Biosafety Manual

And Standard Operating Procedures

For Biosafety Level 2 Agents

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Charlottesville, VA

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SIGNATURE and ACKNOWLEDGEMENT PAGE

Authorization

All members of the Flow Cytometry Core Facility who have signed the list below are approved for entry into Room 7065/7048 while work with BSL 2 agents is in progress. HOWEVER, only those persons who have attended the OSHA Bloodborne Pathogens lecture and are otherwise specifically trained (e.g., the University Biosafety Officer provides a short training program entitled, "Fundamentals of Biosafety") may perform work with samples or cell cultures in Room 7065/7048.

Anyone (including any workers not in the P.I.'s Lab) who uses the Room 7065/7048 lab must sign the disclaimer below.

Disclaimer

We, the undersigned, understand that some agents handled in Room 7065/7048 of Jordan Hall may be infectious to humans. Further, we have read and understood this manual and agree to attend required Bloodborne Pathogens lectures prior to handling samples in Room 7065/7048.

Name	Signature	Date	Agent Vaccination* Yes/No/ Declined?
Joanne Lannigan			Hep B
Mike Solga			Hep B

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

Purpose

This document provides a comprehensive source for all matters covering the use of Biosafety Level 2 (BL2) contaminants handled in the laboratory Jordan Hall Room **7065/7048**. 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 timely and accurate.

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

As a Core Facility, the type of work performed and agents used will vary with investigators. Types of samples anticipated may be from animal tissues or cell lines, human cell lines and primary human tissues. Agents may involve the use of viruses to genetically engineer cells or samples may contain known infectious agents. The details of the types of samples and agents will be provided with each sample submitted to the facility through the use of a Biosafety Questionnaire for BSL determination.

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 seventh floor of Jordan Hall research building within the University of Virginia's Medical Center complex. No cell culture growth or harvesting occurs in either room. Primary human samples or virally infected samples may be handled in either 7065 or 7048. Samples which will be sorted and potentially generate aerosols will be handled in 7065 **ONLY**.

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

General Lab Rules

1. Work with live human cell cultures will be performed in Jordan Hall, Rooms 7065 and 7048.

2. Specimens of human origin will be collected by individual investigators according to their protocols which have been submitted and reviewed by the IBC. These samples will be transported in a secure secondary container that is clearly labeled and marked with a biohazard symbol. If these samples are to be sorted they will be handled using an Aerosol Containment Hood.

3. NO eating, drinking, smoking, handling contact lenses, or applying cosmetics in Room 7065 or 7048 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).

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

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

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

7. Wash hands after handling biohazardous materials, after removing gloves, and before leaving the laboratory.

8. Razor blades, scalpels, and hypodermic needles ("sharps") should be used only when absolutely needed in Rooms 7065 and 7048.

9. Work surfaces are decontaminated at least once a day and after any spill of viable material with 10% Bleach

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

11. Plasticware should be substituted for glassware whenever possible.

Emergency Phone Numbers and Procedures

A. Emergency Phone Numbers

Fire and Medical Emergencies……924-2012
Police……9 + 911
Principal Investigator's Home Phone……..*540-832-2793*
Employee Health……..924-2013
Hospital Emergency Room……...924-2231
Environmental Health and Safety…….982-4911
David N. Easton, C.I.H., University Biosafety Officer……982-4909

B. Emergency Procedures

In case of fire, push the fire alarm button 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 Joanne Lannigan, 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. Do not attempt to move a non-ambulatory person unless it is absolutely necessary. Inactivate the spill after attending to the injured person.

Remove from the injured person all protective clothing (i.e., labcoat) 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 Aerosol Containment Hood

1. To 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.

2. The air flow meter in the hood should read about 150 FPM with the hood access door open or closed. Speeds between 100-200 FPM are acceptable. The pressure drop reading on the gauge on the Filter/Fan unit should read about 0.4" at the high speed. The low speed reading should be close to zero. When the air flow drops to less than 100 FPM with the door open, or the pressure drop across the filter increases 30% above its initial reading, the HEPA and Dacron prefilter should be changed.

3. Wipe inner surfaces, before and after a sort, with a solution of 10% bleach and allow to dry. Particular attention should be paid to the sample station and the sorting chamber. This should be followed by a DH2O spray and wipe. Always keep a bottle of disinfectant (e.g., bleach, 70% ethanol, etc.) in the cabinet for decontaminating, or in case of a spill.

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

5. Before manipulating infectious materials, try to make sure that you have everything you need in the cabinet. The fewer times you open and close the door of the cabinet, the less disruption of the air flow.

6. For maximum side stream stability, the door on the sorting chamber should be closed while sorting. This provides sufficient airflow into the sort chamber to contain aerosols, but not enough to disturb the side streams.

7. To access the sort chamber, open the aerosol containment hood door and activate the high speed fan switch by locking the door in the activation switch, stop the sort by putting fluidics into standby, wait a few seconds, then open the sorting chamber door.

8. 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.

9. After the cabinet has been emptied, wipe exposed areas 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.

Decontamination Procedures

1. Infectious agents are to remain in the Biosafety Cabinet until they are properly decontaminated or properly prepared for transport from the core facility to the investigator's lab. Infectious material should NEVER be in an open vessel OUTSIDE of the cabinet.

2. Any items used in conjunction with infectious material must be decontaminated by wiping with either 10% (v/v) diluted bleach or 70% ethanol.

3. 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.

4. 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.

5. Decontamination of liquid: Flow Cytometer waste containers should be filled with enough undiluted household bleach to yield a 10% final solution when full. Mix well and allow standing 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.

6. Plasticware and other reusable items: These items are to be chemically decontaminated and rinsed (whenever possible) before removal from Room 7065 and 7048.

Mixtures of Infectious Agents and Radiochemicals

NA – No radioactive materials will be permitted in Rooms 7065 and 7048

Cleaning Procedures

1. Presort and postsort sample holders (inside and out) should be cleaned after every use.

2. Important things to wipe daily: 1) door handles and 2) water faucets on non-foot pedal operated sinks 3) benchtops, and 4) instrument sample lines.

3. 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 within the Health Sciences Center. 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 (catalog # 7135)*].

VALIDATION AND HISTORY FOR BIOSAFETY MANUAL

A. Director's Certification

I hereby certify that I have reviewed the contents of this manual and that it reflects my current operating policy for the laboratories #7048 and #7065 located in Jordan Hall research building.

Joanne Lannigan, M.S.
Director, Flow Cytometry Core Facility

Signature _____ Annual Review Date _____

B. History of Manual's Creation

Date Created:

Author:

Reference Source: Theodore E. Mifflin, Ph.D.
Dr. Tom Sturgill's ('94) Biosafety Manual

APPENDIX

FLOOR DIAGRAM FOR Jordan Hall Room 7048

APPENDIX

FLOOR DIAGRAM FOR Jordan Hall Room 7065

APPENDIX
Biosafety Level 2 Application Documentation

APPENDIX

Shipping Biological and Infectious Substances

The interstate transportation of biohazardous substances is regulated by the U.S. Public Health Service (PHS) and the Department of Transportation (DOT). While these regulations apply to interstate transport by highway, rail, vessel, and air, the "Dangerous Goods Regulations" specified by the International Air Transport Association (IATA) are the guidelines followed by all Domestic and International air carriers. Knowledge and compliance with the IATA regulations ensures that those who ship (the consignees) regulated materials meet or exceed all PHS and DOT requirements.

The IATA Dangerous Goods Regulations are complicated and involved. Knowing how to properly classify, declare, pack, label, and handle biohazardous materials is mandatory and appropriate training must be documented. The following information provides the minimum guidance necessary to perform these activities. The Office of Environmental Health and Safety has a proprietary training module on compact disc which must be completed by any University employee whose job duties include routine shipment of biohazardous substances. Individuals who complete the program must print out the confirmation that they passed the final exam. Attaching the confirmation to this manual is acceptable evidence of technical competence.

IATA Definitions - 1999

3.6.2 Division 6.2 – Infectious Substances

3.6.2.1 Definition

Division 6.2 includes substances which are infectious to humans and/or animals and include genetically modified micro-organisms and organisms, biological products, diagnostic specimens and clinical and medical waste, as described in 3.6.2.1 to 3.6.2.5

3.6.2.1 Infectious Substances

Infectious substances are substances know to contain, or reasonably expected to contain, pathogens. Pathogens are micro-organisms (including bacteria, viruses, rickettsia, parasites, fungi) or recombinant micro-organisms (hybrid or mutant) that are known or reasonably expected to cause infectious disease in humans or animals. Infectious substances are not subject to the provisions of these Regulations for Division 6.2 if they are unlikely to cause human or animal disease. Infectious substances are, however, subject to the provisions of these Regulations for Division 6.2 if they are capable of spreading disease when exposure to them occurs.

Note: Toxins from plant, animal or bacterial sources which do not contain any infectious substances or toxins that are contained in substances which are not infectious substance should be considered for classification in Division 6.1 and assignment to UN 3172.

3.6.1.3 Biological Products

These are those products derived from living organisms, that are manufactured and distributed in accordance with the requirements of national governmental authorities which may have special licensing requirements, and are used either for prevention, treatment, or diagnosis of disease in humans or animals, or for development, experimental or investigational purposes related thereto. They include, but are not limited to, finished or unfinished products such as vaccines and diagnostic products.

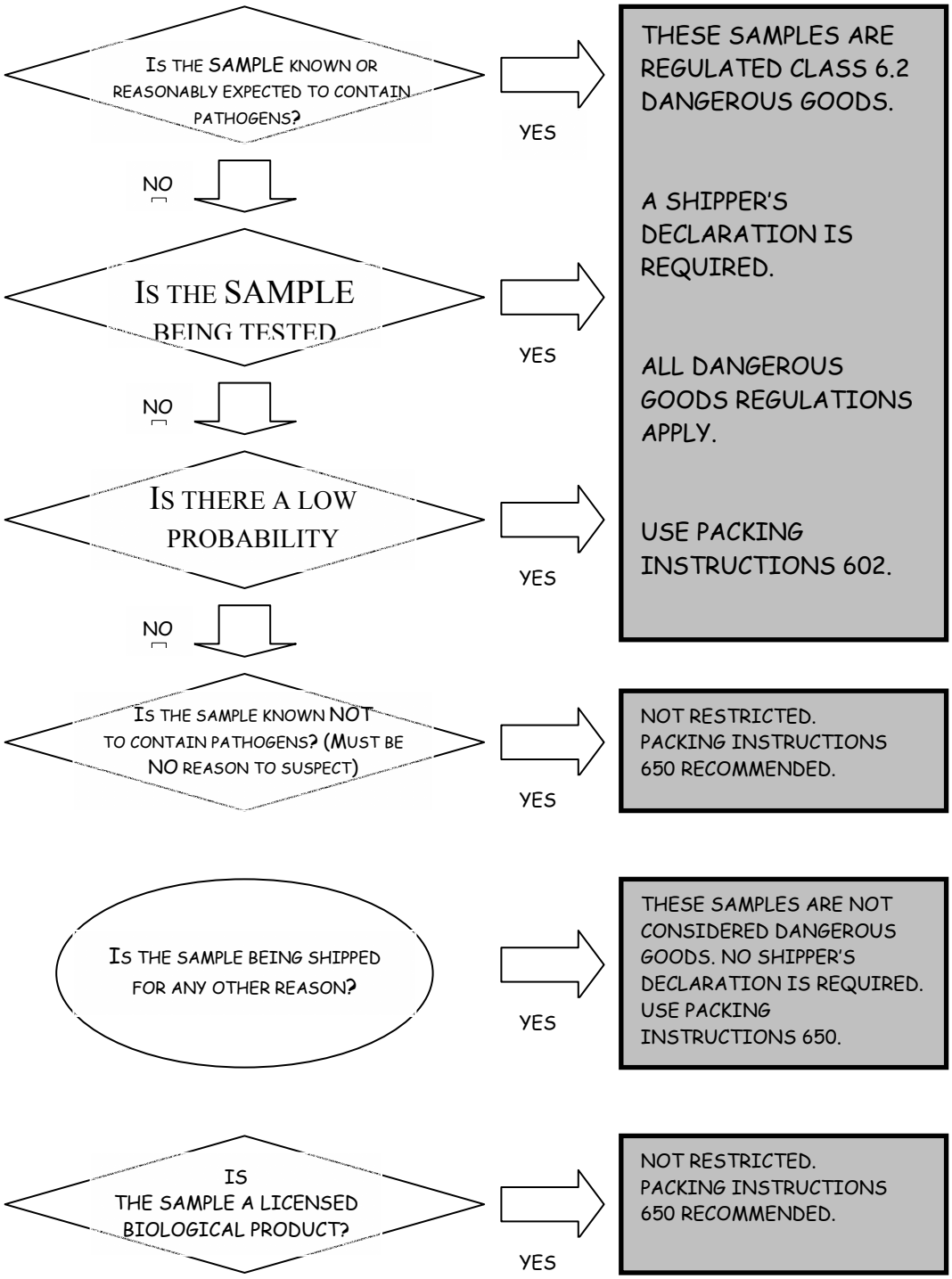
Note: Some licensed biological products may present a biohazard in certain parts of the world only. In that case competent authorities may require these biological products to comply with the requirements for infectious substances or may impose other restrictions.

3.6.1.4 Diagnostic Specimens

Any human or animal material including, but not limited to, excreta, secreta, blood and its components, tissue and tissue fluids, being transported for diagnostic or investigational purposes, but excluding live infected animals.

Dangerous Goods Classification Flow Chart

Infectious Substances - Diagnostic Specimens - Biological Products



COURTESY OF SAF-T-PAK, INC.

Packing Instructions 602
(Infectious Substance)

Packaging

1. Watertight primary
2. Watertight secondary
3. Absorbent material
4. Specimens divided & separated
5. Outer packaging must meet stringent tests
   UN specification mark
6. 4 inch minimum dimension
7. Itemized list
8. Primary or secondary capable of 95 kPa pressure differential (14 psi)
9. Name & phone # of responsible person
10. Prior arrangements with consignee required

Marking the Package

1. Proper shipping name
2. Technical name
3. UN ID #
4. Full address of shipper and consignee
5. Name and phone # of shipper and consignee

Labeling the Package

1. Use US label
2. Must be diamond on point, standard label
3. Only one label of each type

Shipper's Declaration

1. 3 copies
2. Keep on file at least 1 year
3. Carrier keeps one, consignee keeps one

Packing Instructions 650
(Diagnostic Specimen)

Packaging

1. Leakproof primary
2. Watertight secondary
3. Absorbent material
4. Specimens divided & separated
5. Outer packaging capable of passing 1.2 meter drop test
6. 4 inch minimum dimension
7. Itemized list
8. Primary or secondary capable of 95 kPa pressure differential (14 psi)
9. "Packed in Compliance with IATA Packing Instructions 650"

Marking the Package

Each package and the "nature & quantity of dangerous goods" box of the air waybill must show the text "BIOLOGICAL PRODUCTS" or "DIAGNOSTIC SPECIMENS" – PACKED IN COMPLIANCE WITH IATA PACKING INSTRUCTION 650.

Labeling the Package

If no other dangerous goods contained within, no labels are necessary.

Shipper's Declaration

Not required

APPENDIX
MISCELLANEOUS NOTES AND DOCUMENTS