Biosafety

NIST S 7101.50
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1. PURPOSE

The Biosafety suborder provides operational requirements and guidance to enable all NIST personnel to work safely with biohazardous materials.

2. BACKGROUND

None.

3. APPLICABILITY

a. The provisions of this suborder apply to all NIST facilities and to all NIST employees who work with biohazardous materials, with the exceptions noted in NIST O 710, Occupational Safety and Health.

b. Breast milk that is collected for nursing purpose is excluded from the requirements of this suborder.

4. REFERENCES

a. National Institutes of Health, *NIH Guidelines for Research Involving Recombinant DNA Molecules*.

b. 42 CFR Part 73, Health and Human Services (HHS) *Possession, Use, and Transfer of Select Agents and Toxins*; Final Rule.

c. 7 CFR Part 331 and 9 CFR Part 121, Department of Agriculture (USDA), Agricultural Bioterrorism Protection Act of 2002; *Possession, Use, and Transfer of Biological Agents and Toxins*; Final Rule.

d. 29 CFR 1910.1030, *Bloodborne Pathogens*.

5. APPLICABLE NIST DIRECTIVES

a. NIST S 7101.51: *Bloodborne Pathogens*

b. NIST S 7101.60: *Chemical Management*

c. NIST S 7101.20: *Work and Worker Authorization Based on Hazard Reviews*

d. NIST S 7101.22: *Hazard Signage*

e. NIST S 7101.24: *Incident Reporting and Investigation*

f. NIST S 7101.23: *Safety Education and Training*

6. REQUIREMENTS

NIST S 7101.20: Work and Worker Authorization based on Hazard Reviews, requires the OUs to conduct hazard reviews of all activities prior to the initiation of those activities to ensure that proper precautions have been taken and proper practices are being followed to enable the safe conduct of the work by NIST employees. When activities involve the use of biohazardous materials, the OU must, as part of its overall hazard review process, submit a Biohazardous Materials Registration and Authorization Request to the BSO for review and approval. Many of the elements in this section pertain to the content of Biohazardous Materials Registration and Authorization Requests.

a. General Biosafety Requirements

(1) Registration and Authorization of the Use of Biohazardous Materials

(a) A Biohazardous Materials Registration and Authorization Request shall be completed and submitted to the BSO for review and approval for each activity that involves biohazardous materials.

(b) Research requiring BSL-3 and BSL-4 facilities is not currently permitted at NIST.

(2) Acquisition of Biohazardous Materials

(a) Acquisition of biohazardous materials from commercial vendors, other government agencies, and/or academic institutions shall take place only after a Biohazardous Materials Registration and Authorization Request has been approved by the BSO and Division line management, and in the case of human cell lines, the IRB.

(3) Biosafety Risk Assessment and Assignment of RGs and BSLs

(a) A risk assessment shall be conducted by the researcher for each activity involving the use of biohazardous materials. For factors that should be considered during the risk assessment process, see Appendix B.

(b) Each biohazardous agent shall be assigned an RG and proposed BSL. This information must be documented on the Biohazardous Materials Registration and Approval Request.

i. RG assignments for specific agents may be found in various sources, including Appendix B of the *NIH Guidelines* (Classification of Human Etiologic Agents on the Basis of Hazard) and the American Biosafety Association (ABSA) Risk Group Database. For selected examples of RG assignments, see Appendix C.

ii. For descriptions of BSL classifications, see Appendix D.

(c) The BSO shall be available to assist researchers in performing risk assessments and assigning RGs and BSLs.

(4) Certification of BSL 2 laboratories

(a) Laboratories shall be inspected and certified by the BSO to be BSL-2 compliant per the BMBL guidelines prior to the start of biological experiments. For requirements, refer to 8b.(1)(3), c(1)(2), and d(2) below.

(5) Inspection of Laboratories

(a) BSL 1 and BSL 2 laboratories shall be inspected annually in a manner to be determined by the BSO. Inspections shall be announced, and an inspection checklist shall be provided.

(6) Select Agents and Toxins

(a) The CSO, acting as the NIST Responsible Official in accordance with the requirements of *Possession, Use, and Transfer of Select Agents and Toxins*, Final Rule, must approve the acquisition of any select agent or toxin prior to that agent or toxin being acquired. Furthermore, laboratories must be registered with and be inspected by the CDC or the USDA prior to working with non-exempted select agents and toxins. A list of the select agents and permissible toxin amounts can be found at www.selectagents.gov.

(7) Recombinant DNA Experiments

(a) NIST researchers who receive funding from the NIH for rDNA research must adhere to the NIH rDNA Guidelines.

(b) The acquisition and use of all rDNA materials shall be reviewed and approved by the NBC.

(8) Animal Work

(a) Work involving animals may expose workers to zoonotic agents in a variety of ways such as wound infections, inhalation of aerosols (e.g., dust from animal bedding), and animal bites or scratches. Work with animals must be subjected to the hazard review process in which appropriate controls are identified in accordance with the requirements of the NIST Hazard Analysis and Control Suborder.

(b) Section VIII of BMBL provides agent summary statements for zoonotic agents. Division Chiefs shall ensure that appropriate equipment and measures are utilized to ensure that NIST personnel are protected during tasks involving animals.

b. Laboratory Practices

The following laboratory practices shall be observed by all employees.

(1) General Practices for All Biological Laboratories

(a) Access to the laboratory must be controlled in accordance with the requirements in 8.d.(1) and 8.d.(2) of this document;

(b) Laboratory personnel must wash their hands after working with potentially hazardous biological materials and before leaving the laboratory;

(c) Eating, drinking, smoking, handling contact lenses, applying cosmetics, and storing food for human consumption are not permitted in laboratory areas;

(d) Mouth pipetting is prohibited; mechanical pipetting devices must be used;

(e) Sharps precautions shall be observed in accordance to the NIST Bloodborne Pathogens suborder;

(f) All procedures shall be designed to minimize the creation of splashes and/or aerosols;

(g) Work surfaces shall be decontaminated with appropriate disinfectant after completion of work and after any spill or splash of potentially infectious materials;

(h) All potentially infectious biological materials shall be decontaminated using an effective method prior to disposal. In addition:

i. Materials to be decontaminated outside of the immediate laboratory must be placed in a durable, leak-proof container and secured for transport to the space in which the decontamination will take place.

ii. Materials to be removed from NIST for decontamination must be packed in accordance with OSHE Environment Management Group's procedures, which are in compliance with DOT (49 CFR Part 171-180) and OSHA (29 CFR Part 1910.1030) regulations.

(i) A sign incorporating the universal biohazard symbol must be posted at the entrance of any BSL-1 or BSL-2 laboratory;

i. for BSL-2 laboratories, the sign must include the laboratory's BSL, the supervisor's or laboratory contact's information, agents' information, and instructions for entering and exiting the laboratory;

ii. the signs must be removed when biohazardous materials are no longer present; and

iii. the signs must comply with the requirements of the NIST Hazard Signage Suborder;

(j) The universal biohazard labels must be posted on refrigerators, freezers, and incubators where biohazardous materials are stored, and on containers that are used to transport biohazardous materials.

(k) All stored biohazardous materials must have labels containing the following information:

i. Name of material;

ii. Acquisition or production date; and

iii. Initials of user.

For storage containers that are too small to capture all the labeling requirements listed above, a numbering system documented with the corresponding information is acceptable.

(l) All biohazardous materials must be inventoried for storage.[1]

(2) Special Additional Practices for BSL 1 Laboratories

(a) No special additional practices are required.

(3) Special Additional Practices for BSL 2 Laboratories

(a) All persons entering the laboratory must be advised of the potential hazards using general and specific NIST hazard signs;

(b) Laboratory personnel must be provided medical surveillance, as needed, and offered available immunizations for agents handled or potentially present in the laboratory;

(c) A laboratory-specific biosafety manual must be prepared (using, e.g., the NIST Requirements found in this document as a template), adopted as policy, and made available in hard copy in the laboratory;

[1] The on-line biohazardous materials database will have the capability of meeting this requirement. For biohazardous materials that either exhibit hazardous chemical properties or mixed with other hazardous chemicals, the hazardous chemicals shall be registered separately in the Chemical Inventory System (CISPro) in accordance with the requirement of the NIST Chemical Management suborder.

(d) Proficiency in standard and special microbiological practices must be demonstrated by laboratory personnel before they are authorized to work with BSL-2 agents;

(e) Potentially infectious materials must be placed in a durable, leak proof container during collection, handling, processing, storage, or transport within a facility;

(f) Laboratory equipment should be routinely decontaminated, especially after spills, splashes, or other potential contamination;

(g) Equipment must be decontaminated by the owner before repair, maintenance, or removal from the laboratory;

(h) Spills involving infectious materials must be contained, decontaminated, and cleaned up by staff properly trained and equipped to work with infectious materials; and

(i) For incidents that result or could have resulted in exposure to infectious materials:

i. a report must be made to the first-level supervisor or group leader and a report must be filed to the NIST Incident Reporting and Investigation System (IRIS) in accordance to the requirements of the NIST Incident Reporting and Investigation Suborder;

ii. medical evaluation, surveillance, and treatment should be provided; and

iii. appropriate records shall be maintained.

c. Safety Equipment

Appropriate containment devices and PPE shall be available in all biological laboratories.

(1) Containment Devices

(a) Containment devices such as Biological Safety Cabinets (BSCs) are not required in BSL-1 laboratories.

(b) Properly maintained BSCs shall be used in BSL-2 laboratories whenever procedures with the potential for creating aerosols or splashes are performed. Such procedures may include, but are not limited to, pipetting, centrifuging, vortexing, homogenizing, and sonicating. Activities involving both biohazardous materials and hazardous volatile chemicals could be conducted in specific types of BSC or properly maintained fume hoods after a thorough risk assessment has been conducted by the

researcher and approved by the BSO. A centrifuge with sealed rotor or sealed safety buckets shall also be made available, if needed.

(c) BSCs shall be located away from doors and from high traffic areas in the laboratory to reduce disruption of air flow in the BSCs.

(d) BSCs must be tested and certified annually or after installation, repair, or relocation. The certification must be performed by vendors certified by the National Sanitation Foundation.

(e) For recommended practices for working in a BSC, see Appendix E.

(2) Personal Protective Equipment (PPE)

(a) Once a biological and/or procedural hazard has been identified, the required PPE shall be determined as part of the hazard review process. Minimum PPE for working with biohazardous materials includes gloves, protective eyewear, laboratory coats, closed-toe and closed-back shoes, and long pants.

(b) Area-specific PPE requirements shall be established and posted on the laboratory entrance in accordance with NIST Hazard Signage Suborder.

(c) In BSL-2 laboratories, when it is anticipated that potentially infectious biological materials could splash or splatter during work performed outside a BSC, appropriate face protection shall be worn. Such protection would include, but is not limited to, goggles, side-shielded safety glasses, and full-face shields. Bench-top splash shields can be used instead of full-face shields.

(d) Long-sleeved lab coats or gowns shall be worn to protect skin and personal clothing from contamination. Protective clothing shall not be worn outside of laboratory. Reusable lab coats or gowns shall be laundered on-site or by a laundering service at least once a month or whenever gross contamination occurs. Personnel shall not launder lab coats or gowns at home. Disposable gowns shall be used when on-site laundering service is not available. Disposable gowns shall be replaced at least once a month or whenever gross contamination occurs.

(e) Gloves must be worn when handling biohazardous materials. Use of standard nitrile or powder-free latex gloves is considered adequate for handling most biohazardous materials. Non-latex glove alternatives shall be made available to researchers who are allergic to latex gloves. Gloves shall be considered single use only and disposed

of at the conclusion of the task as infectious/medical waste. Hands shall be washed immediately after the removal of gloves at the conclusion of the task.

(f) When significant splash and splatter are anticipated, disposable shoe-covers/booties shall be worn. Prior to exiting the laboratory, these must be removed and be disposed of as infectious waste.

(g) If gross contamination occurs, PPE shall be removed immediately and replaced. PPE shall be removed and be disposed of with other biohazardous waste before exiting the laboratory.

d. Facilities

(1) BSL-1 Laboratories

(a) Laboratories should have lockable doors for access control.

(b) Laboratories must have a sink for hand washing.

(c) The laboratory should be designed so that it can be easily cleaned. Carpet and rugs in laboratories are not appropriate.

(d) Laboratory furniture must be capable of supporting anticipated loads and uses. Spaces between benches, cabinets, and equipment should be accessible for cleaning.

(e) Bench tops must be impervious to water and resistant to heat, organic solvents, acids, alkalis, and other chemicals.

(f) Chairs used in laboratory work must be covered with a non-porous material that can be easily cleaned and decontaminated with appropriate disinfectant.

(2) BSL-2 Laboratories

(a) Laboratory doors should be self-closing and have locks. Only authorized personnel shall have access to the laboratories.

(b) Laboratories must have a sink for hand washing. The sink may be manual, hands-free, or automatic. It should be located near the exit door.

(c) The laboratory should be designed so that it can be easily cleaned. Carpet and rugs in laboratories are not allowed.

(d) Laboratory furniture must be capable of supporting anticipated loads and uses.

(e) Spaces between benches, cabinets, and equipment should be accessible for cleaning.

(f) Bench tops must be impervious to water and resistant to heat, organic solvents, acids, alkalis, and other chemicals.

(g) Chairs used in laboratory work must be covered with a non-porous material that can be easily cleaned and decontaminated with appropriate disinfectant.

(h) Biosafety cabinets, where applicable, should be installed so that fluctuations of the room air supply and exhaust do not interfere with proper operations. BSCs should be located away from doors, heavy-traffic areas, and other possible airflow disruptions.

(i) Vacuum lines should be protected with an inline HEPA filter and liquid disinfectant traps.

(j) An eyewash station must be available in the laboratory or be in accessible locations that require no more than 10 seconds to reach. The eyewash station shall be located on the same level as the hazard and the path of travel shall be free of obstructions that may inhibit the immediate use of the equipment.

(k) A method for decontaminating all biohazardous wastes should be available in divisional laboratories (e.g., autoclave, chemical disinfection, incineration, or other validated decontamination method).

e. Disinfectants, Decontamination, and Biohazardous Spill Clean-up

Appropriate decontamination supplies such as disinfectants, absorbent pads/wipes, biohazardous waste bags, gloves, and tongs or forceps to pick up broken glass shall be available in all biological laboratories.

(1) Disinfectants

(a) Chemical disinfectants that are registered as EPA tuberculocidal disinfectants are suitable for surface decontamination, equipment decontamination, spill cleanup, and liquid waste disinfection.

(b) Commonly used chemical disinfectants such as 70% ethanol solution, freshly prepared 10% bleach solution, and Cavicide are acceptable for use in biological laboratories.

(c) A 10% bleach solution or an equally effective formulation shall be used for surface decontamination after working with human specimens and cleaning up spills involving human specimens. Each bleach solution container must be labeled with either a made-on date or an expiration date, which is 24 hours after the day the solution was made.[2] Check the production date on the commercial bleach container before use.

(2) Decontamination

(a) All bench surfaces and equipment used in experiments shall be decontaminated when work is completed.

(b) Prior to decontaminating contaminated equipment, see the user's manual for compatible disinfectants.

(3) Biohazardous Spill Cleanup

(a) For recommended procedures for cleaning up spills of biohazardous materials, see Appendix F.

f. Biohazardous Waste Management

(1) On-Site Waste Treatment

(a) Steam sterilization (autoclaving) is an acceptable method for treating solid and liquid biohazardous waste generated on site at NIST. Only autoclavable biohazardous waste bags shall be used for autoclaving. For onsite autoclaving procedures, see Appendix G.

(b) Chemical disinfection

i. Chemical disinfection is an alternative treatment option for liquid biohazardous waste.

[2] When bleach and water are mixed to create a disinfecting solution, the solution begins to lose its disinfecting properties after 24 hours. Furthermore, commercial bleach begins to degrade approximately 20% each year after being stored for six months at temperatures between 10 and 21 degrees Celsius (50 and 70 degrees Fahrenheit).

ii. Chemical disinfectants that are registered as EPA tuberculocidal disinfectants are acceptable for liquid biohazardous waste disinfection. A freshly prepared 10% bleach solution is an effective disinfectant, particularly for human specimens. A minimum contact time of 20 minutes is recommended for a 10% bleach solution disinfection.

iii. Properly disinfected liquid biohazardous waste (with no hazardous chemicals) can be disposed of down the drain.

(c) Solid and liquid hazardous microbiological and molecular waste, human specimens, and tissue culture waste must be autoclaved before disposal. As an alternative, liquid biohazardous waste can be disinfected by chemical disinfectants.

(d) Pipets and pipet tips that have come in contact with risk groups 2 and human specimens should be placed in a pipet container, and when three-quarters full, the container should be autoclaved before disposal. Alternatively, contaminated pipets and pipet tips can be chemically disinfected. Chemically disinfected pipets/pipet tips can then be placed in a biohazard waste receptacle.

(e) Disposable gloves, gauze, parafilms, vials, test tubes, and other laboratory supplies that have come in contact with risk groups 2 agents and human specimens must be autoclaved before disposal.

(f) Potentially infectious materials (risk group 2 agents, biological toxins, and human specimens) and associated laboratory materials that have not been disinfected can be disposed directly into the biohazard waste receptacles, as long as the materials are contained and the receptacles are covered with lids. Lids are not required for biohazard waste receptacles such as cardboard waste boxes that contain disinfected or properly contained (e.g., in closed pipet containers or closed waste bags) infectious materials.

(g) Laboratory supplies (non-sharps) that have come in contact with only risk group 1 agents can be disposed of in regular trash.

(2) Off-site Waste Treatment and Disposal

(a) For NIST Gaithersburg and Boulder researchers without access to an autoclave, an off-site biohazardous waste treatment and disposal option is available through OSHE. All solid and liquid biohazardous waste shall be properly contained before disposal

into a red biohazardous waste bag. When the red biohazard bag is three-quarters full, it should be tied off and placed in a biohazardous waste cardboard box, which should then be taped closed. The bags and boxes should be handled only when wearing gloves and lab coat. Contact OSHE for pick-up of the biohazardous waste cardboard boxes.

(b) All sharps (e.g., needles, syringes with attached needles, capillary tubes, slides and cover slips, scalpel blades, razor blades, and broken contaminated glassware) must be disposed in a rigid, puncture-resistant, and leak-proof sharps container with a universal biohazard label. When the container is three-quarters full, contact OSHE for hazardous waste pick up.

g. Transportation and Shipping of Biohazardous Materials

(1) Intra-Campus and Local Transfers of Biohazardous Materials

(a) Biohazardous materials to be transferred intra-campus should be placed in a closable primary container. Absorbent material should be placed around the primary container. The primary container and absorbent materials are then placed into a closable secondary container. A universal biohazard label shall be placed on the secondary container. The secondary container should be disinfected routinely.

(2) Shipping of Biohazardous Materials

(a) The International Air Transportation Association's (IATA) Dangerous Goods Regulations (DGR) govern all international and domestic air transport of biohazardous materials. IATA classifies biohazardous materials into Category A Infectious Substances, Category B Biological Substances, and Exempt Human Specimens. These categories have different packaging and labeling requirements. All personnel involved with the shipping of Category A infectious substances are required to receive training on the applicable requirement. Contact the BSO for assistance in shipping biohazardous materials out of NIST.

h. Importation of Etiologic Agents

(1) In general, a permit from the United States Public Health Service Division of Quarantine is required for the importation of any infectious agent known to cause disease in humans. Contact the BSO prior to requesting such a permit.

i. Emergency Response

(1) All BSL 2 laboratories shall establish emergency response procedures based on the biohazardous materials used. Notify OSHE personnel when a spill or exposure to a biohazardous agent occurs outside of primary containment such as the BSC and report the incident to IRIS.

(2) Refer to Appendix H for a limited list of emergency response procedure examples.

j. Medical Surveillance

(1) Occupational Health and Immunizations

(a) The NIST Health Unit will provide immunization consultations and occupational health support for incidents involving exposure to biological hazards.

(2) Injuries and Illnesses Involving Biohazardous Materials

(a) Injuries and illnesses resulting from exposure to a hazardous biological agent shall be reported using the NIST Incident Reporting and Investigation System.

k. Decommissioning of Biological Laboratories

(1) Biological laboratories shall be decommissioned in accordance with the following procedures when biological work in them is terminated:

(a) All biohazardous materials must be removed from the laboratory by disposing of them according to the requirements of this suborder, shipping them to another facility following approved shipping regulations, or transferring them with proper documentation to another NIST responsible party.

(b) All biohazardous waste shall be properly decontaminated and disposed of in accordance with the requirements of this suborder.

(c) All equipment that has come in contact with the biohazardous materials shall be properly decontaminated.

(d) All bench-tops or other work surfaces where biohazardous materials were manipulated must be wiped down with an approved disinfectant.

(e) All BSCs must be properly decontaminated.

(f) All other hazards in the laboratory shall be handled in accordance with other NIST OSH suborders.

(g) The steps taken to decommission the laboratory shall be documented by the OU responsible for the laboratory and reviewed and approved by the BSO.

l. Training

(1) Training shall be provided, documented, and recorded in accordance with the requirements of the NIST Safety Education and Training Suborder.

(2) BSL 2 laboratory supervisors shall complete a one-time supervisory biosafety training course developed by OSHE. Prior participation and completion of the NIST Biosafety and Biocontainment Training BSL 2 Supervisory Training satisfies this requirement.

(3) All new employees who will be working with biohazardous materials shall complete an OSHE-instructor-led biosafety training course prior to working with biohazardous materials.

(4) All current employees who will be working with biohazardous materials for the first time shall complete an OSHE-instructor-led biosafety training course prior to working with biohazardous materials. Prior participation and completion of the NIST Biosafety Basics and Compliance Training satisfies this requirement.

(5) All employees who work with biohazardous materials shall complete an OSHE-developed on-line biosafety training refresher course every two years.

(6) All employees who work with biohazardous materials at BSL 2 shall complete an OSHE-provided one-time, hands-on biosafety techniques training course. After completing the training and obtaining concurrence from the BSO, parties designated by their OUs as responsible for the safety of activities in laboratories in which such work is conducted may provide this training to other laboratory personnel.

(7) All employees who ship Category A infectious substances shall receive applicable DOT and IATA training.

m. Biosecurity

(1) Biosecurity safeguards that may be used at NIST include, but are not limited to, risk and threat assessments, facility security plans, laboratory access policies, and biohazardous material inventories.

(2) Suspected thefts of RG 2 agents shall be reported to OSHE and local law enforcement officials immediately.

7. DEFINITIONS

a. Autoclave – Equipment with a chamber used to sterilize items by applying wet heat (i.e., high-pressure steam) at temperatures above the normal boiling point of water and pressures above normal atmospheric pressure.

b. Biohazard – A biological material or agent that presents potential risk to the health of humans or other organisms either directly through infection or indirectly through damage to the environment. Biohazards include, but are not limited to, bacteria; fungi; viruses; parasites; rickettsia; biological toxins; prions; non-human mammalian cell lines and tissues; human specimens such as human blood, serum, plasma, blood products, primary and continuous human cell lines, unfixed human tissues, fecal materials, semen, vaginal secretions, cerebrospinal fluid, synovial fluid, pleural fluid, pericardial fluid, peritoneal fluid, amniotic fluid, saliva, tears, sweat, breast milk, and urine; and recombinant DNA materials such as inserts or vectors that are known to express toxins, oncogenes, and/or virulent factors.

Non-toxic proteins and commercially available enzymes, cell culture medium and supplements, reagents such as monoclonal antibodies, and random DNA base pairs are not considered biohazards.

c. Biohazardous – Describes a biological agent/material with the risk of posing a potential hazard.

d. Biohazardous Material – See definition of *biohazard*.

e. Biohazardous Materials Registration and Authorization Request – A NIST form submitted by the Organizational Unit (OU) to the Biosafety Officer (BSO) for review and approval as part of the OU hazard review process for activities that involve the use of biohazardous materials.

f. Biohazardous Waste – Waste that includes, but is not limited to, discarded microbiological cultures, stocks and all associated materials, discarded human specimens and all associated

materials, discarded tissue cultures and stocks, discarded live and attenuated vaccines, discarded molecular waste, and contaminated sharps.

g. Biological Agent – A biological organism or material that is often directly responsible for producing an effect (e.g., disease). Agent examples include bacterium, fungus, parasite, Rickettsia, virus, proteinacious infectious particle (prion), or biological toxin.

h. Biological Materials – A broad range of microbiological agents, recombinant DNA materials, non-human mammalian cell lines and tissues, human blood and blood products, and other materials of human, animal, and plant origins.

i. Biosafety Cabinet or Biological Safety Cabinet (BSC) – A cabinet with built-in high-efficiency particulate air (HEPA) filters that provides personnel, environmental, and sample protection when appropriate practices and procedures are followed. When combined with appropriate microbiological techniques, the three classes of BSC provide different levels of protection:

(1) Class I BSCs, which are rarely used in biological laboratories, provide protection to personnel and the environment only, not the sample;

(2) Class II BSCs, which are the most commonly used BSCs at NIST at the current time, provide personnel, environmental, and sample protection; and

(3) Class III BSCs, which are used when working with agents in Risk Groups 3 and 4, provides maximum personnel, environmental, and sample protection.

j. Biosafety Level (BSL) – Also known as a level of containment, a combination of standard laboratory practices and techniques, safety equipment, and facility design specifications for containing biohazardous materials. The CDC distinguishes the following four levels:

(1) Biosafety Level 1 (BSL 1), for working with well-characterized agents not consistently known to cause disease in healthy adult;

(2) Biosafety Level 2 (BSL 2), for working with agents associated with human disease for which the routes of transmission include percutaneous injury, ingestion, and mucous membrane exposure;

(3) Biosafety Level 3 (BSL 3), for working with indigenous or exotic agents that may cause serious or potentially lethal disease as a result of exposure by the inhalation route; and

(4) Biosafety Level 4 (BSL 4), for working with dangerous and exotic agents that may pose a high individual risk of aerosol-transmitted laboratory infections that are frequently fatal, for which there are no vaccines or treatments.

k. Biosafety Officer (BSO) – Also known as the Biological Safety Officer, a person appointed by the Chief Safety Officer as the OSHE Safety Program Manager for the NIST Biosafety Program.

l. Biosecurity – A set of preventive measures designed to reduce the risk of loss and/or intentional removal (theft) of valuable and/or regulated biohazardous materials.

m. Decontamination – The process of reducing or inactivating biohazardous contaminants or components to an acceptable level to reduce or eliminate the possibility of transmission of pathogens to undesired hosts such as laboratory workers, the general public, and other organisms in the environment.

n. Disinfectant – A chemical germicide agent that is applied to inanimate objects to kill microbes, but is not capable of killing endospores, some viruses, or mycobacterium. Disinfectants are typically chemical germicides. Common chemical disinfectants include 10% diluted household bleach and 70% ethanol.

o. Disinfection – A process of eliminating nearly all recognized pathogenic microorganisms but not necessarily all microbial forms (e.g., bacterial spores) from inanimate objects (e.g., work surfaces, equipment).

p. Etiologic – An adjective that means disease-causing.

q. Fixed – A biological material that has been chemically treated for preservation. Certain fixatives such as paraformaldehyde or gluteraldehyde are capable of rendering the biological materials inactive.

r. High-Efficiency Particulate Air (HEPA) Filter – A medium composed of pleated borosilicate fiber sheets capable of trapping at least 99.97% of airborne mono-dispersed particles of 0.3 micrometers (μm) in diameter.

s. Human Specimens – Human blood, serum, plasma, products made from blood, primary and continuous cell lines, tissues, fecal materials, semen, vaginal secretions, cerebrospinal fluid, synovial fluid, pleural fluid, pericardial fluid, peritoneal fluid, amniotic fluid, saliva, tears, sweat, breast milk, and urine.

t. Infectious Substances – Materials known to be, or suspected to contain, an animal or human pathogen, and that must be transported according to Department of Transportation (DOT) and the International Air Transport Association (IATA) shipping guidelines. There are two categories of infectious substances:

(1) Category A – Materials capable of causing permanent disability or a life threatening or fatal disease in humans or animals, and that must be transported according to DOT and IATA shipping guidelines.

(2) Category B – Infectious materials that do not fall within Category A but still must be transported following DOT and IATA shipping guidelines.

u. Medical or Infectious Waste – Infectious human or animal waste generated or produced as a result of research, a medical diagnosis, treatment, or immunization.

v. Recombinant DNA (rDNA) – The NIH rDNA Guidelines define rDNA as 1) molecules that are constructed outside living cells by joining natural or synthetic DNA segments to DNA molecules that can replicate in a living cell, and 2) molecules that result from the replication of molecules described in 1).

w. Risk Group (RG) – A system adopted by the CDC and NIH that classifies biohazardous agents by the health risk they present to individuals and surrounding communities. The system comprises risk groups numbered 1 through 4, with higher numbers corresponding to higher risks. More specifically:

(1) Risk Group 1 (RG 1) agents are not associated with disease in healthy adult humans;

(2) Risk Group 2 (RG 2) agents are associated with human diseases that are rarely serious, and often have preventive or therapeutic interventions available;

(3) Risk Group 3 (RG 3) agents are associated with serious or lethal human disease for which preventive or therapeutic interventions may be available (high individual risk but low community risk); and

(4) Risk Group 4 (RG 4) agents are likely to cause serious or lethal human disease for which preventive or therapeutic interventions are not usually available (high individual risk and high community risk).

Agents not listed in Risk Groups 2, 3, or 4 are not implicitly classified in RG 1. Refer to Appendix B for relationship between RG and BSL.

x. Select Agents and Toxins – Specific pathogenic agents and toxins strictly regulated by the CDC and USDA (i.e., under 7 CFR 331, 9 CFR 121, and 42 CFR 73) because they may be used as agents of mass destruction or pose a severe threat to human, animal, and plant health; or they are specific genetic elements, recombinant nucleic acids, or recombinant organisms that are related to the list of select agents and toxins as described in the regulations.

y. Sharp – An object that can penetrate the skin. A sharp is often a tool, device, or material that typically has a sharp edge or point such as a needle, scalpel, blade, razor, broken glass, broken capillary tube, or an exposed end of a wire.

z. Standard Microbiological Practices - Administrative controls listed as BSL containment practices in BMBL and the *NIH Guidelines* to protect workers and the environment.

aa. Sterilization - The process of destroying all living microorganisms and viruses on an object. Common sterilization methods include autoclaving and incineration.

8. ACRONYMS

a. APHIS – Animal and Plant Health Inspection Service

b. BMBL – Biosafety in Microbiological and Biomedical Laboratories

c. BSC – Biological Safety Cabinet or Biosafety Cabinet

d. BSL – Biosafety Level

e. BSO – Biological Safety Officer

f. CDC – Centers for Disease Control and Prevention

g. CFR – Code of Federal Regulations

h. DNA – Deoxyribonucleic acid

i. DOT – Department of Transportation

j. HHS – Health and Human Services

k. IATA – International Air Transport Association

l. IRB – Institutional Review Board

m. IRIS – Incident Reporting and Investigation System

n. NBC – NIST Biosafety Committee

o. NIH – National Institutes of Health

p. OSHA – Occupational Safety and Health Administration

q. PPE – Personal Protective Equipment

r. rDNA – Recombinant DNA

s. RG – Risk Group

t. RO – Responsible Official

u. USDA – United States Department of Agriculture

9. RESPONSIBILITIES

a. The Chief Safety Officer is responsible for:

(1) Appointing an OSHE staff member to serve as the NIST Biological Safety Officer (BSO) to carry out the responsibilities for this position delineated below;

(2) Serving as the Responsible Official in accordance with the requirements of *Possession, Use, and Transfer of Select Agents and Toxins*, Final Rule.

b. Division Chiefs (or Equivalents) are responsible for:

(1) Ensuring that staff have adequate supplementary instructions and guidance regarding specific practices and procedures unique to the work being conducted in their organization's laboratories; and

(2) Ensuring that BSL-2 laboratories in their organizations have specific biosafety manuals.

NOTE: Some NIST OUs do not have Division Chiefs; these OUs should designate other individuals to carry out these responsibilities.

c. Employees are responsible for:

(1) Completing and submitting Biohazardous Materials Registration and Authorization Requests to OSHE; as part of the OU hazard review process for activities that involve the use of biohazardous materials.

d. Biological Safety Officer (BSO) is responsible for:

(1) Pursuant to discussions with OU personnel as appropriate, reviewing and approving Biohazardous Materials Registration and Authorization Requests as part of the OU hazard review process for activities that involve the use of biohazardous materials, or, in the case of activities that involve rDNA, present significant new or unique risks, or involve the non-exempted use of select agents or toxins, referring those requests to the NIST Biosafety Committee for review and approval;

(2) Performing annual inspections of BSL-1 and BSL-2 laboratories;

(3) Reviewing plans for new BSL-2 laboratories and renovations and providing recommendations on ventilation and design; and

(4) Assisting the CSO in serving as the Responsible Official in accordance with the requirements of *Possession, Use, and Transfer of Select Agents and Toxins*, Final Rule.

e. NIST Biosafety Committee is responsible for:

(1) Advising the CSO on the status of the NIST Biosafety Program;

(2) Reviewing and approving Biohazardous Materials Registration and Authorization Requests not approved by the BSO, at the request of the submitting OU; and

(3) Reviewing and approving Biohazardous Materials Registration and Authorization Requests for activities that involve rDNA, present significant new or unique risks, or involve the non-exempted use of select agents or toxins.

f. Chief Facilities Management Officer is responsible for:

(1) Implementing an effective and integrated NIST pest management program.

10. AUTHORITIES

There are no authorities specific to this suborder alone.

11. DIRECTIVE OWNER

Chief Safety Officer

12. APPENDICES

a. Appendix A. Revision History

b. Appendix B. Risk Assessment Factors

c. Appendix C. Risk Groups: Selected Examples

d. Appendix D. Biosafety Level Classifications

e. Appendix E. Recommended Practices for Working in Biological Safety Cabinets

f. Appendix F. Recommended Procedures for Cleaning Up Spills of Biohazardous Materials

g. Appendix G. Recommended Procedures for Autoclaving Biohazardous Materials

h. Appendix H. Emergency Response Procedures - Examples

Appendix A. Revision History

Revision	Date	Responsible Person	Description of Change
None	03/18/13	Wing Wong	None – initial document.
1	1/5/2021	April Camenisch	Updated suborder and CFR links.

Appendix B: Risk Assessment Factors

(1) Risk groups correlate with but do not necessarily equate to biosafety levels. For example:

(a) When a significant amount of aerosol is generated from working with RG-1 agents, the aerosol-generating step should be conducted in a BSL-2 containment device such as a biological safety cabinet.

(b) Work with a known RG-3 agent such as the Human Immunodeficiency Virus can be conducted in a BSL-2 laboratory, depending on the amount of the agent being used.

(2) Risk assessment determines the degree of correlation between an agent's risk group classification and biosafety level. Factors to be considered during the risk assessment process include, but are not limited to:

(a) Material pathogenicity;

(b) Route of transmission;

(c) Infectious dose;

(d) Quantity;

(e) Experimental protocol; and

(f) Availability of preventive measures and treatments.

Appendix C: Risk Groups - Selected Examples

Agents not listed in Risk Groups (RG) 2, 3, and 4 in what follows are not implicitly classified in RG 1.

(1) Risk Group 1 (RG1) agents are not associated with disease in healthy adult humans.

(a) Bacterial agents: *Bacillus subtilis* and *Escherichia coli* K-12

(2) Risk Group 2 (RG2) agents are associated with human diseases that are rarely serious, and often have preventive or therapeutic interventions available.

(a) Bacterial agents: *Bacillus anthracis*, *Escherichia coli* O157:H7, *Legionella* species, *Staphylococcus aureus*, *Streptococcus pneumonia*, and *Vibrio cholera*.

(b) Fungal agents: *Blastomyces dermatitidis* and *Cryptococcus neoformans*.

(c) Parasitic agents: *Entamoeba histolytica* and *Giardia* species.

(d) Viral agents: Hepatitis viruses, Cytomegalovirus, Epstein Barr virus, and Parvoviruses.

(3) Risk Group 3 (RG3) agents are associated with serious or lethal human disease for which preventive or therapeutic interventions may be available (high individual risk but low community risk).

(a) Bacterial agents: *Brucella* sp., *Coxiella burnetii*, and *Mycobacterium tuberculosis*.

(b) Fungal agents: *Coccidioides immitis* and *Histoplasma capsulatum*.

(c) Viral agents: Hantaviruses and human immunodeficiency viruses.

(4) Risk Group 4 (RG4) agents are likely to cause serious or lethal human disease for which preventive or therapeutic interventions are not usually available (high individual risk and high community risk).

(a) Viral agents: Ebola virus and Monkey B virus.

Appendix D: Biosafety Level Classifications

(1) Biosafety Level 1 (BSL-1) laboratories are suitable for work involving fixed/inactivated biological materials and well-characterized agents not known consistently to cause disease in healthy adults and that present minimal potential hazard to laboratory personnel and the environment. Work is typically conducted on open bench tops using standard microbiological practices. Special containment equipment or facility design is not required, but may be used as determined by an appropriate risk assessment. Some examples of BSL-1 activities include handling of inactivated human specimens and working with RG 1 agents.

(2) Biosafety Level 2 (BSL-2) laboratories are suitable for work involving agents that pose moderate hazards to personnel and the environment. With good microbiological techniques and the appropriate safety equipment and facility designs, these agents can be used safely in activities conducted on the open bench, provided the potential for producing splashes and aerosols is low. These agents are typically transmitted by cuts, ingestion, or mucous membrane exposure. Some examples of BSL 2 activities include handling of human blood and blood products, handling of human and non-human primate cell lines and/or tissues, and working with Risk Group 2 agents.

(3) Biosafety Level 3 (BSL-3) laboratories are suitable for work involving agents with a potential for respiratory transmission, and which may cause serious and potentially lethal infection. Microorganisms such as *Mycobacterium tuberculosis* and *Coxiella burnettii* are manipulated at BSL-3.

(4) Biosafety Level 4 (BSL-4) laboratories are suitable for work involving dangerous agents that post a high individual risk of life-threatening disease, which may be transmitted via the aerosol route and for which there is no available vaccine or therapy. Viruses such as Marburg or Congo-Crimean hemorrhagic fever are manipulated at BSL-4.

Appendix E: Recommended Practices for Working in Biological Safety Cabinets

(1) If the cabinet has been shut down, the blower should be operated for at least 10 minutes to allow the cabinet to purge before work begins again.

(2) The work surface, the interior walls, and the interior surface of the window sash should be wiped with 70% ethanol or a freshly prepared 10% bleach solution before and after work. When bleach is used, a second wiping with 70% ethanol or sterile water is needed to remove the residual chlorine to prevent corrosion.

(3) The front and rear perforated grills should be clutter free.

(4) Overcrowding inside the BSC should be avoided.

(5) Sudden movements in and out of and sweeping across the front grille of the BSC should be avoided.

(6) Flame sources should not be used in the BSC.

(7) All work materials including aerosol-generating equipment should be placed as far back in the cabinet as practical.

(8) Biohazard waste bags should not be taped to the side of the cabinet.

(9) Upright pipet collection containers should not be used in a BSC nor placed on the floor outside the cabinet. Only horizontal pipet discard trays containing an appropriate chemical disinfectant or disposable pipet container should be used inside the cabinet.

Appendix F: Recommended Procedures for Cleaning Up Spills of Biohazardous Materials

(1) Spills inside the BSC

Allow the BSC to run during clean-up. Cover spill with disinfectant-soaked paper towel or other absorbent materials. Carefully pour additional disinfectant solution around the edges of the spill and then into the spill. Avoid splashing. Allow a 20 minute contact period. Use paper towels or other absorbent materials to wipe up the spill, working from the outer edges into the center. Discard clean-up materials and gloves into biohazardous waste bin for autoclaving.

(2) Spills in the laboratory, outside the BSC.

Alert personnel in the immediate area of spill. Remove any contaminated clothing and place in biohazardous waste bin. Keep the BSC running or turn it on. Leave the area for approximately 30 minutes for the aerosols to settle before re-entering. Re-enter with disposable gown, shoe covers, face shield or eye protection and N-95 mask, and gloves. Cover spill with disinfectant-soaked paper towel or other absorbent materials. Carefully pour additional disinfectant solution around the edges of the spill and then into the spill. Avoid splashing. Allow a 20 minute contact period. Use paper towels or other absorbent materials to wipe up the spill, working from the outer edges into the center. Discard clean-up materials, disposable gown, gloves, and shoe covers into biohazardous waste bin for autoclaving.

(3) Spill in a centrifuge without safety buckets

A spill inside a centrifuge has the potential for multiple infections from a single incident. Aerosols are generated when fluid escapes from the rotor or cup while the centrifuge is operating at high speed. All opening of centrifuges must be performed slowly. If a centrifuge tube breaks while the centrifuge is running, turn off the motor. Allow the machine to be at rest for 30 minutes before opening. If breakage/leakage is observed after the centrifuge has stopped, re-close the lid immediately and allow the machine to be at rest for 30 minutes. Unplug centrifuge before initiating clean-up. Don puncture resistant gloves, lab coat, face shield or eye protection and N-95 mask before proceeding with clean-up. Flood the centrifuge bowl with disinfectant. Remove buckets and rotors to BSC for thorough chemical disinfection with a minimum contact time of 20 minutes. Discard clean-up materials and gloves into biohazardous waste bin for autoclaving. The use of sealable safety buckets in centrifuge is strongly recommended.

(4) Spill in a centrifuge with safety buckets

Transfer the sealed bucket to a BSC before opening. Remove leaked tube and dispose into biohazardous waste bin inside the BSC. Soak bucket with disinfectant for a minimum contact time of 20 minutes. Discard clean-up materials and gloves into biohazardous waste bin for autoclaving.

(5) Spill outside the laboratory during transport on campus

Alert personnel in the immediate public area of the spill. Do not attempt to clean-up the spill without appropriate PPE. Return with disinfectant, absorbent materials, and a disposable biohazardous waste bag. Don lab coat, shoe covers, face shield or eye protection and N-95 mask, and gloves. Cover spill with disinfectant-soaked absorbent materials. Carefully pour additional disinfectant solution around the edges of the spill and then into the spill. Avoid splashing. Allow a 20 minute contact period. Use absorbent materials to wipe up the spill, working from the outer edges into the center. Discard clean-up materials, gloves, and shoe-covers into the disposable biohazardous waste bag. Return the disposable biohazardous waste bag into the lab for proper disposal.

Appendix G: Recommended Procedures for Autoclaving Biohazardous Materials

(1) The autoclavable biohazardous waste bag should be autoclaved when three-quarters full.

(2) Bags should be handled while wearing gloves and lab coat.

(3) When removing the bags from the waste collection bins, the bags should be immediately knotted or tied off.

(4) The temperature of the autoclave must be at least 121°C (250°F) with a minimum pressure of 15 psi. The waste must be treated for a minimum of 45 minutes in a liquid cycle.

(5) A sterilization indicator strip should be run with each cycle.

(6) Routine autoclave efficacy monitoring using a biological indicator such as *Bacillus stearothermophilus* ampoule should be conducted at least monthly.

(7) Once the waste has been treated, it can be placed in a regular household garbage bag, tied up, and picked up by custodians.

(8) Date of treatment, name of person who performs the treatment, method/conditions of treatment, and verification of operating parameters or biological monitoring should be properly documented.

Appendix H: Emergency Response Procedures – Examples

(1) Biohazardous spills on body

(a) Flood exposed skin with running water from faucet or safety shower for at least 15 minutes.

(b) Remove all contaminated clothing and shoes and dispose of them in a biohazard waste receptacle.

(c) Seek medical attention if needed.

(d) Report the incident to a supervisor as soon as possible and file a work-related injury report via **IRIS.**

(2) Biohazardous materials splashed in the eye

(a) Immediately rinse eyeball and inner surface of eyelid continuously with water for 15 minutes.

(b) Hold the eyes open to effectively wash behind eyelids.

(c) Seek medical attention if needed.

(d) Report incident to supervisor as soon as possible and file a work-related injury report via **IRIS**.

(3) Needle sticks/cuts

(a) Clean the puncture site with soap and flush it with water for at least 15 minutes.

(b) Seek medical attention if needed.

(c) Report incident to supervisor as soon as possible and file a work-related injury report via **IRIS.** Also fill out the OSHA Sharps Injury Log.