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BSL1 and BSL2 Biological Waste Management Guideline

1.0 Purpose and Scope

The purpose of this Guideline is to describe the procedures for handling medical and/or biological waste generated involving RG1 and RG2 agents in *BSL1 and BSL2* Laboratory and clinical laboratories to final disposal and destruction.

This Guideline is developed to assist personnel who utilize microbiological laboratories at the Boston University Charles River Campus (CRC) in the proper disposal of biological waste generated by their operations. This Guideline establishes the minimum requirements for RG1 and RG2 biological waste management in *BSL1and BSL2 laboratories*.

2.0 References

2.1 Regulations
105 CMR 480 State Sanitary Code – Commonwealth of Massachusetts

2.2 Supplementary Documents
Boston University Biosafety Manual

3.0 Definitions

3.1 Biological Waste – The following are defined as medical or biological waste, per 105 CMR 480.000:

3.1.1 *Blood and Blood Products*: Discarded bulk human blood and blood products in free draining, liquid state; body fluids contaminated with visible blood; and materials saturated/dripping with blood. Blood and Blood Products shall not include: feminine hygiene products.

3.1.2 *Pathological Waste*: Human anatomical parts, organs, tissues and body fluids removed and discarded during surgery, autopsy, or other medical or diagnostic procedures; specimens of body fluids and their containers; and discarded material saturated with body fluids other than urine. Pathological waste shall not include: Teeth and contiguous structures of bone without visible tissue, nasal secretions, sweat, sputum, vomit, urine, or fecal materials that do not contain visible blood or involve confirmed diagnosis of infectious disease.

3.1.3 *Cultures and Stocks* of RG1 or RG2 Infectious Agents and their Associated Biologicals: All discarded cultures and stocks of infectious agents and their associated biologicals, including culture dishes and devices used to transfer, inoculate, and mix cultures, as well as discarded live and attenuated vaccines intended for human use, that are generated in:

3.1.3.1 Laboratories involved in basic and applied research;
3.1.3.2 Laboratories intended for educational instruction; or
3.1.3.3 Clinical laboratories

3.1.4 *Contaminated Animal Waste*: Contaminated carcasses, body parts, body fluids, blood or bedding from animals known to be:

3.1.4.1 Infected with agents of the following specific zoonotic diseases that are reportable to the Massachusetts Department of Agricultural Resources, Bureau of Animal Health pursuant to 105 CMR 300.140:

3.1.4.1.1 African swine fever,
3.1.4.1.2 Anthrax,
3.1.4.1.3 Avian influenza – H5 and H7 strains and any highly pathogenic strain,
3.1.4.1.4 Bovine spongiform encephalopathy (BSE),
3.1.4.1.5 Brucellulosis,
3.1.4.1.6 Chronic wasting disease of cervids,
3.1.4.1.7 Foot and mouth disease,
3.1.4.1.8 Glanders,
3.1.4.1.9 Exotic Newcastle disease,
3.1.4.1.10 Plague (*Yersinia pestis*),
3.1.4.1.11 Q Fever (*Coxiella burnetti*),
3.1.4.1.12 Scrapie,
3.1.4.1.13 Tuberculosis,
3.1.4.1.14 Tularemia (*Francisella tularensis*); or

3.1.4.2 Infected with diseases designated by the State Epidemiologist and the State Public Health Veterinarian as presenting a risk to human health; or

3.1.4.3 Inoculated with infectious agents for purposes including, but not limited to, the production of biologicals or pharmaceutical testing.

3.1.5 *Sharps*: Discarded medical articles that may cause puncture or cuts, including, but not limited to, all needles, syringes, lancets, pen needles,

pasteur pipettes, broken medical glassware/plasticware, scalpel blades, suture needles, dental wires, and disposable razors used in connection with a medical procedure.

3.1.6 *Biotechnology By-Product Effluents:* Any discarded preparations, liquids, cultures, contaminated solutions made from microorganisms and their products including genetically altered living microorganisms and their products.

3.2 RG1 Microorganism - RG1 agents are not associated with disease in healthy adult humans. Examples of RG1 agents include asporogenic *Bacillus subtilis* or *Bacillus licheniformis*; adeno- associated virus (AAV) types 1 through 4; and recombinant AAV constructs, in which the transgene does not encode either a potentially tumorigenic gene product or a toxin molecule and are produced in the absence of a helper virus. A strain of *Escherichia coli* is an RG1 agent if it (1) does not possess a complete lipopolysaccharide (*i.e*., lacks the O antigen); and (2) does not carry any active virulence factor (*e.g*., toxins) or colonization factors and does not carry any genes encoding these factors.

3.3 RG2 Microorganism: RG2 agents are associated with human disease which is rarely serious and for which preventive or therapeutic interventions are often available. See Appendix C of this document for the NIH list of RG2 organisms.

4.0 Roles & Responsibilities

4.1 Laboratory Staff manages biological waste within the BSL1 and BSL2 laboratories per the BU Biosafety Manual requirements. Laboratory Staff:

4.1.1 Manages supplies, biohazard bags, biohazard boxes, sharps containers, autoclave bags and disinfectant within the laboratory,
4.1.2 Collects biological waste in suitable waste containers,
4.1.3 Segregates biological waste from other wastes,
4.1.4 Notifies EHS when full boxes are generated using the online pickup request form,
4.1.5 Chemically disinfects liquids,
4.1.6 Loads, operates and unloads the autoclave if applicable,
4.1.7 Packages biological waste in a double-bagged biohazard waste box and/or sharps container in the laboratory,
4.1.8 Attaches any required labels to the box, and
4.1.9 Maintains the laboratory record-keeping log for autoclaved biological waste.

4.2 Environmental Health and Safety (EHS) supports the Laboratory Staff through collection of full containers, record keeping, training, auditing of procedures, vendor selection/audit and emergency response. EHS:

4.2.1 Provides Biological Waste training to all Laboratory Staff conducting work in BSL1, BSL2 and Clinical labs. Training includes a review of this procedure and Massachusetts requirements.

4.2.2 Provides for the pickup and staging of full boxes of biological wastes from CRC laboratories,

4.2.3 Participates in the vetting and selection of biological waste disposal vendors,

4.2.4 Manages the paperwork involved with shipping biological waste,

4.2.5 Maintains the record-keeping log for all medical or biological waste shipped off-site for treatment. The log includes:

(a) The exact date of each shipment;
(b) The total number of containers;
(c) The type of waste;
(d) The total combined weight or volume;
(e) The name of the transporter with shipping identification number (if applicable)

4.2.6 Reviews and approves biological waste storage rooms/locations,

4.2.7 Maintains a current listing of approved storage rooms/locations (*Appendix A*),

4.2.8 Reviews and approves the use of autoclaves for the sterilization of BSL1 and BSL2 waste,

4.2.9 Maintains a current listing of approved autoclaves (*Appendix B*),

4.2.10 Provides bound Record-Keeping Log books where required by this procedure,

4.2.11 Verifies that shipping papers generated are matched with corresponding medical waste tracking forms for each shipment,

4.2.12 Audits Laboratory Staff procedures and operations to assure compliance with procedures and regulatory requirements, and

4.2.13 Provides clear instructions to generators of biological waste through training and on the EHS web site.

4.3 Biohazardous Waste Contractor (Stericycle)

4.3.1 Provides pickup from designated storage locations

4.3.2 Notifies EHS of discrepancies

5.0 Special Requirements

5.1 Equipment and Supplies Required
- **5.1.1** Biohazard waste red bag liners
- **5.1.2** Biohazard boxes (cardboard)
- **5.1.3** Sharps Containers (plastic)
- **5.1.4** Red/Orange autoclave bags (Fisherbrand polypropylene biohazard autoclave bag or equivalent)
- **5.1.5** Packing tape
- **5.1.6** Nitrile Gloves
- **5.1.7** PPE per the BU Biosafety Manual

5.2 Safety Requirements
- **5.2.1** Specified in the Laboratory-Specific SOPs and the BU Biosafety Manual

5.3 Training
- **5.3.1** Laboratory staff will receive annual Biological Waste training provided by the EHS (may be incorporated into Laboratory Safety Training).
- **5.3.2** Anyone who signs shipping papers will receive annual biosafety and triennial DOT shipment of biological waste training provided by EHS

5.4 Personnel Protective Equipment (PPE)
- **5.4.1** Specified in the BU Biosafety Manual for BSL1 and BSL2 agents

5.5 Medical Surveillance
- **5.5.1** Specified in the BU Biosafety Manual for the agents handled.

5.6 Other Prerequisites
- **5.6.1** Laboratory Safety Training (includes biological waste) is required annually for all laboratory staff generating biological waste. This training includes Bloodborne Pathogens Training.
- **5.6.2** Biological Waste Shipping Training (DOT) is required every three years for those who sign biological waste shipping papers.

6.0 Applicable Locations

6.1 Waste Generation
- **6.1.1** All Boston University Charles River Campus BSL1 and BSL2 laboratories.

6.2 Waste Storage
- **6.2.1** See *Appendix A* for Listing of Boston University Charles River Campus Biological Waste Storage locations.

7.0 Biological Waste Storage Areas

7.1 Storage areas shall be in an uncarpeted room or area with impervious, cleanable, non-absorbent flooring. The space must be used exclusively for waste storage. Additionally:

7.1.1 The storage area must have prominent signage indicating the space is used for the storage of regulated medical or biological waste;

7.1.2 The space must be designed or equipped to prevent unauthorized access;

7.1.3 The accumulation area must be located to protect the waste from the elements and prevent access by vermin;

7.1.4 There must be sufficient space to allow for clear separation of regulated medical or biological waste from any other waste.

7.1.5 The space must be adequate to accommodate the volume of regulated medical or biological waste generated prior to removal of waste for either waste transport off-site or on-site treatment, and

7.1.6 Be maintained such that there is no putrescence or off-site odors, using refrigeration when necessary.

7.1.7 Sharps shall segregated from other biological wastes

8.0 Compactors or Grinders - Shall not be used to process medical or biological waste until it has been rendered noninfectious and safe for disposal in accordance with 105 CMR 480.150.

9.0 Accumulation Time Limit - All medical or biological waste must be treated on-site or transported offsite for treatment at a minimum once per calendar year.

10.0 Solid Biological Waste Collection

10.1 General Laboratory Solid Biological Waste – Includes all non-liquid, non-sharp, non-animal, and non-pathological wastes which are contaminated with RG1 or RG2 agents from a BSL1 or BSL2 laboratory. Any solid waste contaminated with genetically-altered RG1 or RG2 microorganisms is considered biological. This description includes Petri dishes, plastic pipettes, soiled gloves and bench chucks. Unsoiled, uncontaminated gloves, bench chucks, and other materials must not be disposed of as solid biological waste. RG3 and RG4 contaminated wastes are managed under the provisions of a separate document.

10.1.1 Laboratory personnel obtain cardboard ‘burn boxes’ and plastic biohazard bags from EHS. Both boxes and bags must be labeled with the biohazard symbol and the word ‘biohazard’.

10.1.2 Laboratory personnel use tape to construct ½ of the box, and line the open end of the box with two biohazard bags.

10.1.3 Contaminated solid biological waste is placed into the box by laboratory staff as it is generated.

10.1.4 Full bags are taped or tied closed (each bag), and the top of the box closed and sealed with tape.

10.1.5 Laboratory staff write the building and room number on the box using a permanent marker (building letter codes are OK).

10.1.6 The box is staged for pickup by EHS who transports the closed box to a designated storage room.

10.1.7 Laboratory personnel must immediately fill out an on-line pickup request form to notify EHS that a box is ready for storage.

10.1.8 Improperly closed, overfull, or leaking boxes will not be picked up, and must be repackaged by laboratory personnel.

10.1.9 Full, closed boxes are stored in designated storage areas until they are shipped for proper final disposal via a third-party vendor.

10.2 Contaminated Animal Wastes – Animal carcasses, parts, tissues and bedding which are infected with an agent as described in section 3.1 (4) of this document.

10.2.1 Collection by laboratory staff as decribed in 10.1.

10.2.2 In addition, laboratory staff must place a yellow 'incinerate only' sticker on full, closed boxes containing contaminated animal wastes.

10.2.3 Laboratory animals which do not meet the definition in 3.1 (4) of this document should still be collected as biological waste. In these cases, the yellow 'incinerate only' sticker is not necessary.

10.2.4 Pickup is as described in 10.1 above.

10.2.5 Animal carcasses which are disposed of at the Laboratory Animcal Care Facility (LACF) are stored in double-lined red bags in a dedicated freezer until picked up for disposal.

10.3 Pathological Wastes – Biological wastes including human parts, tissues organs as described in section 3.1 (2) of this document.

10.3.1 Collection by laboratory staff as decribed in 10.1.

10.3.2 In addition, laboratory staff must place a yellow 'incinerate only' sticker on full, closed boxes containing contaminated animal wastes.

10.3.3 Pickup is as described in 10.1 above

11.0 Liquid Biological Waste Management

11.1 All liquid biological wastes (excluding RG3 and RG4 materials from BSL3 and BSL4 laboratories) are decontaminated and disposed of inside the laboratory. Any liquid containing genetically-altered micro-organisms is considered biological waste.

11.2 Liquid waste is collected by the laboratory in labeled waste flasks or beakers. If a vacuum system is used, a double-flask with HEPA filter between the vacuum line and outlet is employed.

11.3 Active liquid waste flasks or containers must have secondary containment adequate to hold their entire contents.

11.4 Prior to disposal, waste must be disinfected using an EPA registered agent per the laboratory SOP and BU Biosafety Manual. If bleach is used, a final bleach solution of at least 10% must be made, with at least 20 minutes of contact time.

11.5 After complete disinfection, waste liquid is discharged into a laboratory sink drain.

11.6 All liquid biological wastes must be disinfected at least daily.

11.7 The Institutional Biosafety Committee shall review these practices at least annually.

12.0 Mixed Wastes

12.1 Biological wastes which contain radioactive or chemical contamination must be disinfected in the laboratory.

12.2 Chemical disinfectant, such as bleach, must be added to completely destroy potential pathogens that exist in the waste. Care must be used to prevent potential chemical reactions between the chemical or radioactive waste substance and the chemical disinfectant.

12.3 Biological waste with chemical or radioactive contamination must NEVER be autoclaved unless authorized by EHS.

12.4 Once a biological waste with chemical or radioactive contamination is disinfected, it must be managed as a radioactive or chemical waste.

13.0 Sharps Waste Management

13.1 Sharps, as defined in section 3.1(5) of this document, are collected in rigid, plastic containers provided to laboratories by EHS.

13.2 Containers are orange or red/orange in color, shatter-proof, leak-proof and puncture-proof, and marked with the universal biohazard label and the word 'biohazard'.

13.3 Laboratory staff place used sharps, whether contaminated or not, into these containers. When containers become full they are closed and staged for pickup.

13.4 An on-line pickup request should be made to EHS to collect the full sharps container and replace it with an empty one.

13.5 Overfull or open sharps containers will not be picked up until the laboratory has corrected the overfilling or closed the container.

14.0 Autoclaving Waste

14.1 Registration – As a rule, autoclaves are not to be used to disinfect RG1 or RG2 wastes from BSL1 or BSL2 laboratories. However, if the Institutional Biosafety Committee (IBC) determines that an RG1 or RG2 organism used in a protocol should be autoclaved prior to being sent for disposal as biological waste, the proposed autoclave unit must be registered with OEHS. See *Appendix B* for a listing of autoclaves authorized for the sterilizing of BSL1 and BSL2 biological waste.

14.2 Standard Operating Procedure – A written Standard Operating Procedure for the sterilization of waste (may include animals and sharps) must be developed by the laboratory supervisor responsible for the use of the autoclave. The SOP must be in accordance with the BU Biosafety Manual and submitted to EHS as a part of the approval process.

14.3 Records – for each load or cycle including cycle time, pressure and temperature must be recorded in the Record-Keeping Log book provided by EHS.

14.4 Quarterly Autoclave Validation - The autoclave(s) used for sterilizing BSL1 and BSL2 waste will be validated quarterly by Laboratory Staff using a biological indicator. The Laboratory –specific SOPs shall include validation procedures.

14.5 Annual Calibration: Laboratories shall arrange for annual calibration/maintenance of autoclaves used for decontamination of biological waste.

14.6 Autoclaved Waste Transfer - All autoclaved waste will be removed immediately from the autoclave at the completion of the run. According to the laboratory-specific SOP, autoclaved waste will be packaged, labeled and stored as described in section 10.1 of this document.

14.7 Animal Waste - Infected animal carcasses and tissues are to be autoclaved only if allowed by the approved SOP. Autoclaved animals are repackaged for incineration according to 10.2 of this document.

15.0 Records and Forms

15.1 Autoclave Decontamination Logs

15.1.1 Standardized forms available through EHS,

15.1.2 Kept in binders at the site of the autoclaves. Forms must be consecutively numbered inside the binders.

15.1.3 Completed and maintained by the users of the autoclave,

15.1.4 Must include record of annual calibration, and

15.1.5 Kept on-site for 3 years.

15.2 Off-site Disposal Logs

15.2.1 Standardized forms available through EHS

15.2.2 Managed by EHS, and kept in binders in the EHS office.

15.3 Shipping Papers

15.3.1 Required by the Department of Transportation to be included with every shipment of biological waste offsite.

15.3.2 Shipping papers are provided by the vendor of biological waste disposal services.

15.3.3 Signed by custodial leads at the time of shipment, and are given to EHS for retention for 3 years.

15.3.4 When wastes are transported by EHS from one building to a storage area, a shipping paper is generated and kept on file by EHS.

15.4 Medical Waste Tracking Documents

15.4.1 Provided by the biological waste service provider as a receipt of final disposal of biological waste.

15.4.2 EHS verifies destruction of each shipment by receipt of Medical Waste Tracking Documents within 30 days of shipment.

15.4.3 Shipments for which Medical Waste Tracking Documents are not received within 30 days are reported to the Department of Public Health, and are investigated and resolved by EHS.

15.5 Institutional Biosafety Committee Membership List and Meeting Minutes

15.5.1 The Institutional Biosafety Committee meets on a monthly basis.

15.5.2 Minutes of the meeting are kept by the Institutional Biosafety Committee office,

15.5.3 At least annually, the Institutional Biosafety Committee reviews this waste policy, and the minutes from this meeting are kept by EHS.

15.5.4 A list of Biosafety Committee members is also kept by EHS.

15.6 Training Records

15.6.1 Training records are managed by EHS and kept electronically or in hard copy form.

16.0 SOP Revision History

Version	Section / Paragraph Changed	Changes Made	Effective Date
V.1.1	Throughout.	SOP placed in new SOP format. Specific responsibilities incorporated.	
V.2	Throughout	Incorporated specific guidance on waste collection techniques based on DPH seminar.	
V.3	Formatting throughout	Corrected Microsoft-related formatting issues. Verified IBC comments.	1/09
V.4	No significant changes	No significant changes	2/2010
V.5	No significant changes	No significant changes	2/2011
V.6	Appendix A	Added 590 Comm storage room	4/2012

APPENDIX A

Listing of Boston University CRC Waste Storage locations

Location	Service Area	Account Code	Stericycle Pickup	Capacity (boxes)	Description
OEHS Garage	Campus		At least monthly	100	A room has been constructed inside the garage for the storage of biological waste boxes.
LACF Freezer	LACF		At least monthly	5	Animal wastes from LACF are stored in this freezer.
590 Comm Ave Basement	590 Comm Ave		At least monthly	25	Dedicated waste storage room in an EHS-controlled section of the basement.

APPENDIX B

Listing of Boston University CRC autoclaves authorized for the sterilizing of BSL1 and BSL2 waste.

Location	Laboratories Serviced	EHS Registration Number	Responsible Laboratory Supervisor

APPENDIX C: http://oba.od.nih.gov/oba/rac/Guidelines/NIH_Guidelines.pdf

Risk Group 2 (RG2) - Bacterial Agents Including Chlamydia

--*Acinetobacter baumannii* (formerly *Acinetobacter calcoaceticus*)
--*Actinobacillus*
--*Actinomyces pyogenes* (formerly *Corynebacterium pyogenes*)
--*Aeromonas hydrophila*
--*Amycolata autotrophica*
--*Archanobacterium haemolyticum* (formerly *Corynebacterium haemolyticum*)
--*Arizona hinshawii* - all serotypes
--*Bacillus anthracis*
--*Bartonella henselae, B. quintana, B. vinsonii*
--*Bordetella* including *B. pertussis*
--*Borrelia recurrentis, B. burgdorferi*
--*Burkholderia* (formerly *Pseudomonas* species) except those listed in Appendix B-III-A (RG3))
--*Campylobacter coli, C. fetus, C. jejuni*
--*Chlamydia psittaci*, *C. trachomatis*, *C. pneumoniae*
--*Clostridium botulinum*, *C. chauvoei, C. haemolyticum, C. histolyticum, C. novyi, C. septicum, C. tetani*
--*Coxiella burnetii* – specifically the Phase II, Nine Mile strain, plaque purified, clone 4
--*Corynebacterium diphtheriae, C. pseudotuberculosis, C. renale*
--*Dermatophilus congolensis*
--*Edwardsiella tarda*
--*Erysipelothrix rhusiopathiae*
--*Escherichia coli* - all enteropathogenic, enterotoxigenic, enteroinvasive and strains bearing K1 antigen, including *E. coli* O157:H7
--*Francisella tularensis* specifically *F. tualrensis* subspecies *novocida* [aka *F. novocida*], strain Utah 112; *F. tularensis* subspecies *holartica* LVS; *F tularensis* biovar *tularensis* strain ATCC 6223 (aka strain B38)
--*Haemophilus ducreyi, H. influenzae*
--*Helicobacter pylori*
--*Klebsiella* - all species except *K. oxytoca* (RG1)
--*Legionella* including *L. pneumophila*
--*Leptospira interrogans* - all serotypes
--*Listeria*
--*Moraxella*
--*Mycobacterium* (except those listed in Appendix B-III-A (RG3)) including *M. avium* complex, *M. asiaticum, M. bovis* BCG vaccine strain, *M. chelonei, M. fortuitum, M. kansasii, M. leprae, M. malmoense, M. marinum, M. paratuberculosis, M. scrofulaceum, M. simiae, M. szulgai, M. ulcerans, M. xenopi*
--*Mycoplasma*, except *M. mycoides* and *M. agalactiae* which are restricted animal pathogens
--*Neisseria gonorrhoeae*, *N. meningitidis*
--*Nocardia asteroides, N. brasiliensis, N. otitidiscaviarum, N. transvalensis*
--*Rhodococcus equi*
--*Salmonella* including *S. arizonae, S. cholerasuis, S. enteritidis, S. gallinarum-pullorum, S. meleagridis, S. paratyphi,* A, B, C, *S. typhi*, *S. typhimurium*
--*Shigella* including *S. boydii, S. dysenteriae,* type 1, *S. flexneri, S. sonnei*
--*Sphaerophorus necrophorus*
--*Staphylococcus aureus*
--*Streptobacillus moniliformis*

--*Streptococcus* including *S. pneumoniae, S. pyogenes*
--*Treponema pallidum, T. carateum*
--*Vibrio cholerae, V. parahemolyticus, V. vulnificus*
--*Yersinia enterocolitica*
--*Yersinia pestis* specifically *pgm(–)* strains (lacking the 102 kb pigmentation locus) and *lcr(–)* strains (lacking the LCR plasmid).

Risk Group 2 (RG2) - Fungal Agents

--*Blastomyces dermatitidis*
--*Cladosporium bantianum, C. (Xylohypha) trichoides*
--*Cryptococcus neoformans*
--*Dactylaria galopava (Ochroconis gallopavum)*
--*Epidermophyton*
--*Exophiala (Wangiella) dermatitidis*
--*Fonsecaea pedrosoi*
--*Microsporum*
--*Paracoccidioides braziliensis*
--*Penicillium marneffei*
--*Sporothrix schenckii*
--*Trichophyton*

Risk Group 2 (RG2) - Parasitic Agents

--*Ancylostoma* human hookworms including *A. duodenale, A. ceylanicum*
--*Ascaris* including *Ascaris lumbricoides suum*
--*Babesia* including *B. divergens, B. microti*
--*Brugia* filaria worms including *B. malayi, B. timori*
--*Coccidia*
--*Cryptosporidium* including *C. parvum*
--*Cysticercus cellulosae* (hydatid cyst, larva of *T. solium*)
--*Echinococcus* including *E. granulosis, E. multilocularis, E. vogeli*
--*Entamoeba histolytica*
--*Enterobius*
--*Fasciola* including *F. gigantica, F. hepatica*
--*Giardia* including *G. lamblia*
--*Heterophyes*
--*Hymenolepis* including *H. diminuta, H. nana*
--*Isospora*
--*Leishmania* including *L. braziliensis, L. donovani, L. ethiopia, L. major, L. mexicana, L. peruvania, L. tropica*
--*Loa loa* filaria worms
--*Microsporidium*

--*Naegleria fowleri*
--*Necator* human hookworms including *N. americanus*
--*Onchocerca* filaria worms including, *O. volvulus*
--*Plasmodium* including simian species, *P. cynomologi, P. falciparum, P. malariae, P. ovale, P. vivax*
--*Sarcocystis* including *S. sui hominis*
--*Schistosoma* including *S. haematobium, S. intercalatum, S. japonicum, S. mansoni, S. mekongi*
--*Strongyloides* including *S. stercoralis*
--*Taenia solium*
--*Toxocara* including *T. canis*
--*Toxoplasma* including *T. gondii*
--*Trichinella spiralis*
--*Trypanosoma* including *T. brucei brucei, T. brucei gambiense, T. brucei rhodesiense, T. cruzi*
--*Wuchereria bancrofti* filaria worms

Risk Group 2 (RG2) - Viruses

Adenoviruses, human - all types

Alphaviruses (Togaviruses) - Group A Arboviruses
--Chikungunya vaccine strain 181/25
--Eastern equine encephalomyelitis virus
--Venezuelan equine encephalomyelitis vaccine strains TC-83 and V3526
--Western equine encephalomyelitis virus
Arenaviruses
--Junin virus candid #1 vaccine strain
--Lymphocytic choriomeningitis virus (non-neurotropic strains)
--Tacaribe virus complex
--Other viruses as listed in the reference source (see Section V-C, *Footnotes and References of Sections I through IV*)
Bunyaviruses
--Bunyamwera virus
--Rift Valley fever virus vaccine strain MP-12
--Other viruses as listed in the reference source (see Section V-C, *Footnotes and References of Sections I through IV*)

Caliciviruses

Coronaviruses

Flaviviruses - Group B Arboviruses
--Dengue virus serotypes 1, 2, 3, and 4
--Japanese encephalitis virus strain SA 14-14-2
--Yellow fever virus vaccine strain 17D

--Other viruses as listed in the reference source (see Section V-C, *Footnotes and References of Sections I through IV*)

Hepatitis A, B, C, D, and E viruses

Herpesviruses - except Herpesvirus simiae (Monkey B virus) (see Appendix B-IV-D, *Risk Group 4 (RG4) - Viral Agents*)
--Cytomegalovirus
--Epstein Barr virus
--*Herpes simplex* types 1 and 2
--*Herpes zoster*
--Human herpesvirus types 6 and 7

Orthomyxoviruses
--Influenza viruses types A, B, and C (except those listed in Appendix B-III-D, *Risk Group 3 (RG3) - Viruses and Prions*)
--Tick-borne orthomyxoviruses

Papilloma viruses
--All human papilloma viruses

Paramyxoviruses
--Newcastle disease virus
--Measles virus
--Mumps virus
--Parainfluenza viruses types 1, 2, 3, and 4
--Respiratory syncytial virus

Parvoviruses
--Human parvovirus (B19)

Picornaviruses
--Coxsackie viruses types A and B
--Echoviruses - all types
--Polioviruses - all types, wild and attenuated
--Rhinoviruses - all types

Poxviruses - all types except Monkeypox virus (see Appendix B-III-D, *Risk Group 3 (RG3) - Viruses and Prions*) and restricted poxviruses including Alastrim, Smallpox, and Whitepox (see Section V-L, *Footnotes and References of Sections I through IV*)

Reoviruses - all types including Coltivirus, human Rotavirus, and Orbivirus (Colorado tick fever virus)

Rhabdoviruses
--Rabies virus - all strains

--Vesicular stomatitis virus non exotic strains: VSV-Indiana 1 serotype strains (*e.g.* Glasgow, Mudd-Summers, Orsay, San Juan) and VSV-New Jersey serotype strains (*e.g.* Ogden, Hazelhurst)

Rubivirus (Togaviruses)

--Rubella virus