THE BIOSAFETY LEVEL 2 FACILITY SAFETY MANUAL

Wesleyan University

The Department of Environmental Health, Safety and Sustainability

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FOREWORD

This manual is designed to provide general operating and emergency procedures for working safely in Wesleyan University’s Biosafety Level 2 (BSL-2) Lab (henceforth The Lab). This manual also defines the duties and responsibilities of all individuals using or operating The Lab.

The Lab, located on the first floor of Hall-Atwater Laboratories, is designed to meet federal requirements for a BSL-2 containment laboratory. Containment of biohazards is achieved through the use of primary control equipment, facility design, and laboratory procedures. Not all laboratories in Hall-Atwater are classified as BSL-2. Laboratories or animal rooms considered BSL-2 will be clearly marked at their entrance.

Biohazard containment in this facility will function properly if operational procedures are followed. The operational procedures described in this manual have been designed to provide a high degree of protection to our workers and to the community from potentially infectious materials that may be used in this facility. We would like to emphasize that even the best containment facility and equipment is rendered ineffectual with improper laboratory procedures. Persons working in the biohazard facility must be trained and proficient in microbial practices and techniques before handling infectious or potentially infectious materials. The Principal Investigator is directly responsible for ensuring that each employee has received appropriate training and experience before beginning work in The Lab.

The operating procedures detailed in this manual shall apply to all staff, support personnel, and to any authorized visitors to the facility. It is essential that all personnel entering any BSL-2 laboratory read and comply with this manual.
I. ROLES AND RESPONSIBILITIES:

INSTITUTIONAL BIOSAFETY/CHEMICAL HYGIENE COMMITTEE

The Institutional Biosafety Committee (IBC) approves or rejects all proposals and research conducted within the biohazard facility (BHF) and also designates the biosafety level required. It is also responsible for: reviewing activities which raise health and safety issues, reviewing the activities of the Environmental Health, Safety and Sustainability Department (EHS&S) as it pertains to infectious agents, assess containment levels, establishes a medical surveillance plan for all appropriate personnel and reviews any changes, challenges or grievances concerning research within the facility.

BIOSAFETY OFFICER

The Biosafety Officer (BSO) is the Director of EHS&S. The BSO has the authority to: determine if an employee is unable to work within the facility, deactivate any malfunctioning containment equipment, and insure compliance with governmental health and safety regulations. The BSO is responsible for coordinating meetings of the IBC, provide technical guidance and training materials to personnel regarding laboratory safety, revise day-to-day procedures as experience dictates, insure that workers follow procedures and practices, advise Principal Investigators as to the proper functioning of their workers, initiate and supervise any needed emergency response, investigate and report to the IBC and Principal Investigator, any significant violations within the facility, accompany authorized visitors or maintenance workers around and into The Lab.

PRINCIPAL INVESTIGATOR

The Principal Investigator (PI) is immediately responsible to insure that the purpose of this manual and all other applicable guidelines are fulfilled. They should verify that all staff members conducting research within The Lab are properly trained, have read the BSL-2 manual, and follow the specific protocols and policies related to the containment laboratories. The PI and their specific research projects must inform the laboratory staff of any potential hazards associated with their work including, biological, chemical, and radioactive hazards. The PI is responsible for investigating and reporting to the BSO, in writing, any accidents or incidents involving their staff in The Lab. The PI must also notify the BSO and the IBC of new employees who will be working in The Lab. All new employees must meet with the BSO before they are assigned to work in The Lab.

INDIVIDUAL LABORATORY WORKER

All personnel assigned to The Lab must have an exam by the Davison Health Center Medical Director (or designee), shall read and comply with the procedures of this manual and meet with the BSO before starting work. In addition they should be clearly instructed by their PI as to the procedures they must follow while performing research in The Lab. The laboratory worker is responsible for properly labeling all biological, chemical and radioactive materials within the facility. Any unsafe act or malfunctioning equipment should be brought to the immediate
attention of the BSO and the PI. Employees should report to their PI and the BSO any
instances which constitutes an exposure to biological, chemical or radioactive materials.
 Individuals who are pregnant or immunocompromised should seek medical advice before
working in a BSL-2 laboratory.

AUTHORIZED USER

An authorized user of The Lab is an individual who has sufficient training and experience to
work safely in this facility. The authorized user is selected by the Principal Investigator
assigned to The Lab and is approved or authorized to work in the area by the BSO and the IBC.
This authorization can be rescinded. Periodically, the Principal Investigator will provide a list
of all authorized users permitted in The Lab.

INSTITUTION

The Institution assumes the responsibility for insuring compliance with all guidelines within
this manual and other applicable guidelines including:


CDC Guidelines for protection against Viral Hepatitis and Hepatitis B prevention.

CDC/NIH: Primary Containment for Biohazards: Selection, Installation and Use of Biological

NIH Guidelines on recombinant DNA Molecules, October 1997.


CDC guidelines to prevent T.B. exposure.
II. PHYSICAL CONTAINMENT FEATURES AND ZONE CLASSIFICATION:

The term "containment" is used in describing safe methods for managing infectious agents in the laboratory environment where they are being handled or maintained. The purpose of containment is to reduce or eliminate exposure potential or risk for laboratory personnel and others, and to prevent escape of potentially infectious agents to the outside environment.

Primary containment, the protection of personnel and the immediate laboratory environment from exposure to infectious agents, is provided by good microbiological technique and the use of appropriate safety equipment. Secondary containment, the protection of the environment external to the laboratory from exposure to infectious materials, is provided by the combination of facility design and operational practices.

The biological safety cabinets (BSC) are among the most effective, as well as the most commonly used, primary containment devices in laboratories working with infectious agents. These cabinets when used in conjunction with good microbiological techniques provide an effective containment system for safe manipulation of moderate and some high-risk microorganisms.

The type of laminar flow biological safety cabinets utilized in The Lab is the Class II type B cabinets. This BSC is designed for work with low to moderate risk agents. The design features of this hood include an air barrier along the work opening to prevent the escape of biological agents into the laboratory, High Efficiency Particulate Air (HEPA)-filtered supply air across the work surface, and HEPA-filtered exhaust air.

The air-handling system in the facility is balanced such that the facility is negative to the rest of the building, the laboratories are negative with respect to the corridor, and the biological safety cabinets are negative with respect to the laboratories. Thus, the direction of the air flow is always toward the area of increasing hazard. Exhaust air from the biological safety cabinet is HEPA-filtered before being released to the environment.

Personnel access and egress for The Lab is through the door located at the north end of the room. This access door may also be used for the introduction of materials and equipment into The Lab.

The facility is divided into two zones, potentially contaminated zone and non-contaminated zone. Potentially contaminated zone is the primary containment zone, which includes the class II laminar flow biological safety cabinets. All work requiring BSL-2 containment must be conducted within these cabinets.

The open laboratory consists of the space exterior to the biological safety cabinet which is also considered potentially contaminated and constitutes the secondary containment zone. All work conducted in this space shall be in accordance with BSL-2 requirements. Another potentially contaminated zone also includes: the interior of the facility beyond access door, the ventilation system up to and including the HEPA filters and the sewage system.
Hall-Atwater room 171 acts as a transition zone between the potentially contaminated and non-contaminated zones. The non-contaminated zone is associated with the area "external" to The Lab. Entry into The Lab is restricted to authorized personnel.
III. FACILITY ASSIGNMENT PROCEDURES:

Any investigator desiring use of the facility shall submit a written research proposal to the IBC and the BSO. This proposal must have the signature of the individual’s departmental chairman. Space in The Lab is allocated on a temporary basis and only to those persons who have completed authorized user certification. This certification includes, but is not limited to, an orientation to The Lab and a review of procedures while working in the facility. The IBC will approve use of biohazard materials within The Lab. Biohazard material may not be used for any purpose, or in any other location, other than that originally approved by the IBC. Should a new project be initiated, a new written proposal should be submitted to the IBC and BSO. Personnel changes are to be reported to the BSO as they occur. Once a year the BSO will generate a list of certified personnel and request that the PI verify that it is current.

Upon completion of a project, the investigator is required to decontaminate and remove all materials and equipment in accordance with established procedures. The BSO will ensure that all materials and equipment have been decontaminated prior to removal from the BHF laboratories.
IV. MEDICAL CONSIDERATIONS:

A. PRE-PLACEMENT

All persons working in The Lab have the option of participating in a pre-placement/annual serum collection program. Please discuss this option with the Principal Investigator or the Biosafety Officer.

B. MEDICAL RESTRICTIONS

Pregnant women, person’s on steroid therapy, or immunosuppressive drugs shall not work in the facility prior to a thorough evaluation of the risks involved. The decision to allow these persons to work in the facility is to be made by the University’s Medical Director and the responsible PI with notification to the BSO.

Persons with a fresh or healing laceration or skin lesions should not work with infectious material unless the injury is completely protected. Personnel with injuries of this type must notify their PI and the BSO prior to working in the facility.

C. REPORTING

Emergency telephone numbers must be posted near entrances and at the telephones in every laboratory. Telephone numbers must include the PI, lab contact person, EHS&S and an emergency number.

Any illnesses or symptoms known to be associated with organisms, chemicals being used, or any change in medical condition should be immediately reported to the PI and BSO.

An incident report is to be completed by the investigator and forwarded to the Biosafety Officer as soon as possible after the occurrence of the following:

- Physical injury - e.g., cut, burn, broken bone, slip/fall, other.
- Hazardous materials exposure - contamination through parenteral exposure to a biohazardous material, exposure to a hazardous aerosol, ingestion of a contaminant, or exposure to a carcinogenic compound by inhalation, ingestion, or skin absorption.
- Any spill involving a hazardous, chemical, biological, or radioactive material.

D. MEDICAL PROTECTION

Cholera is an acute bacterial disease caused by an enterotoxin produced by Vibrio cholerae. Two serogroups exist: 01 (classical and El Tor strains) and 0139 (Bengal strains). Humans are the only known natural host. Cholera is caused by consumption of the organism, typically in contaminated water or food. The disease can range from mild, asymptomatic cases to severe, highly symptomatic cases characterized by profuse, dehydrating diarrhea with significant loss
of electrolytes. Under normal circumstances, laboratory workers who follow proscribed precautions are at virtually no risk of infection. Cholera is treatable with oral or parenteral rehydration and, in severe cases, antibiotics.

The BSO shall provide to all authorized users of The Lab, current information on the availability of any medical protection (i.e., vaccines) appropriate for the infectious agents used in the facility. This protection shall be offered to all "at-risk" personnel.

E. MEDICAL FOLLOW UP

In the event of an accidental exposure to Cholera, perform immediate first aid and promptly report to Davison Health Center. The University’s Medical Director will determine and establish the appropriate medical interventions necessary to treat the exposure.
V. GENERAL LABORATORY SAFETY:

A. EQUIPMENT

Equipment to be utilized in the facility must meet building electrical safety standards, which refer to local and national codes. It is critical that equipment to be selected which:

- Does not contribute to spread of biohazards.
- Does not present a hazard to personnel or facility during operation.

It is essential that all equipment be properly maintained. If equipment malfunctions it should not be used and the BSO should be informed. Any equipment exiting The Lab must be decontaminated under the supervision of the BSO. Responsibility for cost, decontamination, or repair of equipment in individual laboratories falls to the Principal Investigators.

B. PHYSICAL BARRIER SYSTEMS

The facility is equipped with Class II Biological Safety Cabinets which is designed to provide protection for personnel as well as materials within the cabinets.

The facility and its installed equipment are designed to provide physical barriers for personnel against exposure to biological hazards. The ventilation system is designed and adjusted to provide directional flow toward the area of increasing hazard. The general ventilation systems are HEPA-filtered to entrap infectious agents and to prevent dispersion outside of The Lab.

C. BIOLOGICAL AGENTS

All infectious and/or biologically hazardous agents being used in The Lab must have approval of the IBC and be registered with the BSO. A hazard warning sign incorporating the universal biohazard symbol should be posted on all the access doors into the laboratories. The hazard warning sign should identify the infectious agent, list the name and telephone number of the PI or other contact person, and indicate the special requirements for entering the laboratory. In addition, each laboratory will be given a BSL-2 designation on the entrance door. Transportation of infectious materials within the facility must be in a secured leak-proof, unbreakable container with an appropriate label. An absorbent material should be added to the container in case of breakage.

Procedures for handling and disposal of these agents are outlined below.

D. CHEMICALS

The handling of chemicals in the facility requires the exercise of proper controls in terms of the class of chemical and any associated hazard. As a general practice, the quantities of chemicals
introduced into the facility should be kept to the minimum required for immediate operations. Excess storage of hazardous chemicals in The Lab should be avoided.

The disposal of acids, alkalis, and organic solvents into the sewer system is prohibited. Chemical waste should be containerized, surface decontaminated and appropriately labeled. The BSO should be contacted for proper disposal procedures.

Waste materials, (i.e., gloves, absorbent pads, culture flasks, etc.) shall be placed in an appropriate container and either autoclaved or bleached depending on the agent used. Liquid, infectious wastes shall be placed in a sealed container with a disinfectant, appropriately labeled, and surface decontaminated. Notify the BSO for proper disposal.

E. RADIOISOTOPES

The introduction and the use of radioisotopes in the facility must be in accordance with regulations established by the Radiation Safety Officer (RSO). The Principal Investigator must be authorized to use the radioisotope with approved handling, safety, and waste disposal procedures. Every attempt should be made to minimize the generation of mixed hazardous waste (infectious and radioactive) unless there is a clear, easy, and safe way to inactivate one of the hazardous components. Under no circumstance should mixed radioactive and infectious waste be transferred to the RSO without prior neutralization of the biological agent.


F. ACCIDENTS AND INJURIES

The specific procedures regarding management and reporting of accidents or injuries shall be in accordance with Wesleyan University rules and regulations. The PI should ensure that all personnel associated with the program are cognizant of these procedures. Any questions should be directed to the BSO.

G. FIRE

The specific procedures regarding management and reporting of fire shall be in accordance with Wesleyan University rules and regulations. The PI shall ensure that all personnel assigned to the facility are cognizant of these procedures.
VI. SAFETY PROCEDURES:

The BSL2 facility is specifically designed to conduct work involving biological material of potential hazard. All personnel must be advised of the potential hazards and instructed in the operational procedures of the facility and specific laboratory. Only persons authorized on the basis of program or support needs shall enter the facility. All personnel admitted into the facility shall read, understand, and follow the procedures detailed in the manual. Any questions regarding safety procedures in The Lab must be addressed to the Principal Investigator or Biosafety Officer.

PERSONNEL PRACTICES

At the start, build safety into your procedures to minimize the likelihood of an adverse outcome.

Working in a relaxed, un-anxious, and non-hurried manner may help you to anticipate and avoid problems.

A lab coat shall be worn at all times in The Lab. The BSO may require a disposable Tyvek® suit and other protective equipment to be worn at various other times within The Lab.

Personal clothing (winter coats etc.) must not enter the BSL-2 laboratories. They must be stored in lockers (if available) at student desks or hung on hooks located outside The Lab.

Disposable gloves shall be worn in accordance with OSHA, CDC and NRC guidelines in regard to the specific agent in use, shall be provided by the PI. The gloves could be taped to the cuff of the protective clothing to prevent any skin exposure. Powder-less latex gloves are recommended for use when handling infectious material.

Double gloving is recommended before penetrating the biosafety cabinet and removed on the way out of the cabinet.

Special care shall be taken to avoid skin contamination with infectious materials and this could be a major route of personal exposure.

Hands should be washed frequently during the day using the germicidal soap provided in each laboratory. Ordinary soap and water is also effective in cleansing the hands.

It is mandatory to wash hands:

1. After handling infectious materials.

2. After a spill and appropriate clean up.
3. When removing protective gloves.

4. When exiting the laboratory.

5. When exiting the facility.

Respiratory protection may be recommended when there is the possibility of hazardous aerosol generating procedures or as deemed necessary by the BSO. If you must wear a respirator, then you must be part of the Respiratory Protection Program which includes a medical evaluation and a fit test.

Head coverings may be required, particularly, with long hair. Long hair should be covered or pinned up.

Contact lenses do not provide eye protection. It is strongly recommended that contact lenses not be worn while conducting work in the laboratory. Safety goggles with side shield, goggles, or a plastic face shield should be worn for eye protection.

Smoking, food, beverages, and cosmetics are not permitted in The Lab.

**MOUTH PIPETTING UNDER ANY CIRCUMSTANCES IS PROHIBITED!**

Disposable cleaning tissue should be used rather than personal handkerchiefs.

Personnel must notify the PI and the BSO of any event or incident which may compromise the safety of personnel or work.
VII. EMERGENCY TELEPHONE NUMBERS

EMERGENCY (All Hours) Dial Xt 3333

Includes:

- Fire
- Biohazard Spill
- Chemical Spill
- Radioisotope Spill
- Medical Emergency

State the nature of the emergency and the location. If possible, remain nearby to direct the emergency response team.

Biosafety Officer Xt2771

Health and Safety Office Xt2771

Radiation Safety Office Xt2771

Work Order Xt3400

Public Safety Emergency Xt3333

ALWAYS NOTIFY PUBLIC SAFETY IN AN EMERGENCY OR ACCIDENT!!!
VIII. EMERGENCY PROCEDURES

INJURIES AND ILLNESSES

Serious Injury or Sudden Illness:

- Dial the emergency number 3333, when special first aid, resuscitation, transport, or rescue service is required. Clearly describe the situation and your location.
- Place all contaminated materials in either a biological safety cabinet or appropriate containment so that medical help can enter the facility.
- Notify the PI and BSO.

Minor Injuries:

- Report all incidents to the PI and BSO.
- A first aid kit is located on the wall in the access corridor near the eyewash station. The use of the first aid kit does not preclude a visit to Davison Health Center.
- Obtain an "Accident/Incident Report Form" from the PI, and report all injuries to Davison Health Center for treatment and the BSO for review.

Emergencies include, but are not limited to, a biohazardous or hazardous chemical spill, fire, BSC malfunction, or a total power failure. The primary objective in an emergency is preservation of personal safety and health. Protecting the facility and the experiment are secondary to personal safety. If there is a hazardous spill in your work area contact the Health & Safety Office (2771) immediately, isolate the spill and leave the area.

BASIC PRINCIPLES

Immediate personal safety overrides maintenance of containment. Evacuation takes priority. Get people out of the emergency area. If possible biohazardous materials should be covered and contained. All equipment should be turned off. The BSO must be informed as soon as possible and will take charge of re-entry, clean-up, and other corrective measures.

The Principal Investigator or BSO is responsible for deciding whether to override containment procedures in case of serious injury or sudden illness.

It is essential that the authorized users of The Lab familiarize themselves with the procedures detailed here. Questions about these procedures should be directed to the PI and the BSO. Personnel should be aware of all exits, fire extinguishers, fire alarms, eyewash stations, safety showers, spill and first aid kits. KNOW WHAT TO DO BEFORE AN EMERGENCY OCCURS.
IX. BIOHAZARD SPILL OUTSIDE A BIOLOGICAL SAFETY CABINET (BSC)

If Biohazardous material is spilled in the open laboratory, one must avoid inhaling any airborne infectious material and getting the infectious agent onto your body and clothing. A "spill kit" is available in The Lab. You should always have available, a freshly prepared solution of a disinfectant in the event of a sudden spill. The BSO should be notified once the contaminated laboratory has been evacuated. Others in the area are to be warned against entry.

Immediate Spill Control:

- Evacuate all personnel and close the door.
- Remove contaminated clothing carefully, folding the contaminated area inward. Place clothing in a bag or directly into the autoclave. Thoroughly wash hands and face and any exposed area of the body. Shower, if necessary.
- Notify the BSO and PI.

POST SIGNS WARNING OTHERS NOT TO ENTER CONTAMINATED AREA. NO ONE SHOULD ENTER THE ROOM PRIOR TO EMERGENCY RESPONDER’S ARRIVAL. Time should be taken to formulate a plan to decontaminate. Once all personnel have been removed from the area, there is no need to rush into the contaminated area.

Assist the BSO as necessary. Decontamination will involve treatment of gross contamination by local application of disinfectant and possible gaseous decontamination of the entire working space.

Decontamination of a Spill:

- Dress in protective clothing, including a Tyvek® suit and double gloves. Respiratory protection is strongly recommended and care should be taken during decontamination not to disperse droplets.
- Place paper towels along the outside of the spill, working from the edges in. Pour the germicidal solution (10% solution of sodium hypochlorite (household bleach) or Vesphene II se®) around the spill and allow to flow into the spill. To prevent aerosols, avoid pouring the germicidal solution directly onto the spill. Try covering the spill with an absorbent pad and apply the decontaminant to the absorbent pad.
- Allow to stand for 30 minutes, this will provide enough contact time for adequate disinfection.
- Carefully remove the soaked pads, placing them into an autoclave bag. Working toward the center of the spill, use paper towels to wipe up the spill. Discard paper towels as they are used into an autoclave bag.
- Using paper towels soaked in disinfectant, wipe beyond the area of visible or suspected splashing, including the floor and vertical surfaces. Discard paper towels in the autoclave bag.
- Decontamination is complete when the whole area of suspected liquid contamination has been washed with a disinfectant and all excess decontaminate has been mopped up.
➢ Place all contaminated materials including gloves, shoecovers, and other protective clothing into an autoclavable bag. Sterilize and dispose of this waste in the red bag system as medical waste.

When the above procedure is followed, the spill area is considered to be decontaminated. The BSO and/or the PI will determine whether the entire laboratory area requires gaseous decontamination.
X. BIOHAZARD SPILL INSIDE A BIOLOGICAL SAFETY CABINET (BSC)

A spill that is confined to the interior of the BSC should present minimal or no risk to personnel in the area. However, chemical disinfectant procedures should be initiated at once while the cabinet ventilation system continues to operate to prevent escape of contaminant from the cabinet.

Spray or wipe, wall, work surfaces and equipment with a disinfectant. A 10% solution of sodium hypochlorite (household bleach) or Vesphene II se® type product is recommended. The operator must be properly gloved and gowned during this procedure. Household bleach can penetrate latex gloves and can be corrosive to metal so consider having an alternative available.

Flood the work surface of the BSC with sufficient disinfectant solution to ensure that the drain pans and catch basins below the work surface contain the disinfectant. Allow the disinfectant to work for 30 minutes before it is cleaned up.

Make sure to wipe all surfaces including the front intake grill. Drain the disinfectant into a container.

Repeat above process with distilled water or mild soap and water.

If the disinfectant is suitable for autoclaving place it, the gloves, wiping cloth and sponges into an autoclave bag; the material should be autoclaved and discarded in the red bag system as medical waste.

If not disinfectant is not compatible with autoclaving (like bleach) place all materials in a red biohazard bag and dispose of as biological waste.

This process will not disinfect the filters, blower, air ducts, or other interior parts of the cabinet. The BSO should be consulted to determine if gaseous decontamination of these items is necessary.
XI. BIOLOGICAL SAFETY CABINET MALFUNCTION

A failure of a BSC exhausts system is indicated by a red warning light (insufficient flow) and an alarm. When the alarm sounds, laboratory workers should follow these procedures:

- Terminate work.
- Cover and contain all vessels containing infectious agents and contaminated equipment. Turn off all electrical equipment and services, i.e., gas and vacuum.
- Notify others in laboratory and leave the room.
- Post signs warning others of the malfunction.
- Call the Biosafety Officer Xt 2771 or 860-982-1096.
- Remain available to provide assistance to the Biosafety Officer and support personnel.
XII. ROTOR FAILURE

If the centrifuge bowl is intact:

- Turn speed control to 0 RPM while letting the vacuum pump continue to run to remove any aerosol generated.
- Leave the room and warn others not to enter.
- Notify the BSO in order to initiate decontamination procedures.

Because of the difficulty of safely and easily introducing a disinfectant into the centrifuge chamber, paraformaldehyde decontamination of the entire unit may be required. The BSO will make necessary decontamination arrangements.

The gaseous decontamination will inactivate aerosolized particles and small droplets; however, grossly contaminated areas must still be treated as a biohazards spill. The rotor or rotor fragments will have to be disinfected with a 10% solution of sodium hypochlorite (household bleach) or Vesphene II se® type product.

The vacuum line and pump will also be contaminated. Decontamination may require partial dismantling of the centrifuge and pump. The appropriate centrifuge service-person will be consulted for an acceptable procedure.

If the centrifuge bowl is ruptured:

This is the equivalent of a biohazard spill in the open laboratory. Significant amounts of aerosols will be generated. Personnel should leave the room immediately and warn others not to enter. Contaminated equipment will require treatment with paraformaldehyde.

The BSO will determine if the entire room requires gaseous decontamination. Until decontamination procedures are completed, no one is to enter the room without proper protective clothing and a full face, HEPA filter respirator.
XIV. CHEMICAL EMERGENCY MANAGEMENT

If the chemical spill presents an immediate danger, evacuate all personnel and close the door.

If spilled material is flammable, turn off ignition and heat sources.

Attend to any person who may have been contaminated. Remove contaminated clothing carefully, folding the contaminated area inward. Place contaminated clothing in an autoclave bag.

Thoroughly wash hands and face and any exposed area of the body. Shower, if necessary, emergency shower/eyewash station is located in the corridor of the facility and an eyewash is available in Room 171.

Notify the BSO.

POST SIGNS WARNING OTHERS NOT TO ENTER CONTAMINATED AREA.

If an airborne infectious agent is involved, make sure a HEPA filtered respirator is being worn.

If contamination is aerosolized, leave room and wait one hour to allow materials to settle before commencing clean up.

Do not touch the spill without suitable protective clothing.

Never assume gases or vapors do not exist or are harmless because of lack of smell. Review the Material Safety Data Sheet for the chemical to determine its hazard.

**Minor Spills**

1. Inform all personnel about the spill, evacuate if necessary.
2. Attend to any person who may have been contaminated.
3. Notify the BSO.
4. If spilled material is flammable, turn off ignition and heat sources.

A chemical "spill kit" is located in each laboratory and contains equipment for acid, caustic, and organic spills, along with an instruction booklet.

Please read the instruction booklet and be familiar with the kit before a spill occurs.

In the event that a worker is contaminated by a chemical, an emergency shower/eyewash station is located in the corridor of the facility.
XV. RADIOACTIVE/BIOLOGICAL EMERGENCY IN THE BIOHAZARD FACILITY

Personal Contamination

Stop work and notify supervisor, BSO, and/or EHS&S Department.

If an airborne infectious agent is involved, wear a HEPA respirator during clean up.

If clothing or garment is contaminated remove and place in plastic bag for later handling.

If body is contaminated wash affected area for at least 15 minutes with mild soap and water and survey with a radiation meter. Use an appropriate disinfectant during cleaning, if necessary.

Continue to wash until radiation levels are at background levels.

Call the Radiation Safety Officer (RSO) at x2771 for additional guidance regarding disposal of contaminated materials.

Contact RSO for guidance regarding autoclaving radioactive waste.

Area Contamination

Stop work, evacuate the area and notify the supervisor and/or Environmental Health and Safety Department.

If an airborne infectious agent is involved make sure a HEPA filtered respirator is being worn.

If contamination is aerosolized, leave room and wait one hour to allow materials to settle before commencing clean up.

Wear protective clothing (i.e. Tyvek® suit, gloves, respirator, etc.) and evaluate the area with a survey meter, if possible. Some radioactive material cannot be detected by a survey meter, therefore wipe tests should be performed.

Place absorbent pad over spill area and apply a disinfectant such as 10% solution of sodium hypochlorite (household bleach) or Vesphene II se® type product. Do not use too much disinfectant so that the amount of radioactive waste is limited.

Allow disinfectant to stand for 30 minutes, this will provide enough contact time for adequate disinfection.
Pick up absorbent pad and place it into a plastic bag. If appropriate, survey area with a meter or take wipe tests to determine if area is free of radioactive contamination. Continue to clean area with soap and water until the area is free of contamination.

Contact the RSO for additional guidance on how to dispose of your contaminated waste. Autoclaving radioactive waste should be avoided, if possible.

Wash hands, check shoes, clothing and hands for contamination with a survey meter after clean up.
XVI. FIRE AND ELECTRICAL FAILURE

Practices to be followed in the event of a fire are as follows:

FIRE INSIDE THE CONTAINMENT FACILITY

Immediately alert other personnel in the facility to the danger.

If possible, without endangering yourself, turn off gas burners and laboratory equipment and leave the facility immediately using stairwells or horizontal exits to Church Street patio after notifying other personnel.

DO NOT USE ANY ELEVATORS

Transmit the alarm. The nearest pull boxes are located outside the facility next to each stairwell. Follow up with a phone call to xt 3333.

If the fire appears containable, (e.g., waste basket or on a bench top), use the fire extinguishers located throughout the facility. These fire extinguishers may be used on any type of fire. Be familiar with the operation of the extinguisher before you have to use it.

TO OPERATE A FIRE EXTINGUISHER

Remove the unit from the wall and carry or drag it to the fire area.

Pull pin.

Aim nozzle at base of the fire.

Squeeze handle.

Sweep nozzle from side to side and gradually progress forward as the flames are extinguished.

Once in a position of reasonable safety, notify the BSO/HAZMAT team concerning any biohazards that have been left exposed. This is the responsibility of all personnel who are in the facility at the time of the fire.

Fire Department or other personnel will wear self-contained breathing apparatus when entrance into the facility is necessary under emergency conditions. Upon resolution of the emergency, the BSO/HAZMAT team shall determine if decontamination procedures are required for Fire Department protective clothing and equipment. Prior to resumption of work in the facility, the BSO shall ensure that all systems of the facility are in proper operation mode.
FIRE IN ANOTHER PART OF THE BUILDING

If an alarm sounds indicating fire in another part of the building, personnel should, if possible, turn off gas burners and laboratory-type equipment.

Infectious materials should be placed in an incubator, refrigerator, or freezer.

Leave the facility, if required, by the most direct route after notifying other personnel. We encourage evacuation if a fire is reported on the floor you on which you are working or the floor below. You must evacuate when the mandatory evacuation alarm sounds.

DO NOT USE ANY ELEVATORS

EMERGENCY EVACUATION

Building evacuation may be necessary in certain emergency situations.

ELECTRICAL FAILURE

In the event of power failure in The Lab, all electrical power will be lost for 10 to 15 seconds until the emergency generator is activated. At this time, only those lights and receptacles on the emergency electrical power supply will be reactivated. Power will be lost to all pieces of equipment not connected to the emergency supply. Once a power failure has occurred, individuals should stop work, decontaminate surfaces, bag or containerize contaminated items, store cultures safely, and in general, "secure" the area and leave. Be sure that all doors are closed when exiting.
XVII. OPERATIONAL PROCEDURES

Only supplies and equipment related to the experiment or studies shall be introduced into the facility.

Supplies, equipment, etc., shall not be removed from the facility unless they have been sterilized or decontaminated under the supervision of the BSO.

In order to maintain the established, negative airflow pattern within the facility, all doors shall be kept closed.

Use of hypodermic needles and syringes should be limited to operations or procedures for which there are no alternatives. All sharps should be disposed of in a sharps container.

Safe transportation of infectious materials within the facility requires the use of a secured, labeled non-breakable secondary container.

All activities involving infectious materials are to be conducted in a BSC. Handling these agents on the open bench is NOT permitted.

**Mouth pipetting is not allowed.** Appropriate pipettes and pipetting aids are to be provided by the investigator.

Work surfaces shall be decontaminated daily and immediately following spills of biohazardous agents with Vesphene II se® type product or a designated disinfectant followed by rinsing with distilled water.

In all procedures, care should be taken to minimize the creation of aerosols. Any aerosol generating procedure must be performed in a BSC.

All flasks, test tubes, etc., in which biological agents are grown or stored shall be appropriately covered to contain potential spills.

To protect the house vacuum system, all vacuum lines within the facility must be fitted with an in-line HEPA filter in addition to a secondary liquid disinfectant trap for biological agents.

Primary suction flasks must contain appropriate liquid disinfectant (i.e., 10% household bleach) before use.

A separate solvent trap is required to capture chemicals; these chemicals are then properly disposed of under the supervision of the BSO.
Sinks within this facility are primarily a water source and hand washing station; they are **NOT** a disposal area. Contaminated liquids are not to be poured into the sinks. All waste including tissue culture media, cultures, buffers, etc., must be autoclaved prior to disposal.

Due to the communal nature of the facility, all materials have the potential of being a hazard and should be treated with appropriate caution.
CENTRIFUGATION - PROCEDURES

All centrifugation must be done in capped centrifuge tubes and bottles within sealed centrifuge rotors. All microcentrifuges are to be used within The Lab. The following procedure should be followed at all times.

Review the owner’s manual.

Log in name, speed, time and rotor in the appropriate log book.

Before centrifuging, inspect tubes, bottles and rotors for rough spots, pitting, discoloration or cracks.

Particular care should be given to insuring a proper seal when using high-speed rotors.

Make sure the proper adapters are in place. Remove and disinfect all adapters at the end of a run.

Fill and decant all centrifuge tubes and bottles within the Biological Safety Cabinet. Wipe the outside of tubes with disinfectant before placing in rotor.

Never overfill centrifuge tubes. Leakage invariably occurs when tubes are filled to capacity. For general purpose centrifuges, the maximum capacity should be 3/4 full. Fill ultracentrifuge tubes per manufacturers’ specifications.

Balance centrifuge tubes and bottles carefully and wipe the pans with disinfectant after use.

Never spin uncapped tubes.

Centrifuge speeds are never to exceed the lower speed rating for the rotor and/or the test tube used.

Rotors and/or safety buckets must be opened in The Lab. Wipe the outside surfaces with disinfectant before removing the rotors from the cabinet.

Wipe the centrifuge chamber with disinfectant before and after each spin.

Decontaminate the rotors and/or buckets after use, by wiping it with Vespheine II se® type product. Rinse thoroughly with distilled water when done, to avoid corrosion.

Be sure and store rotors and accessories in appropriately labeled cabinets and containers.
ENVIRONMENTAL SHAKERS - PROCEDURES

Contents of all vessels must be identified with organism, user, and telephone extension.

All vessels containing living organisms must be capped. These vessels are to be manipulated in such a manner as to prevent wetting of the plugs or caps.

Culture flasks or bottles should be held securely in place, to prevent breakage.

The shaker is to be kept closed when in motion.

BLENDERS, HOMOGENIZERS, GRINDERS AND SONICATORS WHEN WORKING WITH INFECTIOUS AGENTS - PROCEDURES

The equipment listed above creates aerosols when processing materials. When working with hazardous agents operate and open the above equipment in a biological safety cabinet. To reduce the amount of aerosol generated, wait 30 seconds or more after the equipment has been turned off before opening and removing the processed material. Disinfect all equipment when procedures are complete.

XVIII. WASTE DISPOSAL - PROCEDURES

Each authorized user is responsible for the autoclaving of his/her own waste materials and subsequent removal from the autoclave. Scheduling is arranged among users.

General Procedures

Solid waste containers (that do not have sodium hypochlorite solutions) are to be clearly labeled "Infectious" and lined with two autoclave bags. Waste materials are placed in the autoclave. The user must log in each autoclave use. When autoclaving is complete the user is responsible for removing the waste from the autoclave, and discarding it in the red barrels provided. Our typical recommended autoclaving cycle is 1 hour at 250°C.

If the autoclave is in use, or malfunctions, return the waste material to the research laboratory. DO NOT leave the untreated waste on the floor of the corridor, or in the autoclave room. Always check the autoclave to make sure the cycle has started properly. Return promptly when the cycle is complete and remove your waste from the autoclave.

Pipettes and pipette tips are to be placed in containers with sufficient disinfectant, to allow disinfectant contact with the entire pipette or tip. Liquid disinfectants are to be used only for
interim decontamination of items. Under no condition does this constitute a final procedure. Sharps disposal containers must contain sufficient appropriate disinfectant.

**Liquid Waste**

Liquid waste should be mixed with disinfectant (e.g., 10% household bleach) in the biological safety cabinet, whenever possible.

Liquid wastes may be disposed of using methods approved by the Biosafety Officer.

- **Never** autoclave liquids in non-autoclave containers.
- **Never** autoclave household bleach or other chemicals.
- **Never** autoclave radioactive waste.

Consult the Biosafety Officer for disposal techniques concerning hybrid waste material (e.g., radioactive/biohazardous waste).

**Solid Waste**

Media bottles, culture flasks, culture tubes, and any other vessels that may have come in contact with potentially infectious material must be decontaminated in the biological safety cabinet with sufficient disinfectant before being placed in biohazard bags and autoclaved.

Contaminated pipettes may be soaked in sufficient disinfectant for at least 30 minutes before autoclaving.

A disinfectant may be used in sharps containers but note that all sharps containers will be autoclaved before disposal.

**Ordinary Waste**

Limit the amount of ordinary trash (e.g., paper, cardboard), brought into The Lab by removing supplies from their outer packaging prior to stocking the laboratory.

Ordinary trash (paper, wrappers, and cardboard) is placed in the hallway for removal by the Housekeeping Staff. All ordinary trash that may be contaminated shall be autoclaved prior to exiting the facility.
XIX. FACILITY OPERATIONS:

The following basic principles underlie the procedures for movement of personnel and equipment when the containment facility is in use:

Medical Survey must be obtained from the Davison Health Center Medical Director before working in The Lab.

Within The Lab, appropriate protective clothing is always worn.

Protective clothing is to be removed when leaving the containment facility.

Equipment and material is "autoclave out" except for items unsuitable for autoclaving.

Alternative methods of decontamination are determined by the BSO.

ADMISSION TO THE FACILITY

Authorized user form must be completed.

Worker must meet with the BSO.

Persons falling under any medical restrictions must satisfy the appropriate requirements.

PERSONNEL ACCESS AND EGRESS

1. Regulations for work inside The Lab

- Disposable lab wear is to be changed weekly or as needed.
- Shoe covers and head covers should be used when necessary.

When required by the BSO, maintenance personnel are to use disposable Tyvek® suits over street clothes. These suits are placed in a biohazard bag when exiting the facility for appropriate autoclaving and disposal.

2. Personnel Entering

- Store personal items in assigned lockers, if available.
- Non-disposable cloth lab coats, as well as other laboratory attire, must not enter The Lab, lab coats may be hung on hooks, provided in The Lab.
- Proceed to your work area in street clothing.
- Don personal protective equipment which may typically consist of: disposable lab coat or Tyvek® suit, shoe covers, head covers and double latex gloves.
3. Personnel Exiting

- Remove outer pair of gloves when work is completed in biosafety cabinet.
- Remove disposable lab coat or Tyvek® suit, place it in infectious waste container or hang it on a coat hook in the facility laboratory.
- Remove second pair of gloves.
- Wash hands thoroughly before exiting.
- Proceed to the corridor in street clothing and exit facility.

**NO LABORATORY ATTIRE IS TO EXIT THE LAB.**

4. Emergency Exit

In an emergency, personnel in the laboratories may leave via the access corridor without changing clothes, if the situation is significantly dangerous.
XX. ACCESS PROCEDURES FOR ENGINEERING AND OTHER SUPPORT STAFF:

Maintenance including routine servicing, repair, and support services will be administered by the BSO. When the laboratory is in use, the laboratory staff will undertake certain items of routine servicing and support. This procedure will minimize the necessity for others to enter a potentially contaminated area. If repairs or replacements in potentially contaminated area are not within the competence of the laboratory staff, conditions for entry of the maintenance staff and requirements for decontamination will be defined by the BSO.

The BSO must be notified at XT 2771 prior to entry into The Lab.

The BSO will approve all entry into The Lab, persons who comply with all entry and exit procedures will be permitted access to BSL 2 laboratories.

All workers will be accompanied by a BSO and be provided with suitable protective clothing (e.g., disposable lab coat or Tyvek® suit, shoe covers, head covers, gloves, face mask, etc. as needed). The BSO will determine the appropriate personal protective equipment for each job.

If entry is required into a BSL 2 lab, all infectious work will be suspended and the area will be declared safe prior to entry.

Work will be performed under the supervision of the BSO or designee.

Upon exiting the lab, protective clothing will be removed and disposed of.

Hands will be washed thoroughly before exiting the laboratory.

All potentially contaminated tools will be decontaminated by the BSO before exiting the lab.

* Eating, drinking, smoking, handling contact lenses, and applying cosmetics are not permitted in The Lab. Persons who must wear contact lenses in laboratories should also wear goggles or a face shield.

* Persons who have immune dysfunction maybe at risk of acquiring infections. Persons who are at increased risk of acquiring infection or for whom infection may be unusually hazardous are not permitted in The Lab.
XXI. ACCESS PROCEDURES FOR EQUIPMENT, AND MATERIALS:

GENERAL

Procedures are basically the same for laboratory equipment (i.e., instruments, glassware), and materials (i.e., media, cultures), all of which are referred to here as "materials" unless there is a special distinction.

MATERIAL INBOUND

The general rule is that no processing is required for inbound materials. Such materials may be introduced via the pathway used by inbound personnel. Special care should be taken when transporting infectious or hazardous materials into the facility. Overpacking the hazardous material with the addition of an absorbent is recommended when transporting infectious or hazardous materials.

MATERIAL OUTBOUND

From laboratories

All materials exiting from the Biohazard Facility must be either in a closed, plastic autoclave bag, closed buckets or containers. Disposable items and trash can be placed in double autoclave plastic bags.

All materials leaving the laboratories are then taken directly to the autoclave for decontamination. Do not leave materials around the laboratory for clean up the following day.

Laboratory Staff

The laboratory staff is to perform routine laboratory housekeeping, such as autoclaving of lab waste, cleaning of the autoclave chamber, cleaning of laboratory equipment, and other such actions which constitute good laboratory practices.

Support Services

The BSO shall arrange for any necessary support services. These services include service and repair of equipment. Repair of equipment not under service contract will be made by:

- BSL-2 personnel
- EHS&S staff
- Outside repair personnel

All major equipment malfunctions must be reported to the BSO.
Testing and certification of the general air HEPA filtration systems is performed annually. Testing and certification of the Class II Biological Safety Cabinets within the facility, is to be performed semi-annually or whenever a cabinet is removed. All major services and repairs must be coordinated through the BSO. Investigators are responsible for paying the cost of their cabinet certification.
XXII. LABORATORY REGULATIONS:

All safety and operating procedures applied within each laboratory fall under the direct responsibility of the investigator and must be in agreement with the general defined policies of this manual.

Proper recording and control of equipment and safety operations of The Lab are done via a series of sign-up sheets. Records are kept for:

All centrifuges in Room 174 (records are kept on top of equipment).
Registration of outside persons authorized to enter The Lab (kept with the BSO).
Certification and testing reports on airflow, HEPA filters, sterilizers, safety cabinets (kept with the BSO).
Autoclave, users, sterilization checks, temperature chart records (kept with the BSO).

REGULATIONS FOR AUTOCLAVE USE

The autoclave is for decontamination purposes only.

Everyone assigned to The Lab must know the operating procedures of the autoclaves. These procedures are posted on the loading side of the autoclave.

All autoclavable material shall leave the laboratory in covered, sealed, and marked containers.

Appropriate materials leaving The Lab, whether to be reused or disposed of, must go through an autoclave decontamination cycle.

Personnel using the autoclave are to complete a sign-up sheet, providing name, laboratory, and extension before operating the autoclave.

The contact of steam with the infectious agent is essential for any decontamination procedure to work properly.

AUTOCLAVE PROCEDURES

The screen in the autoclave drain line at the base of the autoclave is to be removed and cleaned before each use.

At all times, materials must be placed directly into the autoclave and not left outside the autoclave.
Shallow autoclave pans are available for liquid waste containers and reusable items. Separate pans for each are recommended.

Uncover or loosen all tightly closed pans, bottles, and containers when autoclaving.

Double autoclave biohazard waste bags containing waste are to be securely closed individually, before leaving the laboratory to be autoclaved.

Sharps containers are to be covered, placed in double biohazard bags, and autoclaved as indicated for solid waste.

After placing a barrel in the autoclave, remove top to allow steam penetration. The top can also be autoclaved at the same time.

Barrels are to be relined with double autoclave bags before being returned to lab.

Liquid disinfectants are to be used only for interim soaking of items, before autoclaving. Under no condition does this constitute a final procedure.

The BSO will periodically check the efficiency of the autoclave by testing it with a biological indicator.

It is the responsibility of the laboratory personnel or investigator using the autoclave to, load his/her own material, remove it upon completion, and place the autoclaved waste in the red biohazard containers for removal by the custodial staff.
XXIII. LABORATORY HYGIENE:

Housekeeping is to be done daily by those authorized users assigned to the laboratory. Specific scheduling is to be established by the authorized laboratory personnel.

Room Cleanliness

Each investigator and authorized user is responsible for his assigned area. Each laboratory must be kept neat and clean. To help facilitate this, only limited amounts of material needed may be introduced into the facility. No cartons are permitted. Cleanliness and care within common spaces should be observed by everyone.

Biological Safety Cabinet Cleanliness

Particular attention to cleanliness and tidiness is necessary within the BSC, to permit convenient and uncontaminated laboratory manipulations. Equipment and materials should be limited to essential and current needs. Interior surfaces must be wiped down with Vesphene II se® or other disinfectant before and after using the cabinet. Attention to interior and exterior of the windows is necessary to maximize visibility of manipulations.

Tidiness

To prevent spills and to permit frequent cleaning, a minimum number of items should be kept on working surfaces.

Pest Control

An important part of housekeeping in biohazard containment is control of insects. The BSO will coordinate the establishment of this program.
XXIV. PROTOCOL FOR CUSTODIAL STAFF: (See lab cleaning policy attached)

Admittance of the custodial staff into The Lab is arranged with the BSO.

Authorized custodial personnel shall carry out duties in laboratory rooms before research activities have begun, and/or after research activities have ceased.

Appropriate protective clothing (i.e., laboratory coat and disposable gloves) will be provided and will only be worn in The Lab.

The custodial staff shall be responsible for floor, wall care and keeping the environment of the facility as dust-free as possible. All dusting will be achieved with a germicide-soaked cloth. This includes removing dust from the higher reaches of the lab, i.e., on tops of conduits, as well as window ledges, etc.

Only use treated dry-dust mop heads to suppress the aerosolization while cleaning.

Used wet mop heads are to be replaced as needed and are to be autoclaved before discarding. When autoclaving, the autoclave bag should not be tied to allow the steam to penetrate.
XXV. AUTHORIZED USER:

Requirements for BSL-2 personnel:

Complete medical requirements.

Study the Wesleyan University Biohazard Facility Safety and Operations Manual and other guidelines (Chemical Hygiene Plan etc) where applicable.

Receive training in the use of the facility’s containment equipment and proper techniques.

Demonstrate familiarity with facility procedures.

Sign the statement below. Review the Containment Facility’s Safety and Operations Manual semiannually and re-initial this statement.

Receive appropriate keys and locker assignment from BSO.
XXVI. CONTAINMENT FACILITY KEYS:

I understand that the Containment Facility keys issued to me are:

1. my responsibility
2. not, under any circumstances, to be shared, distributed, given on loan and/or reproduced.
3. to be returned to the Biosafety Officer when no longer needed by me.
4. If any Containment Facility keys issued to me are lost, I will notify the Biosafety Officer immediately.

I have read and understand the information contained in the EHS&S Biohazard Facility Safety and Operations Manual. I understand my responsibilities as an authorized user of the Facility.

_____________________________  ______________________________
Date                         Signature

_____________________________  ______________________________
Date                         Biosafety Officer

_____________________________
Date                         Principal Investigator or Designee
XXVII. AUTOCLAVE (Room 208 Hall-Atwater)

OPERATING INSTRUCTIONS FOR AUTOCLAVE (STEAM CYCLE)

1. COMPLETE SIGN-UP SHEET

2. REMOVE DEBRIS FROM CHAMBER AS NEEDED

   **CHECK STRAINER - CLEAN BEFORE EACH USE**

3. MASTER POWER SWITCH - "ON".

4. CHECK JACKET PRESSURE GAUGE: IT SHOULD READ 15 TO 20 POUNDS.

5. LIQUID CYCLE BUTTON SHOULD BE DEPRESSED.

6. PLACE CONTAMINATED MATERIALS IN CHAMBER.

7. CHECK TIMER - 60 MINUTES.

8. LOCK DOOR, RECHECK JACKET PRESSURE.

9. PRESS "CYCLE START" BUTTON.

STERILIZER THEN GOES THROUGH SEQUENCE AUTOMATICALLY.

BUZZER WILL SOUND ON CLEAN SIDE WHEN CYCLE IS COMPLETE.

10. OPEN DOOR AND REMOVE MATERIAL.

**AUTOCLAVE IS NOW READY FOR ANOTHER CYCLE.**

DO NOT USE DRY CYCLE.

DO NOT PRESS THE "CYCLE OFF" BUTTON.
DO NOT LEAVE DOOR ON CONTAMINATED SIDE LOCKED WHEN NOT IN USE.

**ANY QUESTIONS OR PROBLEMS, CALL X2771**

*This step is critical. If strainer is clogged, the steam cannot be exhausted from the autoclave.*
### XXVIII. GENERIC DISINFECTANT CHART:

**ASSESSMENT OF GENERIC DISINFECTANTS ON BASIS OF EFFICACY**

<table>
<thead>
<tr>
<th>DISINFECTANT</th>
<th>Bactericidal Activity</th>
<th>Tuberculocidal Activity</th>
<th>Pseudomonal Activity</th>
<th>Sporicidal Activity</th>
<th>Virucidal Activity</th>
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<td>good</td>
<td>moderate</td>
<td>good</td>
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