Instructions: Use this template to establish a lab-specific biosafety plan for your lab. Input appropriate information in the yellow-highlighted areas. Tailor any other wording to fit your lab's intentions. You may delete this instruction box upon generation of your final draft. Submit draft to chslabsafety@okstate.edu for review.

OKLAHOMA STATE UNIVERSITY
CENTER FOR HEALTH SCIENCES

Biological Safety Procedures

Laboratory XXX

Biosafety Level 2

Principal Investigator:

NAME

Laboratory/Facility Specific Biosafety Manual

The purpose of this laboratory/facility specific biosafety manual is to:

- Protect employees, visitors, and the surrounding community from the health hazards in the laboratory
- Meet applicable federal, state, and institutional guidelines or requirements
- Define responsibilities for laboratory safety
- Provide laboratory specific standard operating procedures
- Provide information about safe work practices, safety equipment, and personal protective equipment
- Serve as a resource for laboratory staff

Responsibilities

Safety in activities involving biohazardous agents ultimately depends on the individual conducting these activities. Motivation and good judgment are essentials in the protection of health and the environment. The NIH Guidelines are intended to help the institution, the IBC, and the PI determine the safeguards that should be implemented. These guidelines will never be complete or final in that all conceivable experiments involving rDNA and other biohazardous agents cannot be foreseen. Therefore, it is the responsibility of each individual employee to adhere to the intent of the Guidelines as well as to their specifics.

Personnel who work with Risk Group 1 agents must have standard training in microbiological practices to ensure proper handling of the agent. **Those personnel working with Risk Group 2 or 3 agents must also have specific training in handling pathogenic microorganisms and those individuals working with Risk Group 3 agents must have specific training in handling potentially lethal agents.**

Risk Group 4 agents are not permitted on the OSU campus and Biosafety Level 4 Facilities are not available.

It may be inadvisable for a person in an immunocompromised condition to work with microorganisms. This includes individuals under systemic corticosteroid therapy, chemotherapy for malignancies, radiation therapy, and those who have certain diseases (e.g., lymphomas, leukemia, and AIDS) which induce severe impairment of immune competence. Medical advice should be sought regarding possible work restrictions.

Additionally, certain microbes such as *Toxoplasma gondii*, rubella virus, cytomegalovirus, and vesicular stomatitis virus pose a hazard to pregnant women who should carefully evaluate the risk of working with or near these agents. Special hazards and exceptions (e.g., individuals vaccinated against rubella virus) must be determined by the Principal Investigator (P)I who is primarily responsible for establishing the safety of personnel under his/her supervision.

Identification of biohazards

- Infectious agents
  - Fungi of the genus *Candida* (e.g. *C. albicans*). *Candida* species are considered **Risk Group 2** microorganisms.
  - *Escherichia coli* (non-pathogenic) - considered **Risk Group 1**
  - Gut microbiota - considered **Risk Group 1 or 2**
  - *Pseudomonas aeruginosa* - considered **Risk Group 2**
- Cell lines – all considered **Risk Group 2**
  - Caco-2
  - HEK-293
- Human and non-human primate body fluid (blood, saliva, etc.) and tissue samples - considered **Risk Group 2**
- Viral – all considered **Risk Group 2**

- **See Safety Data Sheets in Appendix for**
  - **Symptoms of infections**
  - **Risk assessment (including pathogenicity, route of transmission, agent stability)**
  - **Infectious dose**
  - **Prophylaxis**

- **Concentrations used** typically 10^4 to 10^9 cells per ml are used in experiments and stocks.
- **Origins:** Most *Candida* and bacterial laboratory strains are derived from human isolates. They are either commercially available (e.g. ATCC) or originate from prior research projects of the PI.

Cell lines were purchased from ATCC.

Working in lab XXX at Biosafety Level 2

Biosafety Level 2 builds upon BSL-1. BSL-2 is suitable for work involving agents that pose moderate hazards to personnel and the environment. It differs from BSL-1 in that

1. **laboratory personnel have specific training in handling pathogenic agents and are supervised by scientists competent in handling infectious agents and associated procedures;**
2. **access to the laboratory is restricted when work is being conducted; and**
3. **all procedures in which infectious aerosols or splashes may be created are conducted in the Biosafety Cabinet (BSC) or other physical containment equipment (e.g. centrifuge with rotor lid).**

The following standard and special practices, safety equipment, and facility requirements apply to BSL-2 work in the laboratory:

A. *Standard Microbiological Practices*

1. The laboratory supervisor enforces the institutional policies that control access to the laboratory.
2. Persons **must wash their hands after working** with potentially hazardous materials and **before leaving the laboratory**.
3. **Eating, drinking, smoking, handling contact lenses, applying cosmetics, and storing food for human consumption is not permitted in the laboratory areas**. **Food must be stored outside the laboratory area in cabinets or refrigerators designated and used for this purpose.**
4. **Mouth pipetting is prohibited**; mechanical pipetting devices must be used.
5. **Policies for the safe handling of sharps:**
    a. Careful management of needles and other sharps are of primary importance. Needles must not be bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated by hand before disposal.
    b. Used disposable needles and syringes must be carefully placed in the designated sharps containers for disposal.

c. Non-disposable sharps must be placed in a hard-walled container for transport to room XXX for autoclaving.

d. Broken glassware must not be handled directly. Instead, it must be removed using a brush and dustpan, tongs, or forceps. Plastic ware should be substituted for glassware whenever possible.

6. Perform all procedures to minimize the creation of splashes and/or aerosols.
7. Decontaminate work surfaces after completion of work and after any spill or splash of potentially infectious material with appropriate disinfectant (e.g. 10% bleach).
8. Decontaminate all cultures, stocks, and other potentially infectious materials before disposal by autoclaving or another effective method. Depending on where the decontamination will be performed, the following methods should be used prior to transport:

   a. Materials to be decontaminated outside of the immediate laboratory must be placed in a durable, leak proof container and secured for transport to the autoclave room.

   b. Materials to be removed from the facility for decontamination must be packed in accordance with applicable local, state, and federal regulations.

9. Inform the Principal Investigator immediately if any pest (e.g. insects, mice, etc.) is encountered in the laboratory.
10. All laboratory personnel will receive appropriate training by the Principal Investigator (or a designated representative) regarding their duties, the necessary precautions to prevent exposures, and exposure evaluation procedures. Personnel will receive annual updates or additional training when procedural or policy changes occur.
11. Personal health status may impact an individual's susceptibility to infection, ability to receive immunizations or prophylactic interventions. Therefore, all laboratory personnel and particularly women of child-bearing age will be provided with information regarding immune competence and conditions that may predispose them to infection. Individuals having these conditions are encouraged to self-identify to the institution's healthcare provider for appropriate counseling and guidance.

B. *Special Practices*

1. All persons entering the laboratory must be advised of the potential hazards and meet specific entry/exit requirements, i.e. wear personal protective equipment. Visitors must be accompanied by authorized personnel at all times.
2. If clinical samples of human origin are handled in the laboratory, personnel will be provided medical surveillance and offered Hepatitis B immunizations. When appropriate, a baseline serum sample should be stored.

3. The laboratory supervisor will ensure that laboratory personnel demonstrate proficiency in standard and special microbiological practices before working with BSL-2 agents.
4. Potentially infectious materials must be placed in a durable, leak proof container during collection, handling, processing, storage, or transport within the facility.
5. Laboratory equipment should be routinely decontaminated, as well as, after spills, splashes, or other potential contamination. Equipment must be decontaminated before repair, maintenance, or removal from the laboratory.
6. The following procedures are provided as a guideline to **biohazardous spill cleanup.**

Inside the biological safety cabinet (BSC):

a. Wear laboratory coat, safety glasses and gloves during cleanup.
b. Allow cabinet to run during cleanup.
c. Apply disinfectant and allow a minimum of 20 minutes contact time.
d. Wipe up spillage with disposable disinfectant-soaked cloth or tissue.
e. Wipe the walls, work surface and any equipment in the cabinet with a disinfectant-soaked cloth.
f. Discard contaminated disposable materials in appropriate biohazardous waste container(s) and autoclave before discarding as waste.
g. Place contaminated reusable items in biohazard bags or in autoclavable pans with lids before autoclaving and cleanup.
h. Expose non-autoclavable materials to disinfectant and allow 20 minutes contact time before removing from the biological safety cabinet.
i. Remove protective clothing used during cleanup and place in a biohazard bag for autoclaving. If disposable, treat as medical waste.
j. Run cabinet 10 minutes after cleanup before resuming work or turning cabinet off.

In the laboratory, outside the biological safety cabinet:

a. Clear area of all personnel. Wait for aerosol to settle before entering spill area.
b. Remove any contaminated clothing and place in biohazard bag to be autoclaved.
c. Wear a disposable gown, shoe covers, safety glasses and gloves. In a Biosafety Level 3 (BSL3) facility, respiratory protection may be required.
d. Initiate cleanup with disinfectant as follows:
i. Soak paper towels in disinfectant and place over spill.

ii. Encircle the spill with additional disinfectant being careful to minimize aerosolization during pouring while assuring adequate contact. Start from the periphery and work toward the center.

iii. Decontaminate all items within the spill the area.

iv. Allow 20 minutes contact time to ensure germicidal action of disinfectant before passing items to clean area.

v. Wipe equipment with 1:10 bleach, followed by water, then 70% ethanol or isopropanol.

vi. Place disposable contaminated spill materials in appropriate biohazardous waste container(s) for autoclaving.

vii. Place contaminated reusable items in biohazard bags in autoclavable pans with lids or wrap in newspaper before autoclaving and cleanup.

Inside Centrifuge

a. Clear the immediate area of all personnel. Wait 30 minutes for aerosol to settle before attempting to clean up spill. Keep centrifuge closed.

b. Wear a laboratory coat, safety glasses and gloves during cleanup.

c. Remove rotors and buckets to nearest biological safety cabinet for cleanup.

d. Thoroughly disinfect inside of centrifuge.

e. After thorough disinfection of rotor or rotor cups, remove contaminated debris and place in appropriate biohazardous waste container(s) and autoclave before disposing as infectious waste.

Outside laboratory, during transport

a. Transport biohazardous material in an unbreakable sealed primary container, placed inside a second unbreakable lidded container. Label the outer container with the biohazard symbol if material is Risk Group 2 or higher.

b. Should a spill occur in a public area, do not attempt to clean it up without appropriate PPE.

c. As an interim measure, wear gloves and place paper towels, preferably soaked in disinfectant, directly on spilled materials to prevent spread of contamination. To assure adequate contact, surround the spill with disinfectant, if available, taking care to minimize aerosols.

7. Incidents that may result in exposure to infectious materials must be immediately evaluated and treated according to procedures described in this laboratory biosafety safety manual. All such incidents must be immediately reported to the laboratory supervisor. Medical evaluation, surveillance, and treatment will be provided and appropriate records maintained.

8. Animals and plants not associated with the work being performed are not permitted in the laboratory.
9. All procedures involving the manipulation of infectious materials that may generate an aerosol should be conducted within the BSC or other physical containment devices.

C. *Safety Equipment* (Primary Barriers and Personal Protective Equipment)

1. The BSC, other appropriate personal protective equipment, or other physical containment devices must be used whenever:
   a. Procedures with a potential for creating infectious aerosols or splashes are conducted. These may include pipetting, centrifuging, grinding, blending, shaking, mixing, sonicating, opening containers of infectious materials, inoculating animals intranasally, and harvesting infected tissues from animals or eggs.
   b. High concentrations or large volumes of infectious agents are used.
      i. Such materials may be centrifuged in the open laboratory using sealed
      ii. rotor heads or centrifuge safety cups.
2. Protective laboratory coats or gowns designated for laboratory use must be worn while working with hazardous materials. **Remove protective clothing before leaving for non-laboratory areas** (e.g., cafeteria, library, administrative offices**). Protective clothing must be autoclaved before disposal or laundering. Laboratory clothing should not be taken home.**
3. **Eye and face protection** (goggles, mask, face shield or other splatter guard) is used for anticipated splashes or sprays of infectious or other hazardous materials when the microorganisms must be handled outside the BSC or containment device. Eye and face protection must be disposed of with other contaminated laboratory waste or decontaminated before reuse. Persons who wear contact lenses in laboratories should also wear eye protection.
4. **Gloves must be worn** to protect hands from exposure to hazardous materials. Glove selection should be based on an appropriate risk assessment. Alternatives to latex gloves are available. Gloves must not be worn outside the laboratory. In addition, BSL-2 laboratory workers should:
   a. Change gloves when contaminated, integrity has been compromised, or when otherwise necessary. Wear two pairs of gloves when appropriate.
   b. Remove gloves and wash hands when work with hazardous materials has been completed and before leaving the laboratory.
   c. Do not wash or reuse disposable gloves. Dispose of used gloves with other contaminated laboratory waste. Hand washing protocols must be rigorously followed.

5. Eye, face and respiratory protection should be used in rooms containing infected animals as determined by the risk assessment.

D. *Laboratory Facilities (Secondary Barriers)*

1. Laboratory doors should be closed and locked in accordance with the institutional policies.
2. The laboratory has a sink for hand washing. It is located near the exit door.
3. The laboratory should be designed so that it can be easily cleaned and decontaminated. Carpets and rugs are not permitted in the laboratory.
4. Laboratory furniture must be capable of supporting anticipated loads and uses. Spaces between benches, cabinets, and equipment should be accessible for cleaning.
    a. Bench tops must be impervious to water and resistant to heat, organic solvents, acids, alkalis, and other chemicals.
    b. Chairs used in laboratory work must be covered with a non-porous material that can be easily cleaned and decontaminated with appropriate disinfectant.
5. Laboratory windows should not be opened.
6. Vacuum lines should be protected with High Efficiency Particulate Air (HEPA) filters, or their equivalent. Filters must be replaced as needed. Liquid disinfectant traps may be required.
7. An eyewash station is readily available at the sink nearest to the lab entrance.
8. The Class II BSC must be certified annually and operated according to manufacturer's recommendations.

E. Further Requirements and Procedures

1. **Transport of infectious material outside of the approved space:** Transport of biohazardous material **must be authorized by the Principal Investigator and must comply with OSU, local, state, and federal regulations**. A primary and secondary containment must be used for any transport.
2. **Sending and receiving packages containing infectious material: Sending of biohazardous material must be authorized by the Principal Investigator and must comply with OSU, local, state and federal regulations.** Special primary and secondary containments must be used for any shipment.

    For further information and shipping authorization, send an email

request to chslabsafety@okstate.edu.

Receiving of biohazardous material must be authorized by the Principal Investigator and must comply with OSU, local, state and federal regulations. When packages containing biohazardous material are received, the secondary and primary containers should be disinfected using 70% ethanol or 1:10 bleach. Containers should only be opened in the BSC.

F. Further information:

Biosafety in Microbiological and Biomedical Laboratories (BMBL) 5th Edition: http://www.cdc.gov/biosafety/publications/bmbl5/index.htm

Appendix

Safety Data Sheets

See next pages