Laboratory Biosafety Plan Template

For Biosafety Level 1 and 2

[Your Name]

[Your Title]

Winston-Salem State University

[Building Name, Room Number]

Winston-Salem, NC

[Preparation Date]

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SIGNATURE and ACKNOWLEDGEMENT PAGE

Authorization [*This section is based upon your specific laboratory setup*]

All members of the [*Principal Investigator's*] Lab who have signed the list below are approved for entry into Room [*XXXX*] while work with BSL [*1 or 2*] agents is in progress. Anyone (including any workers not in the P.I.'s Lab) who uses the Room [*XXXX*] lab must sign the disclaimer below.

Disclaimer

We, the undersigned, understand that [*the agents used in Room XXXX*] handled in Room [*XXXX*] of [*Building Name*] are infectious to humans. Further, we have read and understood this manual and agree to attend any Laboratory Safety training conducted by the [*Principal Investigator's*] prior to handling samples in Room [*XXXX*] .

Name	Signature	Date	Agent Vaccination* Yes/No/ Declined/NA?

*Agent vaccination in table should be specified, as appropriate to potential exposure in laboratory: HBV, Vaccinia, Influenza, etc.

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Purpose

This document provides a comprehensive source for all matters covering the use of Biosafety Level 1/2 (BL1/2) contaminants handled in the laboratory [*Address of Principal Investigator*]. Specifically, it describes the procedures to be used to insure a safe working environment while working with regulated recombinant DNA systems, infectious microorganisms, or human cell cultures and human body fluids. This manual will be reviewed annually by the Principal Investigator for changes or corrections to ensure that it is accurate.

[The information in the following two sections is an example of the type of specific description of your operations and activities that you should include in your manual. You should discuss detailed procedures and the actual precautions to be taken by those who actually work with the agents found in your laboratory.]

Background

(Description of work to be performed and agents that are to be used as shown in the following example)

[*Human primary cell cultures. Human primary cell cultures are used to investigate hypertension in mammals. Specifically, selected cell cultures derived from tissues obtained from persons with hypertension are examined for biochemical defects that lead to dysfunction in the regulation of blood pressure…*]

Description of Laboratories

[These two laboratories are characterized for arrangement and location of equipment and facilities in the two diagrams. Both laboratories are located on the [floor] of [*Building Name*] building on the campus of Winston-Salem State University, including the PTCRC. ALL cell culture growth and harvesting occurs in Room [*XXXX*]. Human body fluids to be analyzed for pathogenic viruses are processed and handled in Room [*XXXX*].]

Floor diagrams are displayed as appendices at the end of this manual.

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General Lab Rules

NO eating, drinking, smoking, handling contact lenses, or applying cosmetics in [*Room XXXX* or *Room YYYY*] at any time. Persons who wear contact lenses should also wear goggles or a face shield while working with infectious materials (respirators are available if desired).

Gloves must be worn when working with human primary cell cultures and when isolating pathogenic viruses from human body fluids.

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

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

Wash hands

- after handling biohazardous materials,
- after removing gloves, and
- before leaving the laboratory.

Razor blades, scalpels, and hypodermic needles ("sharps") should be used only when absolutely needed in [*Room XXXX*].

Work surfaces are decontaminated at least once a day and after any spill of viable material [*specify the appropriate disinfectant*].

All cultures, stocks, and other regulated wastes are decontaminated before disposal by an approved decontamination method, such as autoclaving.

Plasticware should be substituted for glassware whenever possible.

Emergency Phone Numbers and Procedures

Emergency Phone Numbers

Fire and Medical Emergencies	750-2911
Police	750-2911
Principal Investigator's Home Phone	[*555-5555*]
Health Services	750-3300
Safety Officer, Angela Richardson	750-8620
Compliance Officer, Vernon Shanks	750-3019

Emergency Procedures

In case of fire, pull the fire alarm and evacuate immediately. Appropriate judgment should be exercised in deciding whether to store or contain any hazardous materials prior to evacuation.

Any injury to a laboratory worker shall be reported immediately to [*the Principal Investigator*], and timely and appropriate action shall be taken to evacuate such employee from the laboratory and to obtain appropriate medical treatment. Administer first aid outside of the lab if the injured person is ambulatory.

If an accident involves a biohazardous spill, move the injured person away from the spill. Inactivate the spill after attending to the injured person.
Do not attempt to move a non-ambulatory person unless it is absolutely necessary.

Remove from the injured person all protective clothing (i.e., lab coat) that may have been contaminated, and rip away the protective clothing only if necessary. Do **NOT** move the injured person to remove the protective clothing. Wash any contaminated skin with disinfectant such as Betadine or Envirocide.

Proper Use of Biological Safety Cabinets ("Laminar Flow Hoods")

To assure sterility inside 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 in the biosafety cabinet.

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.

Wipe inner surfaces (especially the pan) with a solution of [*the disinfectant chosen as effective for the agent you are using*] and allow to dry. Always keep a bottle of disinfectant (e.g., 5% bleach, 70% ethanol, etc.) in the cabinet for decontaminating, or in case of a spill.

NEVER place anything over the front or rear grille of a biosafety cabinet.

Disrupting the air flow 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 your experiments!

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

Remember: **"A biosafety cabinet is only as safe as the person using it."**

Before manipulating infectious materials, try to 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.

Work should be performed on 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.

After manipulating infectious agents, make sure all are in tightly closed containers before removing them. Wipe down the surface of all equipment used in manipulations

(pipettors, etc.) with disinfectant before removing from the cabinet. All waste and disposable items should be left in the cabinet until properly decontaminated or contained.

After the cabinet has been emptied, wipe exposed surfaces including the front grille and splash area with disinfectant. Allow the blower to run for a minimum of ten minutes to purge any aerosols from inside the cabinet before shutting off the blower.

NOTE: Class IIA cabinets re-circulate about 70% of the air inside themselves and exhaust the remainder to the lab. Any use of volatile solvents should be kept to a minimum or done elsewhere. Dangerously high levels of volatile fumes can accumulate inside the cabinet and pose a threat of fire or explosion.

Decontamination Procedures

Infectious agents are to remain in the Biosafety Cabinet until they are properly decontaminated. Infectious material should NEVER be in an open vessel OUTSIDE of the cabinet.

Any items used in conjunction with infectious material must be decontaminated by wiping with either 5% (v/v) diluted bleach or 70% ethanol.

Chemical Sterilization: Whenever possible, materials should be immersed in a solution of bleach (NaOCl) (household bleach diluted 1 part with 19 parts water, or 5% (v/v)) for minimum of fifteen minutes before any further handling. Disposables such as pipette tips, test tubes, petri plates, etc. should be immersed, filled, or rinsed with 5% (v/v) bleach and allowed to stand for fifteen minutes before being thrown away in a Contaminated Material Container. Immersion in 70% ethanol may be an acceptable means of decontamination for items that are incompatible with bleach.

Autoclaving: All solid, contaminated waste should be autoclaved in clear autoclavable bags. Following the appropriate sterilization cycle, the clear bag may be disposed of as regular trash.

Decontamination of liquid: This should be done by adding undiluted bleach, NaOCl, (sold as 6% w/v concentration) to a final concentration of AT LEAST 0.3% w/v NaOCl (or 5% dilution). Also rinse the vessel with bleach provided in the wash bottle in the cabinet. Mix well and allow to stand for fifteen minutes before being poured into the drain. Rinse with copious amounts of cold water. Liquid waste that is not compatible with NaOCl should be autoclaved for at least 30 minutes, using slow exhaust before disposal.

Volatile or organic solvents such as fixatives that are, by their nature, toxic to biological materials need not be chemically decontaminated or autoclaved.

Plasticware and other reusable items: These items are to be chemically decontaminated and rinsed (whenever possible) before removal from Room [*YYYY*].

Note: Avoid autoclaving large quantities of bleach solution. Flush decontaminated liquids down the drain and rinse non-disposable items with water before removal from

Room [*YYYY*]. If it is impractical to rinse items, the NaOCl (bleach) should be neutralized by adding 1 mL of 5% sodium thiosulfate per mL of 5% hypochlorite ion.

Mixtures of Infectious Agents and Radiochemicals

The infectious agent should be neutralized first then the whole solution can be treated as radioactive waste. Care should be taken in choosing a chemical disinfectant. Some can cause more problems that they solve. **(e.g., Chlorine compounds such as bleach should NOT be used to disinfect anything containing ^{125}I because the chlorine will cause the volatilization of radioactive iodine!)**

Spills

Inside of a containment device: Please refer to Appendix G of the Biosafety Process Manual.

Leaking tubes in the centrifuge: If the rotor is sealed, and removable, place it in the biosafety cabinet before opening. Before attempting to deal with the leak, prepare a pan of disinfectant solution large enough to immerse the entire rotor in (iodine based solutions are recommended over Bleach because of corrosion). Don any protective gear needed to clean up spills and carefully remove the lid from the rotor. Retrieve unbroken tubes, wipe outside with disinfectant, and leave them in the cabinet, out of the way. The broken tube should be removed with a forceps or other instrument if possible and immersed in a beaker of disinfectant solution for a minimum of fifteen minutes. All instruments and rotor pieces involved in the incident should be chemically decontaminated before re-use.

After proper decontamination, instruments and rotor pieces may be washed with a mild detergent according to the manufacturer's instructions. As an added measure of caution, the inside of the centrifuge (chamber) may be wiped out with a non-corrosive disinfectant.

If a tube has broken in a centrifuge that does not use a containment type rotor, DO NOT open the centrifuge. Turn off the power and allow sufficient time for aerosols that have been created to settle (approximately 30 min.). Don protective garb and respiratory protection before opening the chamber. Decontaminate the inside of the chamber with a noncorrosive disinfectant (70% Ethanol or Wescodyne) by thoroughly soaking the interior. A spray bottle or lab squeeze bottle is sufficient. Large amounts of liquid generated during decontamination may be removed by a disposable pipette attached to suction device attached to a disinfectant trap. Any paper waste generated during clean-up should be bagged and autoclaved.

Note: It is recommended that a sealed rotor or bucket be used when centrifuging infectious materials. If none is available, placing a **smaller** tube inside a larger sealable tube can provide some protection against aerosol creation in the event of breakage.

Spills of infectious material outside of a containment device: to Please refer to Appendices H and I of the Biosafety Process Manual.

Biological/Radioactive emergencies/spills: These types of spills should be handled similarly to spills of infectious agents. Determine if anyone has been contaminated; remove contaminated clothing and wash contaminated skin with soap and water. The lab should be evacuated, especially if an aerosol was generated. Report spill to Safety Director.

The infectious agent should be neutralized first, taking care when choosing a disinfecting agent to avoid chemical incompatibility. For example, hypochlorite (bleach) will volatize radioactive iodine. Refer to the section of this manual that covers clean-up of spills for more details.

All spills of any nature must be described in your laboratory notebook. The description must include: 1) the type of spill, 2) the time and date it happened, 3) the time and date it was cleaned up, and 4) the time and date you autoclaved the waste from the spill.

All major spills must be reported to [*the Principal Investigator*]. A major spill is one in which: 1) hazardous materials contact skin, eyes, etc., 2) a break in the skin occurs, 3) the spill splashes over an area larger than one foot in diameter, 4) the extent of the spill is undetermined, or 5) the spill involves an aerosol.

Cleaning Procedures

Pans and splash guards (inside and out) should be cleaned after every use.

Important things to wipe daily: 1) door handles and 2) water faucets on non-foot pedal operated sinks.

Pipettors and other shared small equipment should be wiped with 70% ethanol after use, ***before*** removing them from the cabinet.

Specimen Transport

Human specimens and diagnostic samples are frequently transported from clinical collection sites to research facilities on campus. A properly labeled (biosafety sticker with specified agent identity) leak-proof transport carrier is available exclusively for this purpose. [*Examples of acceptable containers include either a Playmate® cooler, or the Nalgene® Bio Transport Carrier*].

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VALIDATION AND HISTORY FOR BIOSAFETY MANUAL

Principal Investigator Certification

I hereby certify that I have reviewed the contents of this manual and that it reflects my current operating policy for the laboratories #[*XXXX*] and [*YYYY*] located in [*Location*] research building.

[*Principal Investigator's Name*]
[*Principal Investigator's Title*]

Signature _____ Annual Review Date _____

Date Created:

APPENDIX A – FLOOR DIAGRAM

FLOOR DIAGRAM FOR [*Building Name*] [Room *XXXX*]