Texas Tech University

Standard Operating Procedures Manual

Biosafety Level 2 Laboratory

of
Dr. Boyd Butler

Assistant Professor
Department of biological Sciences
Biology 207/214

September 1, 2012

PURPOSE

This manual applies to all research staff, hosted visitors and guests, volunteers, building staff, and service staff who enter the laboratory. This manual will be reviewed annually by the Principal Investigator or Laboratory Supervisor for changes or corrections to ensure that it is accurate.

I. Introduction to General Safety and Training for the BSL2 Laboratory

A. Required Training

The minimum requirements for qualification to work in the Butler BSL2 lab are:
1) Current Environmental Health & Safety (EH&S) trainings including: Biological Safety, Lab Safety, Bloodborne Pathogen Exposure Control, Chemical Hygiene Training, and Radiation Safety if applicable.

Laboratory personnel shall demonstrate the following:

- Willingness to follow established laboratory safety guidelines and these standard operating procedures

This document will provide the basis of training in conjunction with EH&S training.

Dr. Butler will provide information and arrange for training at the time of an individual's initial assignment to the lab. Dr. Butler will arrange for refresher training at least annually and when there are any changes in processes or procedures.

For more information concerning training, also see the TTU Biosafety Manual on the EH&S web site.

B. Administrative Procedures

It is the responsibility of each employee to carefully consider every action taken in the BSL2 lab and its potential impact on possible exposure or contamination, and to follow established Standard Operating Procedures (SOPs) and protocols diligently and without variance.

All employees will read and adhere to the TTU Biosafety Manual and to the SOP Manual for the Dr. Butler laboratory. All employees will use pertinent sections in the Biosafety in Microbiological and Biomedical Laboratories, CDC-NIH, 5th edition as a guideline and reference.

All employees will take the online Texas Tech University EH&S courses in Laboratory Safety Training and Biological Safety Training. Records of certification will be kept on file by Human Resources and EHS.

No employee will be trained to work in the lab without the express permission of Dr. Butler

New SOPs and protocols must be approved by the PI before initiation.

C. General Laboratory Safety

Work will be performed in Biology 207/214.

1. Laboratory employees must immediately notify the laboratory manager or PI in case of an accident, injury, illness, or overt exposure associated with laboratory activities.

2. No eating, drinking, smoking, handling contact lenses, or applying cosmetics in the lab at any time.

3. No animals or minors (persons under the age of 18), or immuno-compromised persons will be allowed to enter the lab at any time.

4. Food, medications, or cosmetics should not be brought into the lab for storage or later use. Food is stored outside in areas designated specifically for that purpose.

5. No open-toed shoes or sandals are allowed in the laboratory.

6. PPE includes gloves, lab coat, and eye protection.

7. All skin defects such as cuts, abrasions, ulcers, areas of dermatitis, etc. should be covered with an occlusive bandage.

8. Mouth pipetting is prohibited; mechanical pipetting devices are to be used at all times.

9. All procedures are to be performed carefully to minimize the creation of splashes or aerosols.

10. Follow all manufacturer's instructions and SOPs when using any of the laboratory equipment.

11. Wash hands:
    - after removing gloves, and
    - before leaving the laboratory.

12. Razor blades, scalpels, and hypodermic needles ("sharps") should be discarded into the "sharps" container in the biosafety cabinet. DO NOT recap needles.

13. Work surfaces will be decontaminated as needed. Follow manufacturer instructions for contact time.

14. All cultures, stocks, and other regulated wastes are decontaminated by autoclaving before disposal. Liquids (non-organic) can be decontaminated with bleach, bringing the solution to 10% bleach, and discarded in the sink. No other chemicals can be discarded in the sink.

D. General Biosafety Cabinet ("Hood") Safety

1. Reserve time in advance to have priority for use of the BSCs.

2. Turn on the blower in the cabinet at least 10 minutes before placing infectious materials into the hood.

3. Check the certification sticker and Magnehelic gauge to verify that the biosafety cabinet is working properly.

4. Check the air-flow indicator to verify that the air flow is operating properly.

6. Gloves must be worn at all times.

7. DO NOT disrupt the airflow through the hood by placing ANY item on the grills or by opening the door to the corridor.

8. In general, the interior of the hood should be considered to be a contaminated zone, even though every effort is made to keep the surfaces clean, as is consistent with accepted good microbiological practice and sterile technique.

10. Clean the inside surfaces of the BSC with 70% Ethanol.

11. Allow the blower to run for at least 10 minutes following use.

12. The UV light is turned on between procedures (at least 5 minutes) and at the end of the day (all night). UV lights must be turned off before work begins in the hood. **Do not look directly at UV lights as this can cause eye damage**.

E. General Accident Procedures

Spills - Apply paper towels to absorb the spill, and then soak paper towels with 20% Bleach. For spills outside the biosafety hood, alert others in the area. Use N95 mask if there is a possibility of harmful aerosols.

Follow all aspects of the emergency SOPs without exception.

II. Standard Operating Procedures

A. 1000 – Containment Requirements

1001 - Laboratory

1001.1 Entering the lab to begin work

A. Put on required PPE.

B. Gather all materials for the experiment.

1001.2 Exiting Laboratory

Before exiting the lab, be sure that all required documentation has been completed, the hood and work area are clean, all contaminated waste materials are disposed of properly, and stocks have been returned to the proper storage area. Remove PPE and throw away gloves. Wash your hands.

1002 - Specimen Transport

Transport of biological materials to another building or lab within the same building should be done in a covered container. If the samples are infectious, use a secondary container and label it with the contents and a contact person/phone number.

1003 - Work within Laboratory Biology 207/214

During work with blood products persons must be wearing gloves and lab coats.

1003.1 Preparation of primary containers of agent stock solutions and manipulations of primary containers of agents should be conducted in a biological safety cabinet.

1003.2 The user should verify inward airflow of the biological safety cabinet before initiating work by checking the Magnehelic.

1003.3 All work should be done within the operationally effective zone of the biological safety cabinet.

1003.4 Care should be taken to avoid contaminating medium or other cell culture supplies.

1003.5 Discard pipets and tips appropriately. Glass tips are to be placed in glass only containers and plastic tips, flasks and media bottles are to be placed in plastic only containers. Both containers are to be lined with autoclavable biohazard bags.

1003.6 The interior of the hood should be cleaned periodically.

1003.7 When vacuum lines are used with systems containing agents, they will be protected with in-line filters to prevent entry of agents into the lines, and will be protected by a liquid trap containing bleach. **NOTE: No biological agent-containing material should be allowed into the drain of a sink unless the material has been decontaminated with bleach!**

B. 2000 – Proper Use of Equipment

2001 - Biological Safety Cabinets

2001.1 – To assure sterility inside the cabinet and establish proper air flow for containment, the blower should be turned on at least ten minutes before infectious materials are to be put into the biosafety cabinet.

2001.2 – Biosafety cabinets must be certified prior to use. A qualified outside contractor must certify these cabinets annually. Check the certification sticker on the front of the unit to verify your biosafety cabinet's condition.

2001.3 – The biosafety cabinet air flow ("Magnehelic") gauge should be checked (reading is equal to approximately 0.5 inches) to assure proper operation of the cabinet before placing any materials into it. Readings indicate relative pressure drop across the HEPA filter. Higher readings may, therefore, indicate filter clogging. Zero readings may indicate loss of filter integrity. In either of these cases, notify the Laboratory Manager or PI.

2001.4 – NEVER place anything over the front or rear grill of a biosafety cabinet.

2001.5 – Disrupting the airflow into the front grill allows contaminated air from inside the cabinet to blow into the lab or directly at the person sitting at the cabinet. It also allows non-sterile air from the room to blow into the biosafety cabinet over the experiments.

2001.6 – Materials should be placed in the cabinet so as not to block air flow into the rear grill. Leave a few inches for air to flow around objects. Any disruption of the air flow in the cabinet decreases its effectiveness.

2001.7 – Before manipulating infectious materials, make sure that you have everything you need in the cabinet. The fewer times you pull your hands out of the cabinet, the less disruption of the air flow.

2001.8 – Work should be performed in the center of the work surface of the cabinet whenever possible. Work outward progressing from clean to dirty (contaminated). However, infectious agents should not be placed directly adjacent to or directly on the intake grills.

2001.9 – After manipulating infectious agents, make sure all containers are tightly closed.

2001.10 – All waste and disposable items generated by work in the cabinet should be left *(describe where it should be stored)* until properly decontaminated or contained for transport to the autoclave.

2001.11 – After the cabinet has been emptied, wipe inner surfaces with 70% ethanol. Do not shut down the blower.

2001.12 – The bleach in the vacuum traps must be changed after one week of use or when the flask is half full. To discard trap liquid, first treat with fresh bleach for 20 minutes, and then empty it into the sink.

2001.13 – The vacuum filters must be replaced if clogged or if liquid makes contact with the filter. Used filters should be placed in the waste to be autoclaved.

(NOTE: Please see the section on Biological Safety Cabinets in the TTU Biosafety Manual for more information.)

NOTE: Though Class IIB cabinets are hard-ducted (so that all air is removed from the room), Class IIA cabinets recirculate about 70% of the air inside themselves and exhaust the remainder to the lab. Any use of volatile solvents, such as absolute ethanol, should be kept to a minimum or done elsewhere. **Dangerously high levels of volatile vapors can accumulate inside the cabinet and pose a threat of fire or explosion.**

2002 – Incubators

2002.1 – Upright Incubators

A. Incubators are normally set at 37^{o} C.

B. Temperature should be checked each day by all users.

C. Operation manuals are located Biology 214.

D. If an alarm is sounding, check the panel for the identifying blinking light.
   1. If there is no obvious reason for the alarm, contact the PI.
   2. The "CO_2 Low" (or High) message indicates a deviation from 5% CO_2. Check the hose from the wall to the unit.
   3. The "tank farm" must be checked for empty tanks once/week.

E. Decontaminate incubators at least every 2 days.

2002.2 – Water bath

The water bath should be monitored for water level, and filled with distilled water only. To prevent growth of any organisms, water should be treated with.

2003 – Autoclave

See PI for instructions on how to use the autoclaves

2004 – Emergency Equipment

2005.1 – Fire Extinguisher, located Hallway outside Biology 214.

A. Operation

1. Fire extinguishers should be used only if the fire is small and confined to one small area! USE JUDGEMENT IN THIS! DO NOT CREATE A LIFE-THREATENING SITUATION WHILE TRYING TO EXTINGUISH A FIRE!

2. To operate, pull the pin to release the handle.

3. Stand at a safe distance from the fire (as directed on the fire extinguisher).

4. Aim the nozzle at the base of the fire, squeeze the handle to discharge the agent, and sweep completely left and right until a few seconds after seeing no fire.

B. Maintenance

Fire extinguishers are inspected annually by EH&S. Check the gauge periodically to ensure operational status.

C. 3000 – Operational Procedures

3001 – Inventory Control System

Chemical Inventory: All dry chemicals are inventoried in Excel file entitled “Chemical Inventory” Dell Biology 214.

3002 – Working inside the Biosafety Cabinet (BSC)
Protocols are listed below in 3004 and D4000

3003 – Working outside the Biosafety Cabinet

Working outside the hood includes such actions as transporting samples from the hood to a centrifuge, incubator, sonicator, or water bath.

3003.1 – Vials or tubes being transported to the centrifuge, water bath, etc. should be in a stable rack.

3003.2 – No liquid should be allowed into the sink drain unless it contains a final concentration of 10% bleach.

3004 – Removal of Equipment, Viable Samples, and Autoclavable Wastes from the Biosafety Cabinet, Internal Clean-up and Decontamination of the Biosafety Cabinet and Maintenance of Laboratory.

Access to the laboratory is limited to staff, or other persons with permission of the Principal Investigator, when work with BSL-2 pathogens is being conducted.

Laboratory staff and all other persons working with infectious substances must wear gloves. Contaminated gloves must be changed IMMEDIATELY. Under NO CIRCUMSTANCES will gloves be reused.

Laboratory staff must wash hands after handling infectious materials, after removing gloves, and before leaving the laboratory.

Laboratory staff must not eat, smoke, handle contact lenses, or apply cosmetics in the laboratory.

Food for human consumption should be stored outside laboratory work area in refrigerators designated for that purpose only.

Only mechanical pipetting devices should be used in the laboratory.

All sharps must be placed in red (approved) sharps containers. Sharps containers must be turned in when ¾ full.

Forceps, a brush and pan or other mechanical mean should be used to pick up broken glass. Broken glass may not be picked up by hand. Broken glass must be placed in a sturdy cardboard glass box or sharps container.

All non-liquid contaminated cultures, stocks, and other biologically contaminated waste must be placed in biomedical waste containers for disposal.

All infectious liquids must be autoclaved or disinfected prior to being poured into drain.

All procedures must be performed to minimize creation of splashes or aerosols.

All work surfaces must be decontaminated at the completion of work, at the end of the day, or after any spill or splash of viable material.

Persons who are at increased risk of infection, or for whom infection may have serious consequences, must not be allowed to enter laboratory when work with infectious agents are in progress without permission of the Principal Investigator.

All persons working in laboratory must be advised of potential hazards.

All support personnel must be trained or made aware of hazards before working in the laboratory.

Spills and accidents that result in overt exposures to infectious materials must be mmediately reported to the Principal Investigator and appropriate medical evaluation must be provided.

Laboratory equipment, surfaces and other contaminated materials must be decontaminated with an effective disinfectant on a routine basis, after work with infectious materials is finished, and especially after overt spills, splashes, or other contamination.

Contaminated equipment must be decontaminated before the equipment is repaired in the laboratory or sent out of the laboratory for repair, maintenance, or other purposes.

A Biosafety Cabinet must be used for all procedures with potential for creating infectious aerosols or splashes, or whenever handling high concentrations of infectious materials.

Biological Safety Cabinet must have a current (annual) certification.

Biological Safety Cabinet must not be used until it is recertified after relocation, any movement, or dislocation.

Centrifuges used for high concentrations or large volumes of infectious agents must have sealed rotor heads or centrifuge safety cups. Safety cups must be opened only in a Biosafety cabinet.

Face protection (goggles, mask, face shield, or other splatter guard) must be used for all procedures when such procedures could produce splashes or sprays of infectious or other hazardous materials.

Protective clothing must be removed and left in laboratory before going to non-laboratory areas (cafeteria, library, administrative areas).

Protective clothing must be either disposed of in laboratory or laundered by institution. (NEVER taken home!)

Laboratory doors must be locked when the laboratory is not occupied.

Spaces between benches, cabinets, and equipment must be kept accessible for cleaning.

Eyewash must be flushed at least monthly and kept clear of obstructions.

All personnel must know the location of the nearest safety shower and must ensure that area under the shower is kept free of obstructions.

D. 4000 – Experimental Procedures
NK Isolation and Cleanup

When working with whole blood or blood products always wear gloves and work in the Biosafety cabinet

1. Whole blood or peripheral blood leukopacs
   Mix whole blood with plain sterile PBS at a 1:1 ratio.
   Pipette 25 ml of Blood/PBS into 50 ml conical tube
   Underlay with 20 ml Ficoll premium

2. Centrifuge 20 min at 2000rpm at room temp with no brake.

3. Remove by pipetting the sera supernatant and place into a new 50ml conical tube.

4. Remove peripheral blood mononuclear layer by gentle pipetting and place into new 50ml conical tube.

5. Add plain PBS to ml and spin cells at 1750 rpm for 10 min with braking.

6. Remove supernatant by aspiration and resuspend cell pellet in ACK lysis buffer.

7. Incubate in ACK for no more than 1 min. Add plain PBS to 50 ml and centrifuge at 1200 rpm for 10 min with braking.

8. Remove supernatant by aspiration

9. Resuspend cells at 1 x 10^6 cells/ml in Primary NK media

When working with whole blood or blood products always wear gloves and work in the Biosafety cabinet

All pipettes used with blood or blood products must be disposed of in Biohazard container.

Add 10% Bleach to Sera supernatant following step 3. Cap tube and discard in Biohazard container.

Add 10% Bleach to remaining cell pellet following removal of peripheral blood mononuclear layer in Step 4. Cap tube and discard in Biohazard container.

Any exposure of exposed or broken skin to whole blood or blood products:

1. Immediately wash the area with soap and water for 15 minutes.

2. If it is eye exposure, irrigate the eyes at an eyewash station for 15 min.

3. Apply first aid if necessary

4. Notify your supervisor

5. Call UMC occupational medicine (806-775-8200) to arrange a post-exposure evaluation

6. Take a Bloodborne Pathogen Exposure Incident Report to the provider to be completed at the evaluation

E. 5000 – Safety Checks and Emergency Procedures

5001 – Training and Orientation

All employees will take the TTU EH&S courses in Laboratory Safety Training, Biological Safety Training, and Fire Safety/Fire Prevention (Respiratory Protection Training and Radiation Safety Training as needed), and annual refreshers.

5002 – Personal Protective Equipment (PPE)

5002.1 When using a biological safety cabinet, protective clothing, including gloves and a long-sleeved body covering (gown, laboratory coat, smock, coverall, or similar garment) should be worn so that hands and arms are completely covered to prevent contamination of cultures, skin and street clothing.

5002.2 Eye protection should be worn when handling infectious organisms or chemicals.

5002.3 These requirements also apply to anyone working in the area while someone else is working at the biosafety cabinet.

5003 – Waste Removal from the Lab

Biohazardous waste is to be removed by EHS. Fill out online form on the TTU EHS website to schedule pickup of Biohazardous Waste.

5004 – Management of Accidental Exposures

In the event of an exposure to an infectious agent or material:

<u>Intact skin</u>

- Remove contaminated clothing.
- Vigorously wash contaminated skin for 1 minute with soap and water; there is a safety shower in the hallway outside of Biology 214.
- Inform Dr. Butler and seek medical attention at the TTU Campus Health Service.

<u>Broken, cut, or damaged skin or puncture wound</u>

- Remove contaminated clothing.
- Vigorously wash contaminated skin for 5 minutes with soap and water; there is a safety shower in hallway outside of Biology 214.

- Inform Dr. Butler and seek medical attention at the TTU Campus Health Service, or call 911 for assistance. If you are contaminated with an infectious agent, notify the emergency responders of the contamination.

Eye

- Immediately flush eyes for at least 15 minutes with water, using an eyewash by sinks in Biology 207/214
- Hold eyelids away from your eyeball and rotate your eyes so that all surfaces may be washed thoroughly; and
- Inform Dr. Butler and seek medical attention at the TTU Campus Health Service, or call 911 for assistance.

Ingestion or Inhalation

- Inform Dr. Butler and seek medical attention at the TTU Campus Health Service, or call 911 for assistance.

- Do not induce vomiting unless advised to do so by a health care provider.

5005 – Emergency Phone Numbers and Procedures

Emergency 9-911
Ambulance, Fire, Police:
University Medical Center (UMC)(806) 775-8200
Texas Tech Police: Non-emergency (806) 742-3931

Principal Investigator:
Dr. Boyd Butler
Lab: 806-742-2710 ext 256
Office: 806-742-2710 ext 256
Cell: 314-322-0163

Building Contact
Lou Densmore, Chair, Department of Biology
Office: 742-2715
Charlie Barnes, Building Manager
Office: 742-2710 ext 276
Lab Safety Coordinator
Boyd Butler
Office 742-2710 ext 256

Physical Plant
Maintenance Emergencies (806) 742-3301 or after 5pm (806) 742-3328

Information Technology
(806) 742-HELP or for Server-related issues (806) 742-3649

Texas Department of Public Safety: (806) 472-2700

TTU Emergency Management Coordinator University Council: Ronald Phillips: (806) 742-2121

TTUS Executive Director of Public Safety and Emergency Management: Jay Parchman (806) 742-9000

The Office of Communications and Marketing maintains the Emergency Communications Center
212 Administration Building
806-742-2136
Normal Office Hours: 8 a.m. – 5 p.m. Central, Monday - Friday

6001 – Receiving Reagents/Cells/Viruses

All cells and biological samples received must be opened and handled within Biosafety Cabinet as described above in D4000

6002 – Recordkeeping in the Butler Laboratory
All online safety course records are to be kept in Biology 214

6003 – Validation and History for SOP Manual

I hereby certify that I have reviewed the contents of this manual and that it reflects my current operating policy for the laboratories of Dr. Boyd Butler located in Biology 207/214.

Dr. Boyd Butler
Assistant Professor
Texas Tech University
Biological Sciences
Campus Box 4131
boyd.butler@ttu.edu

Signature _____ Annual Review Date _____

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APPENDICES

1. TTU Biosafety Manual
2. Biosafety in Microbiological and Biomedical Laboratories, CDC-NIH, 5th edition.
3. MSDS sheets are located in Biology 214