Required Biosafety Level Three (BSL-3)
Practices, Procedures, Facilities, and Safety Equipment
For BSL-3 and BSL3/2 Laboratories

1) LABORATORY OPERATIONS
   a) Responsibilities: A clear organization is set and each person understands the
      responsibilities of all who work in and support BSL3 work. 1), 3), 7) 1
   
   b) Access Control: Access to the laboratory is controlled by the laboratory director and
      restricted to persons whose presence is required. 1), 3), 5)
      
      i) Access to the laboratory is limited to people who have been advised of (and
         understand) the potential biohazard and who comply with entry requirements
         and/or tests (e.g. immunization, TB skin test).
      
      ii) Mechanism to control access is created and documented.
      
      iii) Persons at increased risk of acquiring infection or for whom infection may have
           unusually serious consequences are informed of their risk and are strongly
           discouraged from entering the laboratory.
      
      iv) Entry and exiting procedures to cover:
         (1) Authorization for laboratory staff, visitors, maintenance, visiting scholars.
         (2) Entry/exit log or other method to monitor authorized entry.
         (3) Required personal protective equipment
         (4) Escort requirements.
         (5) Removal of objects and viable cultures.
         (6) Disinfection procedure prior to maintenance
         (7) Disinfection procedure for exiting
      
   v) Minors under 16 years old are not allowed in the laboratory. 1), 3)

   c) Worker Certification: All personnel must demonstrate proficiency in the practices and
      procedures specific to their responsibilities before being authorized to work in the
      BSL3 containment area.
      
      i) Describe the steps and controls to ensure that a worker is qualified and authorized
         to conduct BSL-3 work.
      
      ii) No one (regardless of previous experience in other laboratories) is permitted to
          work in the laboratory without going through a certification process. 1), 3), 5)

   d) Identifying, reporting and correcting problems. 1), 3), 4)
      
      i) Methods are in place for employees to report violations of procedure, identify co-
         worker failings, express concerns, report problems, and offer suggestions without
         fear of repercussion.

1 References cited are the source for the requirement and are listed in Appendix A.
ii) Procedures are in place to address and correct any breach in the laboratory’s exposure control plan, standard operating procedures and special conditions set by the IBC.

iii) Documentation is maintained to show that those who neglect containment practices and procedures are informed and disciplined as necessary.

2) PRACTICES AND PROCEDURES

a) Handling and Storage of viable material:

i) A biological safety cabinet is used for all activities with open containers of viable material. The Institutional Biosafety Committee must specifically authorize exception to this requirement. 1), 3)

ii) Procedures for biological safety cabinet use are developed or adopted. Personnel are trained on the proper use of the biological safety cabinet. 8)

iii) Methods are in place for safe handling of samples that have been frozen or stored in liquid nitrogen. 7)

iv) Biohazardous materials are stored in labeled secondary containers. 5)

v) Refrigerators, freezers, incubators, etc. that contain biohazardous materials for storage must be labeled with a biohazard symbol. 5)

b) Sharps handling: Sharps use and disposal is carefully planned and safety procedures rigorously followed. Sharps handling methods are reviewed and updated. 1), 3), 5)

i) Sharps are eliminated where possible.

ii) Hazards of use are reduced by using engineered sharps injury protection systems (i.e. needleless systems) whenever practicable. Methods and schedule of evaluation and implementation are in place

iii) If engineered sharps injury protection devices are not used for any reason, written documentation of the evaluation of sharps engineered protection devices and the reasons they are not used must be kept on file.

iv) All sharps waste is placed in sharps containers. Broken glass is handled by tongs, dustpan, etc., but not by hand.

c) Waste handling and disposal: 1), 3), 5)

i) Biohazardous waste handling procedures follow the instructions in the UCSD Biosafety Handbook, Chapter 18.

ii) Instructions are posted.

iii) All biohazardous waste is decontaminated in the facility or building.

iv) Autoclave is maintained, calibrated and tested.
v) Autoclave use records are maintained.

vi) Procedures for disposal of biohazardous waste that also include chemical or radioactive waste are developed in consultation with the Biosafety Officer.

d) Decontamination and housekeeping 1), 3), 5), 10)
   i) Work surfaces daily, after each work session, and upon overt spills/contamination.
   ii) Documentation of the disinfectant’s effectiveness is maintained on file.
   iii) A regular schedule for housekeeping is maintained.

e) Transportation and Shipping:
   i) Transport outside the laboratory: A secondary container for transport viable product outside the biosafety cabinet to preventing leakage if the container is dropped. 1)
   ii) Shipping: Biohazardous materials are shipped in certified packaging, labeled, and shipped in accordance with Department of Transportation (DOT) requirements. 10)

f) Laboratory Shutdown: The laboratory has shutdown and clearance procedures for periods of major maintenance, repair, equipment replacement, etc. 7)

3) EXPOSURE CONTROL PLAN / BIOSAFETY MANUAL
An Exposure Control Plan (i.e.: Biosafety Manual) unique to the laboratory is written to address conditions of the current research, facilities and personnel. 1), 3), 5), 10)
The Manual/Plan should adopt the UCSD Biosafety Handbook and:
   a) Biosafety containment practices and procedures unique to the laboratory are detailed for biohazardous materials that present unusual risks or require special handling.

   b) Biohazardous Materials Use Authorization (BUA): Include a copy of the most recent approved Biohazardous Materials Use Authorization.
      i) “Symptomatology” pages from your approved BUA. (see http://www- ehs.ucsd.edu/bio/Wildtype_Symptomatology.doc )
      ii) A list of the biohazardous materials/agents present, the risks associated with each agent, signs and symptoms of disease, and steps to be taken if an exposure incident is suspected or if a laboratory worker exhibits possible signs of disease.

   c) Verify that your Exposure Control Plan is complete: Reference Appendix “B” “Exposure Control Plan Guidelines”

   d) Biosafety Committee requirements are fulfilled: Specifically address requirements.

   e) Make the Exposure Control Plan available to all who work in the laboratory.

   f) Update the Exposure Control Plan at least annually.
4) SPILLS, EXPOSURES, AND EMERGENCIES
   a) Emergency Plan: An emergency plan is developed or adopted that describes the procedures to be followed if an accident, spill, release, or exposure contaminates personnel or the environment. As part of the planning, identify and limit conditions that contribute to the risk of spills or accidents. 1), 3), 4), 5)
      i) All who work in the vicinity understands the emergency plan.
      
      ii) A spill kit is maintained on hand.

      iii) Biohazard spills are decontaminated, contained and cleaned up by staff properly trained and equipped.

      iv) Spills/accidents that result in potential exposure are immediately reported to the laboratory director and the biosafety officer.

   b) Incident Reports: A written report of any spills, exposure, failures of containment, mechanical breakdown, and maintenance problems is submitted to the Biosafety Officer within five workdays. 3)

   c) Exposure/Incident response procedures: Each worker is knowledgeable of the steps to take if a (probable) exposure occurs. A post-exposure management plan is in place. 5)

5) EQUIPMENT & PERSONAL PROTECTIVE EQUIPMENT
   a) Personal protective equipment 1), 3), 5)
      i) The minimum required protective equipment is clearly defined in terms of what, where, when and how it is to be used and removed.

      ii) Protective solid-front laboratory clothing shall be worn by workers when in the laboratory and shall NOT be worn outside the laboratory.

      iii) An eye protection policy is in place. Eye protection is worn when the chance of eye contamination exists.

      iv) Cleaning and re-use conditions (if permitted) are clearly defined

   b) Gloves: Gloves must be worn. Frequent glove changing and handwashing is standard practice. 1)

   c) Biological Safety Cabinet (BSC) 1), 3), 5), 6), 8), 10)
      i) The BSC is located away from doors, air supply vents and heavily traveled areas.

      ii) BSCs are tested and certified after installation, alterations, or maintenance, and at least every twelve months.

      iii) A maintenance log and certification schedule is maintained.
iv) Biosafety Cabinets are seismically anchored. (Good Practice)

v) A warning sign is placed on the BSC stating that decontamination is required prior to servicing contaminated internal parts.

d) **Precautions when the BSC cannot be used:** When procedures cannot be conducted in the BSC, appropriate personal protective equipment and physical containment devices must be used. The Biosafety Officer and the Institutional Biosafety Committee must approve this work before it commences. 1), 7)

e) **Contaminated Laboratory Equipment** 1)
   i) Equipment is decontaminated before in-place (or removal for) repair or maintenance.

   ii) Contaminated equipment and equipment containing biohazardous materials must have a biohazard sticker affixed in a prominent location.

   iii) Procedures are in place for repair of contaminated equipment.

   iv) Where equipment could create a biohazardous aerosol, a containment procedure is in place

f) **Aerosol-generating equipment:** Equipment that may produce biohazardous aerosols has engineered containment to prevent exposures to people or the environment. Devices are tested at least every twelve months. 1)

g) **Chairs** and lab furniture can be easily decontaminated. 1)

6) **EMPLOYEE EDUCATION** 1), 3), 5)
   a) **Right to know:** All persons (including those not directly involved with the biohazardous work) who must enter or work in the vicinity of a BSL3 laboratory must be informed about: the risks associated with each infectious agent; the signs, symptoms, and significance of infection/disease; required procedures and precautions; and are provided a method to address concerns and questions.

   b) **Annual training:** All laboratory & support personnel receive training on potential hazards and appropriate precautions initially and annually thereafter. 1), 2), 3), 4), 5)

   c) **Training curriculum:** A schedule of training for BSL-3 workers is developed with a list of topics to be covered. An outline for each topic, a scheduled of presentations and presenters is maintained.
d) **Records of training:** A file with written documentation of training for each employee is kept. All teaching opportunities individual or group, scheduled or not can meet training requirements provided that records are kept. Documentation should include an outline of the material, name of presenter, and verification (signature) of each employee’s attendance.

e) **Bloodborne pathogens:** All persons who work with human or primate blood, tissues, fluids, cells, or cell lines have bloodborne pathogens training annually.

7) **HEALTH AND MEDICAL MONITORING**

a) **Immune-suppressed:** Ensure that immune-suppressed people are fully informed and regularly reminded of their increased-risk condition. They should not work in a high-risk environment and should seek the advice of occupational health professionals to help them evaluate their medical risks.

b) **Laboratory-acquired illness:** The signs or symptoms of a laboratory-acquired infection are well known by all who work in the laboratory. Procedures are in place to address situations when (if) a person exhibits those signs or symptoms. The Biosafety Officer is notified if a laboratory-acquired infection is suspected.

c) **Vaccinations** are provided as necessary and made available at no cost to the employee. Identify the agents, vaccinations available, justification for offering (or not offering) the vaccination, and means to receive the vaccination.

d) **Serum storage:** If serum storage is required, a policy must be written to address the reasons for requiring serum storage, collection schedule, methods for collection and storage, conditions under which the serum will be tested, methods for identifying samples while maintaining confidentiality and security measures.

e) **Antibody testing** When antibody testing is required, clearly define who is tested; who collects samples, tests and reports results; what are the conditions/schedule for testing and reporting; what is the response to negative or positive results.

8) **FACILITY**

a) **Design and operation specifications/procedures** for the BSL-3 facility are documented and updated as necessary. The facility is annually verified against these parameters. 1)

b) **Security:** The security of the laboratory/facility is considered. University Police consultation is sought and records are maintained. 1), 2)

c) **Self-closing doors:** Doors are self-closing and lockable. 8), 2), 3)
d) **Directional airflow:** Air flows from clean areas toward contaminated areas. Laboratory staff documents frequent checks of directional airflow. 1), 3), 5)

e) **Ventilation alarm:** Audible alarms are installed to warn workers in the BSL-3 facility of ventilation system failure. 7)

f) **Biosafety Cabinets:** Properly installed and certified. Air curtain is OK.

g) **Vacuum lines:** Liquid disinfectant traps and a hydrophobic HEPA filters are required on all vacuum lines. 1), 3), 5)

h) **Signs:** Hazard warning sign(s) with the universal biohazard symbol are posted indicating the agents present, the name and telephone number of the Principal Investigator, and/or other responsible person(s); as well as any special requirements for entering the laboratory. 1), 3), 5)

i) **Laboratory layout:** Spaces between benches, cabinets & equipment are accessible for cleaning. 1)

j) **Sinks:** Handwashing sinks are hands-free & located near exit of each room. 1), 3), 5)

k) **Eyewash:** Functional eyewash is inside the facility. 1)

l) **Walls, floors & ceiling:** The surfaces of walls, floors and ceiling are smooth, impermeable and easily cleaned and all penetrations are sealed. 1), 3)

m) **Autoclave:** Alternatives to the autoclave in the event of breakdown. 7)

n) **Pest management:** An insect and rodent control program is in effect. 1), 3), 9)
Appendix A

REFERENCES:

1) CDC/NIH. Biosafety in Microbiological and Biomedical Laboratories (BMBL) 4th Ed. May 1999 Section III, “Laboratory Biosafety Level Criteria”, Biosafety Level 3 (BSL-3) (pages 26-36).

2) BMBL Appendix F


4) California Code of Regulations, Title 8, (8CCR) Section 3202 “Injury & Illness Prevention Plan”,

5) 8CCR Section 5193, “Bloodborne Pathogens”,

6) 8CCR Section 4154.2 Ventilation Requirements for Biological Safety Cabinets.

7) Good Practice that has been learned through experience – may be required by the Institutional Biosafety Committee.

8) BMBL Appendix A

9) BMBL Appendix G

10) UCSD Biosafety Handbook