1.0 INTRODUCTION

Spills of biological materials are potentially hazardous not only to the individual, but also to coworkers and people in surrounding areas if not responded to properly. The nature/risks of the biological material, the quantity, and location of the spill greatly affect the steps necessary to properly respond. As part of Weill Cornell Medical College’s (WCMC) Environmental Health and Safety (EHS) Program Manual, these guidelines have been prepared to assist personnel in determining the necessary steps to appropriately respond to a biological spill.

2.0 TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section Heading</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.0 Introduction</td>
<td>1</td>
</tr>
<tr>
<td>2.0 Table Of Contents</td>
<td>1</td>
</tr>
<tr>
<td>3.0 Objective</td>
<td>2</td>
</tr>
<tr>
<td>4.0 Applicability</td>
<td>2</td>
</tr>
<tr>
<td>4.1 You Clean Up The Spill</td>
<td>2</td>
</tr>
<tr>
<td>4.2 EHS Cleans Up The Spill</td>
<td>2</td>
</tr>
<tr>
<td>5.0 Responsibilities</td>
<td>3</td>
</tr>
<tr>
<td>5.1 Environmental Health And Safety (EHS)</td>
<td>3</td>
</tr>
<tr>
<td>5.2 Principal Investigators And Supervisors</td>
<td>3</td>
</tr>
<tr>
<td>5.3 Personnel Using Biological Materials</td>
<td>3</td>
</tr>
<tr>
<td>6.0 Hazard Evaluation Prior To Response</td>
<td>3</td>
</tr>
<tr>
<td>6.1 Classification Of Risk</td>
<td>4</td>
</tr>
<tr>
<td>6.2 Quantity And Nature Of Release</td>
<td>4</td>
</tr>
<tr>
<td>7.0 Responses</td>
<td>5</td>
</tr>
<tr>
<td>7.1 Standard Responses</td>
<td>5</td>
</tr>
<tr>
<td>7.2 EHS Responses</td>
<td>7</td>
</tr>
<tr>
<td>7.3 Extreme Hazards (RG3 Organisms)</td>
<td>8</td>
</tr>
<tr>
<td>8.0 Waste Disposal</td>
<td>8</td>
</tr>
<tr>
<td>9.0 Training</td>
<td>8</td>
</tr>
</tbody>
</table>
3.0 OBJECTIVE

The following guidelines are offered to determine the appropriate response to a biological spill, including, if appropriate, how to clean it up. These guidelines must be followed in the event of all biological spills. Use this document to prepare before a biological spill occurs.

4.0 APPLICABILITY

4.1 YOU CLEAN UP THE SPILL

For biological spills which do not involve injury, are contained, pose little hazard to personnel, and for which you have the proper training and proper protective equipment to do the cleanup, you can clean the spill. A flowchart is available in Appendix A – Evaluation of Risk by Pathogen Type to help determine who should respond to a biological spill.

4.2 EHS CLEANS UP THE SPILL

For all other biological spill situations, including those for which you have any questions or doubts about your ability to clean up the spill, contact Environmental Health and Safety (EHS). A biological spill response team from EHS will clean up the spill. Report all injuries, fires, explosions and potentially life threatening situations first to Security (212-746-0911), then to EHS (646-962-7233).

The Biosafety Officer for WCMC is a member of the EHS staff. The Biosafety Officer is available to provide assistance and training with issues relating to biological spill planning and response as requested.
5.0 RESPONSIBILITIES

5.1 ENVIRONMENTAL HEALTH AND SAFETY (EHS)

Environmental Health and Safety’s responsibilities include:

- Act as an informational resource to WCMC personnel, providing technical assistance and guidance.
- Provides a Spill Response team to clean up spills in the following situations:
  - situations that involve injury;
  - situations that pose a fire hazard;
  - If laboratory personnel do not have proper training and/or protective equipment.
  - If based on a hazard evaluation performed using the criteria set forth in this manual, it is determined that laboratory personnel are unable to adequately respond to the spill.

5.2 PRINCIPAL INVESTIGATORS AND SUPERVISORS

Principal Investigator and Supervisor responsibilities include:

- Ensuring that they and their personnel are familiar with the procedures set forth in this manual and are in compliance with regulations and institutional policies regarding biological spill management.
- Working with EHS to provide the following:
  - biological spill training for themselves and laboratory personnel, as appropriate
  - coordination of appropriate spill response and incident reporting.

5.3 PERSONNEL USING BIOLOGICAL MATERIALS

Personnel using biological materials must:

- Follow the directions provided by their principal investigators and supervisors, and the procedures set forth in this manual.

6.0 HAZARD EVALUATION PRIOR TO RESPONSE

When a biological spill occurs, the following factors must be considered before attempting a clean-up response:

- Pathogenicity of the material
- Mode of transmission for the pathogenic
- Location of spill (within or outside of containment?)
- Form of the material spilled (solid, aerosol, liquid?)
- Quantity of material spilled

These factors will dictate the best course of response to the spill.
6.1 CLASSIFICATION OF RISK

Most pathogens have been evaluated and assigned to a risk group (RG) depending on the predicted outcome of an infection with a particular agent in a normal healthy individual. Pathogens belonging to the following risk groups are used at Weill Cornell Medical College:

- **RG1**: Agents that are not associated with disease in healthy adult humans.
- **RG2**: Agents that are associated with human disease which is rarely serious and for which preventive or therapeutic interventions are often available.
- **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).

RG 4 agents are highly restricted and are not used at Weill Cornell Medical College.

New or emerging pathogens may not be classified into a risk group, and certain strains may develop the ability to become more pathogenic, defying current classification. These exceptions require risk assessment and assignment by the Institutional Biosafety Committee (IBC). Examples include the 1993 *E.Coli* O157:H7 outbreaks and drug-resistant strains of *Mycobacterium Tuberculosis*. Some strains may develop the ability to become more pathogenic, i.e. *E.coli* O157:H7 (often found to cause outbreaks of food poisoning), and drug-resistant strains of *Mycobacterium tuberculosis* that cannot be as effectively treated as the non-resistant strains.

6.1.1 Lists of Known Agents and Associated Risk Groups

The most recent edition of the *NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules* contains a listing of all known agents and the Risk Groups each is assigned to at the time of publication. The NIH listing of agents by Risk Group is available in Appendix C.

6.1.2 Assistance with Risk Assessments

The Biosafety Officer, available via the EHS office (646-962-7233), can offer advice for risk assessment and decision making for selecting appropriate containment levels and PPE selection.

6.2 QUANTITY AND NATURE OF RELEASE

The quantity and nature of the release will determine whether laboratory personnel can perform the spill clean-up procedure, or if Environmental Health and Safety should be contacted for guidance and/or must perform the spill clean-up procedure. The quantity, form (i.e. solid, liquid, aerosol) and location of material released, coupled with the pathogenicity of the agent (refer to section 5.1), must be considered.
6.2.1 Small-Quantity vs. Large-Quantity Spills
Small quantities of known material can be cleaned up by laboratory personnel if they are familiar with the associated hazards, have appropriate personal protective equipment, and are trained in spill-cleanup procedures. Larger quantities are more difficult to effectively manage and contain and may require additional assistance.

6.2.2 Solid vs. Liquid, vs. Aerosol Spills
A spill may pose a greater or reduced hazard depending on whether it occurs in a solidified, liquid or aerosol form. Releases of aerosolized and liquefied material are more easily spread and are difficult to contain outside of engineering control (e.g. biological safety cabinet, centrifuge safety cup), while solidified material can be much more easily contained. The mode of transmission of the material may exasperate or mitigate the hazard of the material depending on its form.

6.2.3 Spills within Containment vs. outside Containment
Spills which occur within containment are less hazardous and more manageable than uncontained spills. Spills which occur outside of containment inherently pose a greater risk, since an engineering control is unable to serve as a primary barrier.

6.2.4 Notification of Biosafety Officer
Notify the Biosafety Officer in the event of a spill to relay any pertinent information. The Biosafety Officer will recommend a course of action based on this information.

7.0 RESPONSES

7.1 STANDARD RESPONSES

A majority of spills are of the type that will be handled by laboratory personnel. For Environmental Health and Safety-assisted spills, the range can run from those incidents requiring only the Biosafety Officer to advise laboratory personnel making a response, to incidents requiring reporting to the National Institutes of Health (NIH) or Centers for Disease Control (CDC) and possible outside assistance.

6.1.1. Minor Spills
Minor spills are those that, based on the hazard evaluation, can be effectively managed and cleaned by laboratory personnel.

   6.1.1.1. Spills within a Biological Safety Cabinet (BSC)
   - Small spills within the operating BSC can be handled immediately by removing the contaminated absorbent paper toweling and placing it into the biohazard bag or receptacle. Any splatter onto items within the cabinet, as well as the cabinet interior, should be immediately cleaned up with absorbent material dampened with an
appropriate decontaminating solution. Gloves should be changed after the work surface is decontaminated and before placing clean absorbent toweling in the cabinet. Hands should be washed whenever gloves are changed or removed.

- Spills large enough to result in liquids flowing through the front or rear grilles require decontamination that is more extensive. All items within the cabinet should be surface decontaminated and removed. After ensuring that the drain valve is closed, decontaminating solution can be poured onto the work surface and through the grille(s) into the drain pan.

- Twenty to 30 minutes is generally considered an appropriate contact time for decontamination, but this varies with the disinfectant and the microbiological agent. Manufacturer’s directions should be followed. The spilled fluid and disinfectant solution on the work surface should be absorbed with paper towels and discarded into a biohazard bag. The drain pan should be emptied into a collection vessel containing disinfectant. A hose barb and flexible tube should be attached to the drain valve and be of sufficient length to allow the open end to be submerged in the disinfectant within the collection vessel. This procedure serves to minimize aerosol generation. The drain pan should be flushed with water and the drain tube removed.

- If the spilled liquid contains radioactive material, Health Physics division of EHS should be contacted for specific instructions (646-962-733); see the Radiation Safety Manual for reference.

6.1.1.2. Spills outside a Biological Safety Cabinet (BSC)

- If potentially hazardous biological material is spilled in the laboratory, avoid inhaling any airborne material by holding the breath and leave the laboratory; warn others in the area.

- If someone is splashed with hazardous material, use an eye wash or emergency shower to immediately rinse the affected area with water for at least 15 minutes.

- Remove and place contaminated clothing in a biohazard waste container. Seek medical attention needed (Workforce Health and Safety during work hours; NYP
ER during off-hours and weekends). Refer to the
*Bloodborne Pathogen Exposure Control Plan* manual
for complete guidance regarding exposure response.

- Re-entry into the laboratory should be delayed for a
  period of 30 minutes to allow reduction of the aerosol
  generated by the spill.
- Utilize a biological spill kit to clean up the spill; this
  should be prepared prior in anticipation of an incident.
- Applicable personal protective clothing should be worn
  when entering the laboratory to clean the spill area.
- Place dry absorbent material (e.g. paper towels) on the
  spill to absorb liquids.
- Carefully pour the disinfectant around and on the visible
  spill and place a second layer of paper towels soaked in
  an appropriate disinfectant over the spill. Be careful to
  avoid splashing.
- Decontaminate any other potentially contaminated
  material within the spill area.
- Allow a minimum of 20 minute contact period.
- Use absorbent material to wipe up the disinfectant and
  spill, working toward the center of the spill. Discard
  spill material in biohazard waste receptacles.

6.1.2. **Major Spills**

Major spills are those that, based on the hazard evaluation, cannot be
effectively managed and require a response by Environmental Health and
Safety.

7.2 **EHS RESPONSES**

In certain irregular or more markedly hazardous situations (e.g. those involving
coincidental radiation or toxic chemical release along with the biohazard
incident) the Biosafety Officer will determine which hazard poses the greatest
immediate risk to all personnel affected and together will develop an
appropriate response plan to address any and all hazards.

A chronology of a sample EHS response to a biological spill is provided in
*Appendix B.*

In most situations the response to multiple hazards will be based on the
following hierarchy of control unless other risks warrant a change in order:
- Radiation Hazard
- Biohazard
- Chemical Hazard
7.3 EXTREME HAZARDS (RG3 ORGANISMS)

Laboratory personnel handling known human pathogens must have the capability and expertise in microbiological practices to design and adhere to an appropriate emergency response procedure for incidents occurring in their own laboratories.

In the event that a response has to be made to an RG3 agent or to a large spill of material, Level A or Level B hazardous material (HazMat) suits will be utilized to provide barrier protection for the response team. A risk assessment will be performed to determine the level of respiratory protection required. If a Level B suit is utilized with a Self Contained Breathing Apparatus (SCBA), it is critical that it allow the containment of the respirator to protect the harness from contamination. In the event of a multiple material spill scenario (e.g., hazardous solvents involved), the response team must ensure that the protective suit is adequately compatible with all material involved to prevent degradation of the suit and exposure to pathogenic agents.

If there is a condition with a high likelihood of infectious aerosols existing, only Level A suits will be permitted. The decontamination procedures will require total scrub down of all potentially contaminated areas with a strong disinfectant.

8.0 WASTE DISPOSAL

After decontamination of spill debris via autoclave treatment or chemical disinfectant, utilize the procedures detailed in the EHS Program Manual, Section 5.2 – Waste Disposal Procedures to properly manage and dispose of the waste material.

9.0 TRAINING

Bloodborne Pathogen Exposure Control Plan is a requirement for anyone who responds to biological spills and handles biological materials. This training has been incorporated into Laboratory Safety Training and Clinical and General Safety Training sessions conducted by EHS and must be completed annually.

9.1 TRAINING RECORDS

Training records are maintained by EHS. Lists of personnel who are currently trained are available online at: http://www.weill.cornell.edu/ehs/training.

10.0 RECORD RETENTION, AVAILABILITY, AND REVISIONS

10.1 AVAILABILITY
Copies of this Procedure must be available to the all employees who handle biological materials and/or have responsibility for responding to biological spills.

10.2 REVISIONS

This Procedure will be revised and updated as necessary to reflect changing regulations and circumstances. The most current copy of this Procedure can be obtained by contacting Environmental Health and Safety or at http://weill.cornell.edu/ehs/forms_and_resources/.

11.0 DEFINITIONS

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

**Risk Group 2 (RG2)** agents are associated with human disease which is rarely serious and for which preventive or therapeutic interventions are *often* available

**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)

**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)

12.0 REFERENCES

**EHS Program Manual**, Section 5.2 – Waste Disposal Procedures


**NIH GUIDELINES FOR RESEARCH INVOLVING RECOMBINANT DNA MOLECULES**
APPENDIX A  EVALUATION OF RISK BY PATHOGEN TYPE FLOWCHART

[Diagram of a flowchart showing decision points for biological spill planning and response, including questions such as 'RG1?', 'RG2?', 'RG3?', 'Spill large?', 'Spill contained?', 'Spill 5 liters?', and actions such as 'Seal-off area', 'Call biosafety consultant', 'Decontaminate and remove contaminated material', 'Follow EHS response procedures', and 'Reports generated'.]
APENDIX B  EHS SPILL CHRONOLOGY

1. Spill occurs

2. Secure area, seal with plastic or duct tape (if necessary)

3. Select appropriate disinfectants / personal protective equipment / self-contained breathing apparatus

4. Charge-up sprayers with appropriate disinfectant (1/10 bleach)

5. Contain and control spilled material on autoclavable media

6. Decontaminate surfaces, individual(s) and clothing (if necessary)

7. Autoclave all spill-contaminated material

8. Dispose waste in accordance with 5.2 – Waste Disposal Procedures

9. Send and check up on individuals who have gone to WCMC Workforce Health and Safety (WHS) or NewYork-Presbyterian Hospital Emergency Department

10. Report incident to:
    ♦ Environmental Health and Safety (EHS)
    ♦ Institutional Biosafety Committee (IBC), Institutional Animal Care and Use Committee (IACAC), Institutional Review Board (IRB), as necessary

11. Report to external authorities if required by law:
    ♦ Center for Disease Control and Prevention
    ♦ National Institutes of Health
    ♦ Occupational Safety and Health Administration
APPENDIX C NIH GUIDELINES (APPENDIX B) – CLASSIFICATION OF HUMAN ETIOLOGICAL AGENTS ON THE BASIS OF HAZARD

This appendix details 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.

This appendix reflects the current state of knowledge and should be considered a resource document. It includes the more commonly encountered agents and is not meant to be all-inclusive. Information on agent risk assessment may be found in the Agent Summary Statements of the CDC/NIH publication, Biosafety in Microbiological and Biomedical Laboratories (see Sections V-C, V-D, V-E, and V-F, Footnotes and References of Sections I through IV). Further guidance on agents not listed in Appendix B may be obtained through: Centers for Disease Control and Prevention, Biosafety Branch, Atlanta, Georgia 30333, Phone: (404) 639-3883, Fax: (404) 639-2294; National Institutes of Health, Division of Safety, Bethesda, Maryland 20892, Phone: (301) 496-1357; National Animal Disease Center, U.S. Department of Agriculture, Ames, Iowa 50010, Phone: (515) 862-8258.

A special committee of the American Society for Microbiology will conduct an annual review of this appendix and its recommendation for changes will be presented to the Recombinant DNA Advisory Committee as proposed amendments to the NIH Guidelines.

Table 1. Basis for the Classification of Biohazardous Agents by Risk Group (RG)

<table>
<thead>
<tr>
<th>Risk Group 1 (RG1)</th>
<th>Agents that are not associated with disease in healthy adult humans</th>
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<tr>
<td>Risk Group 2 (RG2)</td>
<td>Agents that are associated with human disease which is rarely serious and for which preventive or therapeutic interventions are often available</td>
</tr>
<tr>
<td>Risk Group 3 (RG3)</td>
<td>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)</td>
</tr>
<tr>
<td>Risk Group 4 (RG4)</td>
<td>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)</td>
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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))
--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
### Risk Group 2 (RG2) - Fungal Agents

- Blastomyces dermatitidis
- Cladosporium bantianum, *C. (Xylohypha) trichoides*
- Cryptococcus neoformans
- Dactyliaria galopava (*Ochroconis gallopavum*)
- Epidermophyton
- Exophiala (Wangiella) dermatitidis
- Fonseccae pedrosoi
- Microsporum
- *Paracoccidioides brasiliensis*

**Risk Group 3 (RG3) - Mycobacteria**

- *Mycobacterium avium* complex, *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. oiticicaiarum*, *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<sup>−</sup>* strains (lacking the 102 kb pigmentation locus) and *lcr<sup>−</sup>* strains (lacking the LCR plasmid)

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- Francisella tularensis specifically *F. tularensis* subspecies novocida [aka *F. novocida*], 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-II-C-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. 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. oiticicaiarum*, *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<sup>−</sup>* strains (lacking the 102 kb pigmentation locus) and *lcr<sup>−</sup>* strains (lacking the LCR plasmid)
--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
--Coecidia
--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. 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

**RISK GROUP 3 (RG3) AGENTS**

RG3 agents are associated with serious or lethal human disease for which preventive or therapeutic interventions may be available.
RISK GROUP 3 (RG3) - BACTERIAL AGENTS INCLUDING RICKETTSIA

--Bartonella
--Brucella including B. abortus, B. canis, B. suis
--Burkholderia (Pseudomonas) mallei, B. pseudomallei
--Coxiella burnetii (except the Phase II, Nine Mile strain listed in Appendix B-II-A, Risk Group 2 (RG2) - Bacterial Agents Including Chlamydia)
--Francisella tularensis (except those strains listed in Appendix B-II-A, Risk Group 2 (RG2) - Bacterial Agents Including Chlamydia)
--Mycobacterium bovis (except BCG strain, see Appendix B-II-A, Risk Group 2 (RG2) - Bacterial Agents Including Chlamydia), M. tuberculosis
--Pasteurella multocida type B - "buffalo" and other virulent strains
--Rickettsia akari, R. australis, R. canadensis, R. conorii, R. prowazekii, R. rickettsii, R. siberica, R. tsutsugamushi, R. typhi (R. mooseri)
--Yersinia pestis (except those strains listed in Appendix B-II-A, Risk Group 2 (RG2) - Bacterial Agents Including Chlamydia)

RISK GROUP 3 (RG3) - FUNGAL AGENTS

--Coccidioides immitis (sporulating cultures; contaminated soil)
--Histoplasma capsulatum, H. capsulatum var. duboisii

RISK GROUP 3 (RG3) - PARASITIC AGENTS

None

RISK GROUP 3 (RG3) - VIRUSES AND PRIONS

Alphaviruses (Togaviruses) - Group A Arboviruses
--Chikungunya virus (except the vaccine strain 181/25 listed in Appendix B-II-D Risk Group2 (RG2) – Viruses)
--Semliki Forest virus
--St. Louis encephalitis virus
--Venezuelan equine encephalomyelitis virus (except the vaccine strains TC-83 and V3526, see Appendix B-II-D (RG2) – Viruses)
--Other viruses as listed in the reference source (see Section V-C, Footnotes and References of Sections I through IV)

 Arenaviruses
--Flexal
--Lymphocytic choriomeningitis virus (LCM) (neurotropic strains)

Bunyaviruses
--Hantaviruses including Hantaan virus
--Rift Valley fever virus
Coronaviruses
-- SARS-associated coronavirus (SARS-CoV)

Flaviviruses - Group B Arboviruses
-- Japanese encephalitis virus (except those strains listed in Appendix B-II-D Risk Group2 (RG2) - Viruses)
-- West Nile virus (WNV)
-- Yellow fever virus
-- Other viruses as listed in the reference source (see Section V-C, Footnotes and References of Sections I through IV)

Orthomyxoviruses
-- Influenza viruses 1918-1919 H1N1 (1918 H1N1), human H2N2 (1957-1968), and highly pathogenic avian influenza H5N1 strains within the Goose/Guangdong/96-like H5 lineage (HPAI H5N1).

Poxviruses
-- Monkeypox virus

Prions
-- Transmissible spongiform encephalopathies (TSE) agents (Creutzfeldt-Jacob disease and kuru agents)(see Section V-C, Footnotes and References of Sections I through IV, for containment instruction)

Retroviruses
-- Human immunodeficiency virus (HIV) types 1 and 2
-- Human T cell lymphotropic virus (HTLV) types 1 and 2
-- Simian immunodeficiency virus (SIV)

Rhabdoviruses
-- Vesicular stomatitis virus (except those strains listed in Appendix B-II-D Risk Group2 (RG2) - Viruses)

**RISK GROUP 4 (RG4) AGENTS**

RG4 agents are likely to cause serious or lethal human disease for which preventive or therapeutic interventions are not usually available.

**RISK GROUP 4 (RG4) - BACTERIAL AGENTS**
None

**RISK GROUP 4 (RG4) - FUNGAL AGENTS**
None
RISK GROUP 4 (RG4) - PARASITIC AGENTS
None

RISK GROUP 4 (RG4) - VIRAL AGENTS
Arenaviruses
--- Guanarito virus
--- Lassa virus
--- Junin virus (except the candid #1 vaccine strain listed in Appendix B-II-D Risk Group2 (RG2) Viruses)
--- Machupo virus
--- Sabia

Bunyaviruses (Nairovirus)
--- Crimean-Congo hemorrhagic fever virus

Filoviruses
--- Ebola virus
--- Marburg virus

Flaviruses - Group B Arboviruses
--- Tick-borne encephalitis virus complex including Absetterov, Central European encephalitis, Hanzalova, Hypr, Kumlinge, Kyasanur Forest disease, Omsk hemorrhagic fever, and Russian spring-summer encephalitis viruses

Herpesviruses (alpha)
--- Herpesvirus simiae (Herpes B or Monkey B virus)

Paramyxoviruses
--- Equine morbillivirus

Hemorrhagic fever agents and viruses as yet undefined

ANIMAL VIRAL ETIOLOGIC AGENTS IN COMMON USE

The following list of animal etiologic agents is appended to the list of human etiologic agents. None of these agents is associated with disease in healthy adult humans; they are commonly used in laboratory experimental work.
A containment level appropriate for RG1 human agents is recommended for their use. For agents that are infectious to human cells, e.g., amphotropic and xenotropic strains of murine leukemia virus, a containment level appropriate for RG2 human agents is recommended.
Baculoviruses

Herpesviruses
--Herpesvirus ateles
--Herpesvirus saimiri
--Marek's disease virus
--Murine cytomegalovirus

Papilloma viruses
--Bovine papilloma virus
--Shope papilloma virus

Polyoma viruses
--Polyoma virus
--Simian virus 40 (SV40)

Retroviruses
--Avian leukosis virus
--Avian sarcoma virus
--Bovine leukemia virus
--Feline leukemia virus
--Feline sarcoma virus
--Gibbon leukemia virus
--Mason-Pfizer monkey virus
--Mouse mammary tumor virus
--Murine leukemia virus
--Murine sarcoma virus
--Rat leukemia virus

MURINE RETROVIRAL VECTORS

Murine retroviral vectors to be used for human transfer experiments (less than 10 liters) that contain less than 50% of their respective parental viral genome and that have been demonstrated to be free of detectable replication competent retrovirus can be maintained, handled, and administered, under BL1 containment.