

Biosafety Level Work Practice Requirement

Type of cell/ procedure	Exempt BSL-1	BSL-1	BSL-2	BSL2 with 3 practices (BSL2+)
characterization	Well characterized agents Agents. Not known/unlikely to cause disease, in healthy human adults, immunocompetent adults, and present minimal hazard potential, to laboratory personnel and to the environment. Prophylactic treatment available. Example: E. coli (K-12 strains), yeast	Well characterized agents Agents. Not known/unlikely to cause disease, in healthy human adults, immunocompetent adults, and present minimal hazard potential, to laboratory personnel and to the environment. Prophylactic treatment available. Example: E. coli (K-12 strains), yeast	Agents that pose moderate hazard to personnel and environment. Agents that are associated with human disease. Treatments are available. Example: Human or Primate Cells, Blood or any human body fluids, Patient specimens. Herpes Simplex Virus, HIV, Salmonellae, Hepatitis B, Listeria, Infected animals with BSL-2 agents.	Laboratories in which work with microorganisms are conducted in a BSL-2 laboratory with biosafety procedures typically found at BSL-3. The risk assessment process, may determine that safety practices, over and above those required at BSL-2 needed for a research project, yet a more complex BSL-3 laboratory facility is not necessary.
Examples of Cells	Wild type cells from murine or other non-human / non-primate species that have NOT been exposed to any microbial agent (e.g., viral, bacterial, fungal, protozoan, parasitic) and have not been genetically modified	Cells from murine or other non-human / non-primate species that have NOT been exposed to any microbial agent but have been genetically modified using non-viral methods (e.g. cells from transgenic animals or cells treated with nucleic acids).	Cells from human or non-human primates or Cells that have been genetically modified using viral methods or Cells exposed to microbial agents (e.g., viral, bacterial, fungal, protozoan, parasitic)	Cells exposed to microbial agents (e.g., viral, bacterial, fungal, protozoan, parasitic) with moderate increased personal hazard. (e.g. HIV) or Cells that have been genetically modified using viral methods with known oncogenic inserts
Bench-top work	Permitted	Permitted only for low risk procedures	Permitted only for low risk procedures	Not permitted for biohazardous materials
Pathogen (bloodborne pathogens -BBP) Training	Not required.	Not required.	Initial and annual renewal required for work with human cells and other cells exposed to bloodborne pathogens.	Initial and annual renewal required for work with human cells and other cells exposed to bloodborne pathogens.
Biological Safety Cabinet (BSC)	Not required	Not required	Required for all aerosol* generated processes	Required for all work with biohazardous agents

HEPA-filtered vacuum lines	Not required	Recommended	Required	Required
Hand-washing facilities	Required	Required	Required	Required (foot, elbow, or electronic activation preferable)
Physical containment	Not required	Decontaminate equipment immediately after use	Use physical containment devices during procedures that have a high potential to create aerosols* when using biohazardous material; Decontaminate immediately after use	Use physical containment devices (centrifuge safety cup, sealed centrifuge rotor) for all activities using biohazardous material; Open containers in a BSC; Decontaminate immediately after use
Sample Transport	No requirement	Leak-proof secondary container.	Leak-proof secondary container.	Leak-proof secondary container.
Restriction	None.	None.	The door must be closed during the work. Biohazard sign posted on the outside.	The door must be closed during the work. Biohazard sign posted on the outside.
Protective Equipment (PPE)	Closed toe shoes. When manipulating samples (i.e. loading/unloading samples), gloves, lab coat, are recommended .	Closed toe shoes. When manipulating samples (i.e. loading/unloading samples), gloves, lab coat, and goggles are strongly recommended . <i>For Spills: Lab coat, nitrile gloves, goggles/face-shield required.</i>	Closed toe shoes, lab coat and gloves required at all times. When manipulating samples (i.e. loading/unloading samples), goggles /face protection is also required . <i>For Spills: Lab coat, nitrile gloves, goggles/face-shield required.</i>	Closed toe shoes, shoe covers, disposable rear opening gown, double gloves and goggles/face protection is required at all times. <i>For Spills: Lab coat, nitrile gloves, goggles/face-shield required.</i>
Biohazard bag	Required**	Required**	Double bag required**	Double bag required**
Waste	Contaminated materials must be decontaminated prior to disposal.	Gloves and other waste must be disposed of as biohazardous.	Gloves and other waste must be disposed of as biohazardous	Gloves and other waste must be disposed of as biohazardous.

***Procedures include but not limited to: centrifuging, grinding, blending, vigorous shaking or mixing, sonic disruption, opening containers of biohazardous materials after above procedures.**
****Do not seal the bags; leave them loosely closed (opening of at least 5 cm).**

BSL-2 CLASSIFICATION OF HUMAN PATOGENS/AGENTS ON THE BASIS OF HAZARD

This appendix includes those biological agents known to infect humans as well as selected animal agents that may pose theoretical risks if inoculated into humans. Included are lists of representative genera and species known to be pathogenic; mutated, recombined, and non-pathogenic species and strains are not considered. Non-infectious life cycle stages of parasites are excluded.

Definitions:

Risk Groups are classifications that describe the relative hazard posed by infectious agents or toxins in the laboratory.

Biosafety level: A specific combination of work practices, safety equipment, and facilities which are designed to minimize the exposure of workers and the environment to infectious agents.

Basis for the Classification of Biohazardous Agents by Risk Group (RG)

Risk Group 1 (RG1)	Agents that are not associated with disease in healthy adult humans
Risk Group 2 (RG2)	Agents that are associated with human disease which is rarely serious and for which preventive or therapeutic interventions are often available
Risk Group 3 (RG3)	Agents that are associated with serious or lethal human disease for which preventive or therapeutic interventions may be available (high individual risk but low community risk)
Risk Group 4 (RG4)	Agents that 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)

Risk Group 1 (RG1) Agents

RG1 agents are not associated with disease in healthy adult humans.

Examples of RG1 agents include asporogenic *Bacillus subtilis* or *Bacillus licheniformis* (see Appendix C-IV-A*, *Bacillus subtilis* or *Bacillus licheniformis* Host-Vector Systems, Exceptions); adeno-associated virus (AAV – all serotypes); and recombinant or synthetic 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* (see Appendix C-II-A*, *Escherichia coli* K-12 Host Vector Systems, Exceptions) 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.

Those agents not listed in Risk Groups (RGs) 2, 3 and 4 are not automatically or implicitly classified in RG1; a risk assessment must be conducted based on the known and potential properties of the agents and their relationship to agents that are listed.

Risk Group 2 (RG2) Agents

RG2 agents are associated with human disease which is rarely serious and for which preventive or therapeutic interventions are often available.

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)(in the - NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules (April 2019)
- 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. tularensis subspecies novicida [aka F. novicida], strain Utah 112;

**F. tularensis* subspecies *holarctica* LVS; **F. tularensis* biovar *tularensis* strain ATCC 6223 (aka strain B38)

*For research involving high concentrations, BL3 practices should be considered (see Appendix G-IIC-2. Special Practices (BL3)).

--*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. chelonae*, *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*

--*Pseudomonas aeruginosa*

--*Rhodococcus equi*

--*Salmonella* including *S. arizonae*, *S. choleraesuis*, *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. parahaemolyticus*, *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 marneffeii
- 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. granulosus, 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. peruviana, 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. cynomolgi, 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

--229E (☐ coronavirus)

--NL63 (☐ coronavirus)

--OC43 (☐ coronavirus)

--HKU1 (☐ coronavirus)

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, MuddSummers, Orsay, San Juan) and VSV-New Jersey serotype strains (e.g. Ogden, Hazelhurst)

Rubivirus (Togaviruses)

--Rubella virus

The list was taken from the- NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules (April 2019)

https://osp.od.nih.gov/wp-content/uploads/NIH_Guidelines.pdf