual or entity should consider: The "NIH Guidelines for Research Involving Recombinant or Synthetic Molecules," (NIH Guidelines). This document is available on the National Select Agent Registry Web site at <a href="http://www.selectagents.gov">http://www.selectagents.gov</a> .				

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<b>CFR: Section 12(d)</b>	The biosafety plan must include an occupational health program for individuals with access to Tier 1 select agents and toxins, and those individuals must be enrolled in the occupational health program.				
<b>CFR: Section 12(e)</b>	The plan must be reviewed annually and revised as necessary.				
<b>CFR: Section 12(e)</b>	Drills or exercises must be conducted at least annually to test and evaluate the effectiveness of the plan.				
<b>CFR: Section 12(e)</b>	The plan must be reviewed and revised, as necessary, after any drill or exercise and after any incident.				
<b>42 CFR 73: Section 13 (a)</b>	An individual or entity may not conduct or possess products (i.e., select agents that are not known to acquire the resistance naturally, if such acquisition could compromise the control of disease agents in humans, veterinary medicine, or agriculture, or recombinant and/or synthetic nucleic acids containing genes for the biosynthesis of select toxins lethal for vertebrates at an LD[50] < 100 ng/kg body weight) resulting from, a restricted experiment with a HHS select agent or toxin unless approved by and conducted in accordance with any conditions prescribed by the HHS Secretary.				
<b>42 CFR 73: Section 13 (a)</b>	In addition, an individual or entity may not conduct or possess products (i.e., select agents that are not known to acquire the resistance naturally, if such acquisition could compromise the control of disease agents in humans, veterinary medicine, or agriculture, or recombinant and/or synthetic nucleic acids containing genes for the biosynthesis of select toxins lethal for vertebrates at an LD[50] < 100 ng/kg body weight) resulting from, a restricted experiment with an overlap select agent or toxin unless approved by and conducted in accordance with any conditions prescribed by the HHS Secretary, after consultation with Administrator.				

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<b>9 CFR 121: Section13(a)</b>	An individual or entity may not conduct or possess products (i.e., select agents that are not known to acquire the resistance naturally, if such acquisition could compromise the control of disease agents in humans, veterinary medicine, or agriculture, or recombinant and/or synthetic nucleic acids containing genes for the biosynthesis of select toxins lethal for vertebrates at an LD[50] < 100 ng/kg body weight) resulting from, the following experiments unless approved by and conducted in accordance with the conditions prescribed by the Administrator:				
<b>NIH: Q-I-B-1</b>	When an animal 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.				
<b>NIH: Q-I-B-2</b>	A permanent record shall be maintained of the experimental use and disposal of each animal or group of animals.				
<b>NIH: Q-II-D-1-a-(1)</b>	Individuals under 16 years of age shall not be permitted to enter the animal area.				
<b>NIH: Q-II-D-1-a-(2)</b>	The containment area shall be locked.				
<b>NIH: Q-II-D-1-a-(3)</b>	The containment area shall be patrolled or monitored at frequent intervals.				
<b>NIH: Q-II-D-1-a-(4)</b>	The containment building shall be controlled and have a locking access.				
<b>NIH: Q-II-D-1-a-(5)</b>	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.				
<b>NIH: Q-II-D-1-a-(6)</b>	Individuals shall enter and exit the animal facility only through the clothing change and shower rooms.				

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NIH: Q-II-D-1-a(7)	Personnel shall use the airlocks to enter or exit the laboratory only in an emergency.				
NIH: Q-II-D-1-a(8)	Animal room doors, gates, and other closures shall be kept closed when experiments are in progress.				
NIH: Q-II-D-1-b(1)	All contaminated liquid or solid wastes shall be decontaminated before disposal.				
NIH: 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, plastic-backed paper toweling shall be used on nonporous work surfaces to facilitate clean-up.				
NIH: Q-II-D-1-b(3)	All wastes from animal rooms and laboratories shall be appropriately decontaminated before disposal in an approved manner.				
NIH: Q-II-D-1-b(4) and NIH: 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.				
NIH: 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.				
NIH: 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.				

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<b>NIH: Q-II-D-1-b-(7) and NIH: Q-II-D-1-f-(3)</b>	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.				
<b>NIH: Q-II-D-1-b-(8)</b>	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.				
<b>NIH: Q-II-D-1-b-(9)</b>	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. 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 every 30 days with an indicator organism. 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.				
<b>NIH: Q-II-D-1-c-(1)</b>	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.				

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<b>NIH: Q-II-D-1-d-(1)</b>	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.				
<b>NIH: Q-II-D-1-d-(2)</b>	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.				
<b>NIH: Q-II-D-1-d-(3)</b>	Appropriate respiratory protection shall be worn in rooms containing experimental animals.				
<b>NIH: Q-II-D-1-e-(1)</b>	Documents regarding experimental animal use and disposal shall be maintained in a permanent record book.				
<b>NIH: Q-II-D-1-e-(2)</b>	A system shall be established for: (i) reporting laboratory accidents and exposures that are a result of overt exposures to organisms containing recombinant DNA, (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/OBA, and other appropriate authorities (if applicable). 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). Medical evaluation, surveillance, and treatment shall be provided as appropriate and written records maintained. If necessary, the area shall be appropriately decontaminated.				
<b>NIH: Q-II-D-1-e-(3)</b>	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.				

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<b>NIH: Q-II-D-1-e-(4)</b>	A permanent record book indicating the date and time of each entry and exit shall be signed by all personnel.				
<b>NIH: Q-II-D-1-f-(2)</b>	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.				
<b>NIH: Q-II-D-1-g-(1)</b>	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.				
<b>NIH: Q-II-D-1-g-(2)</b>	Eating, drinking, smoking, and applying cosmetics shall not be permitted in the work area.				
<b>NIH: Q-II-D-1-g-(3)</b>	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.				
<b>NIH: Q-II-D-1-g-(4)</b>	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.				
<b>NIH: Q-II-D-1-g-(5)</b>	Animal holding areas shall be cleaned at least once a day and decontaminated immediately following any spill of viable materials.				
<b>NIH: Q-II-D-1-g-(6)</b>	All procedures shall be performed carefully to minimize the creation of aerosols.				

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NIH: 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.				
NIH: Q-II-D-1-g(8)	The containment area shall be in accordance with state and Federal laws and animal care requirements.				
NIH: 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 <i>suit</i> area.				
NIH: 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 syringe-needle 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.				
NIH: 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.				
NIH: 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.				
NIH: Q-II-D-1-g(13)	Vacuum lines shall be protected with high efficiency particulate air / HEPA filters and liquid disinfectant traps.				

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<b>NIH: Q-II-D-2-a</b>	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.				
<b>NIH: Q-II-D-2-b</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.				
<b>NIH: Q-II-D-2-c</b>	Windows in the animal facility shall be closed, sealed, and breakage resistant (e.g., double-pane tempered glass or equivalent).				
<b>NIH: Q-II-D-2-d</b>	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.				
<b>NIH: Q-II-D-2-e</b>	Access doors to the containment area shall be self-closing.				
<b>NIH: Q-II-D-2-f</b>	All perimeter joints and openings shall be sealed to form an arthropod-proof structure.				
<b>NIH: Q-II-D-2-g</b>	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.				
<b>NIH: Q-II-D-2-h</b>	A necropsy room shall be provided within the BL4-N containment area.				

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<b>NIH: Q-II-D-2-i</b>	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. 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 every 30 days with an indicator organism. 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.				
<b>NIH: Q-II-D-2-j</b>	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.				
<b>NIH: Q-II-D-2-k</b>	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.				
<b>NIH: Q-II-D-2-l</b>	All high efficiency particulate air / HEPA filters frames and housings shall be certified to have no detectable smoke [dioctylphthalate] 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 <sup>-3</sup> micrograms per liter for 0.3 micrometer diameter dioctylphthalate 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).				

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<b>NIH: Q-II-D-2-m</b>	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 1x10 <sup>8</sup> 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 <i>Bacillus subtilis</i> var. <i>niger</i> or <i>Bacillus stearothermophilis</i> . The operating temperature of the incinerator shall be continuously monitored and recorded during use.				
<b>NIH: Q-II-D-2-n</b>	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.				
<b>NIH: Q-II-D-2-o</b>	Each animal area shall contain a foot, elbow, or automatically operated sink for hand washing. The sink shall be located near the exit door.				
<b>NIH: Q-II-D-2-p</b>	Restraining devices for animals may be required to avoid damage to the integrity of the containment animal facility				
<b>NIH: Q-II-D-2-q</b>	The supply water distribution system shall be fitted with a back-flow preventer or break tank.				
<b>NIH: Q-II-D-2-r</b>	All utilities, liquid and gas services, shall be protected with devices that avoid back-flow.				
<b>NIH: Q-II-D-2-s</b>	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.				

Comments continued:

Inspector summary and comments:

Lead inspector:

Date:

Other inspectors present:

Date:

Lead inspector signature: \_\_\_\_\_

Date: \_\_\_\_\_