

Exposure Control Plan for: *Pseudomonas aeruginosa*

Agent Risk Group: Risk Group 2

Containment Level: BSL 2

General Characteristics

Motile gram-negative aerobic bacteria, 2 – 4 µm long plump-shaped rods, with polar flagella which have an important role in pathogenicity. They are non-spore forming and can produce pigments, such as pyocyanine (green-blue) and pyorubrin (yellow-green) fluorescence. *P. aeruginosa* can produce a large variety of extracellular toxins, including exotoxin A and enterotoxins. Other substances such as hydrocyanic acid, proteolytic enzymes, toxic surface slime, and haemolytic substances may also contribute to the pathogenicity of this species. Toxins combined with harmful substances are determinant factors in the high virulence of *P. aeruginosa* in a variety of different hosts.

Hazards

Contaminated lab equipment. Transportation of samples. Interactions with infected personnel or animals.

Modes of Transmission

P. aeruginosa have been found to survive within droplet nuclei and can remain in aerosols for long periods of time, thus there is evidence of potential airborne transmission. Contact with contaminated water is also a major route, but since the oral infectious dose is thought to be very high, routes that pose the greatest health risk are skin exposure (for example, in contaminated hot tub water) and lung exposure from inhaling aerosols discharged from infected respiratory tracts. The bacterial can often enter the body through injuries and wounds. The use of contaminated mechanical respiratory ventilators in hospital settings is also a common source of nosocomial infections.

Engineering Controls

Currently certified biosafety cabinet, safety-enabled centrifuges, biohazard sharps container. Biohazard Sharps Containers shall be available to dispose of sharps waste, including broken glass, needles, blades, etc. An in-line HEPA filter must be used for vacuum aspiration of spent media.

Administrative/ Work Practice Controls

- Access to the lab shall be restricted while work is in progress, doors shall remain closed during experimentation
- A sign incorporating the universal biohazard symbol shall be posted at the entrance of the laboratory or tissue culture room where agent is used (see last page)
- All lab personnel must be informed of the hazards of agent
- All lab personnel must be trained in proper handling, use, and disposal prior to working agent
- All students must be directly supervised at all times while handling agent

- All lab personnel are advised to avoid rubbing eyes as a precautionary measure against eye infections
- All lab personnel will remove lab coat, discard gloves, and wash hands before exiting the lab

Personal Protective Equipment (PPE)

Lab coat shall be worn while working in the lab

Safety glasses or goggles shall be worn when handling agent

Disposable gloves shall be worn while working in the lab

Disinfection

Benchtops and non-disposable equipment shall be disinfected with 10% bleach or other approved disinfectant. The UWM BMS labs commonly use germicidal disposable wipes for disinfection of bench tops and equipment. Allow disinfectant to remain wet on surface for 10 minutes and dry with paper towel.

Disposal

Disposable items that have been contaminated will be disposed of as biohazardous waste. Non-sharps shall be disposed of in biohazardous bag, while any sharps waste (including glass slides) shall be disposed of in biohazardous sharps box.

Recombinant DNA and Biohazardous Spill Guidelines for BSL-1 and BSL-2 Research and Teaching Laboratories at UWM

The University of Wisconsin-Milwaukee, as a receiving grant institution from the National Institutes of Health (NIH) is required to adhere to the NIH Guidelines for Research Involving Recombinant and Synthetic Nucleic Acid Molecules ([NIH Guidelines, April 2016](#)). Part of the compliance is to maintain a set of spill guidelines for researchers and instructional staff to follow in the event of biohazardous spill or exposure event. All spills/ exposures involving rDNA must be reported to the NIH Office of Science Policy (OSP). The implementation, communication, and training of these guidelines is the responsibility of the principle investigators, deans, directors, lead course teaching faculty, or lead clinical laboratory managers overseeing personnel in a laboratory setting. They are also responsible for ensuring a biohazardous spill kit is available for such events and properly maintain their spill kits, or to designate a responsible person to oversee this task.

BMS labs spill procedures can be found at <https://uwm.edu/bms-labs/spill-response-and-exposures/>

Injury Reporting

BMS Labs injury reporting procedures can be found at <https://uwm.edu/bms-labs/accident-incident-and-injury-reporting/>

If an incident involved an employee or student being injured, the following forms must be completed and submitted within 24 hours of the incident if possible.

- ☠ Supervisor must complete the following forms:
 - [Employer's First Report of Injury or Disease](#)
 - [Supervisor's Accident Analysis and Prevention Report and Evaluation of Repetitive Motion and/or Materials Handling Activities](#)
- ☠ Employee must complete:
 - [Employee's Work Injury and Illness Report](#)
- ☠ If injured party is not an employee, but is a student, student must complete:
 - [General Incident Report 300A2 for student injuries or incidents](#)

Biohazardous spills and accidental release events must be reported promptly. Spills can create additional risks to surrounding personnel, the public, and/ or the environment. Spill events and accidental release incidents can initially be reported via: <https://uwm.edu/safety-health/first-report-of-biological-exposure-or-release-event/>. If there is a large spill/ event, contact the biosafety program office at (414) 588-4261 or uwm-biosafety@uwm.edu to assist with clean-up.

Incident Reporting

PIs are asked to consider what the consequences of exposure the biological hazards they are working with may have and have a developed response procedure for this potential exposure on file with their protocol and/ or registration form. Complete the First Report of Biological Exposure or Release Event Form online. Information that should be kept on file in case of accidental exposure should include the following (UW Biosafety, 2017):

- A description of the pathogen(s), including signs and symptoms of an infection from this pathogen.
- Distinct characteristics of the strain(s) used in the laboratory, including antibiotic resistance, transmissibility, atypical tissue tropism, foreign genes that alter pathogenicity, etc.
- Recommendations for treatment, including effective medications, quarantine, etc.
- A detailed record of a history of exposure to the agent(s) in question for some pathogens from start of employment (work with BSO to determine need for this)
- Completion of an accidental exposure/ release form within 24 hours of the incident, submitted to the BSO. This form must be used when any of the following occur:
 - Potential exposures or releases of organisms or biological toxins on the UWM campus and UWM off-site facilities.
 - Reporting must be completed within 24 hours of the event and is the responsibility of the Principal Investigator to report the event.
 - Potential exposures include needle sticks, animal bites, aerosol exposures, and other incidents potentially resulting in disease.

- Potential releases include spills outside of primary containment as well as potential releases to the environment.
- Unauthorized releases of transgenic animals or plants should also be reported on this form.
- After completing this form, select “Submit” at the bottom of this form. The information on this form will be sent to designated individuals at the UWM Biological Safety Program.
- Information on this form is used to determine how our offices may help you and your laboratory and for mandatory federal reporting purposes.
- The submitter will be contacted for incident follow-up.
- If you need assistance completing this form or reporting an incident, please call the BSO at 414-588-4261.

Spill Outside of Containment

- If a spill happens outside of containment (such as a biosafety cabinet or BioBubble), aerosol generation is a possibility, as well as other exposure risks to the personnel in the laboratory. If a spill happens outside of containment, the following are the guidelines:
- **ALERT** co-workers and **EVACUATE** lab area if in a BSL-2 or higher laboratory. If it is an exposure incident, wash exposed area or flush mucous membranes with copious amounts of water. Post a **DO NOT ENTER** sign on door.
- **ASSESS** personnel, if medical attention is needed contact 9911 if on a campus phone or (414) 229-9911 if using cell or off campus phone.
- **REMOVE AND DECONTAMINATE** all contaminated garments- they must be autoclaved prior to washing.
- **WASH HANDS:** Even if you were not directly exposed, wash hands after removing PPE.
- **CONTACT** the Biosafety Program and Environmental Protection. If necessary, call 9911 for campus police and emergency personnel from a campus phone or 911 from a cellular or other non-campus phone.
- **WAIT 30 MINUTES:** Wait at least 30 minutes before re-entry to allow aerosols to dissipate.
- **DON PPE:** Wear PPE upon re-entry (disposable gown, mask/eye protection, double gloves). Only trained personnel should clean up the spill.
- **DISINFECT:** Cover spill with disinfectant soaked paper towels and pour an appropriate disinfectant solution around spill (1:10 dilution of bleach). Take care not to create aerosols while pouring. Let stand for at least 20 minutes.
- **REMOVE BROKEN GLASS:** Use tools (tongs, broom & dustpan) rather than hands to pick up sharps and broken glass, and contaminated materials whenever possible.
- **DECONTAMINATE BIOHAZARDS:** Transfer all contaminated materials into an autoclave bag for decontamination.
- **FINAL DISINFECTION:** Wipe down entire area with disinfectant (including autoclave bags, disinfectant container and other contact surfaces).
- **DOFF PPE:** Remove and discard PPE (autoclave prior to disposal).

- **DECONTAMINATE SELF:** Shower or wash hands with soap/antimicrobial agent and water.
- **DECONTAMINATE WASTE:** Autoclave all contaminated materials.
- **REPORT INCIDENT:** Report incident to the PI (if not already contacted) and the BSO (Biological Safety Officer) using the online [First Report of Biological Exposure or Release](#) form (on UWM Biosafety site) within 24 hours.

Spill Inside Containment (i.e. Biological Safety Cabinet, BIObubble, enclosed centrifuge, etc.)

When a spill occurs inside of containment, there is a lower hazard to the personnel present in the lab space. However, the proper disinfection and decontamination of the containment area is necessary to ensure that personnel are not exposed after the event and to maintain future function of the containment engineering controls.

A properly functioning and up-to-date BSC should contain potentially hazardous biological aerosols from spills within its unit on its own. Therefore, it is extremely important to have your biosafety cabinet checked annually. It is the responsibility of the PI to have a well-developed SOP in place for operation and cleanup of a BSC, as well as spill procedures, which are required in any approved research protocol. The following are general procedures:

- ☠ **STOP WORK:** Immediately stop all work but leave BSC or hood blower fan on during clean-up.
- ☠ **COMMUNICATE SPILL:** Notify other personnel in lab of spill in containment.
- ☠ **DON PPE:** Wear PPE (gloves, lab coat, eye protection).
- ☠ **FLOOD SPILL WITH DISINFECTANT:** Cover spill with disinfectant soaked paper towels and pour an appropriate disinfectant solution around spill.
- ☠ **WIPE DOWN SURFACES:** Using paper towels and appropriate disinfectant detergent, wipe down walls, work surfaces and equipment.
- ☠ **DECONTAMINATE DRAIN PAN:** Flood work surface and drain pan (Type II BSC) with disinfectant and let stand for at least 20 minutes. Wipe up all excess disinfectant and empty drain pan into a collection vessel with disinfectant. Flush drain pan with water and remove drain tube.
- ☠ **DECONTAMINATE BIOHAZARDS:** Transfer all contaminated materials into an autoclave bag for decontamination. Wipe down autoclave bag, disinfectant container and other contact surfaces with disinfectant.
- ☠ **REMOVE AND DISCARD PPE:** Remove PPE (autoclave prior to disposal) and place in biohazard bag.
- ☠ **WASH HANDS:** Wash hands thoroughly with soap/ antimicrobial agent.
- ☠ **REPORT INCIDENT:** Report incident to the PI (if not already contacted) and the BSO (Biological Safety Officer) using the online [First Report of Biological Exposure or Release](#) form (on UWM Biosafety site) within 24 hours.

Recommended Clean-Up Materials for Lab Facilities

The following should be kept in the laboratory, and all personnel should be trained in where it is housed, how to use it, and provide the plan in place for accidental spills.

- ☠ **Disinfectants:** Selection should be made based on the biological agent(s) it would be used against (See Table 4). If dilutions are made, such as with bleach, fresh solutions should be made on a schedule depending on the materials used and the manufacturer's recommendations.
 - **Recommended:** Highlight™ by Kinnos, which turns fresh bleach solutions blue, which can be sprayed on surfaces, and then changes color when effectively decontaminated.
- ☠ **Absorbent materials:** There should be, at a minimum, a sufficient quantity of paper towels on hand to soak up the maximum volumes handled in the laboratory. There are other absorbent pads available, but paper towel will suffice.
- ☠ **Extra PPE:** This is dependent upon the biological agent, but when handling a spill, a gown, gloves, and eye protection should always be worn to prevent additional accidental exposure from occurring. Respiratory protection should be available.
- ☠ **Signage:** Signage available for posting until aerosols have settled after a spill.
- ☠ **Autoclavable Bags:** To autoclave contaminated materials after clean-up.
- ☠ **Sharps container:** To collect any sharps in the spill incident.
- ☠ **Biohazard stickers:** To label contaminated materials as biohazardous.

Exposure Response

Vaccine Recommendation: There is no vaccine for *Pseudomonas aeruginosa*. Practicing good personal hygiene including frequent hand washing and staying up-to-date on recommended vaccinations including yearly flu vaccine can lower your risk of contracting an infection.

At-Risk Personnel: Anyone handling *Pseudomonas aeruginosa*, or encountering a carrier are at risk for contracting *Pseudomonas aeruginosa*. Immunocompromised hosts are at increased risk.

Incubation Period: 24-72 hrs

Signs/ Symptoms: As opportunistic pathogens, *Pseudomonas* spp. often invades the host tissue and cause infection and bacteremia in immunocompromised hosts (e.g., HIV/AIDS, cystic fibrosis, bronchiectasis, and severe chronic obstructive pulmonary disease, burns, malignancy, or diabetes mellitus). The common site of infection is the lower respiratory tract, and severity ranges from colonization without immunological response to severe necrotizing bronchopneumonia;.

Treatment/ Post-Exposure: If exposure occurs or there is a development of any of the above signs and symptoms while experimenting with *Pseudomonas aeruginosa* or while caring for persons infected with *Pseudomonas aeruginosa* seek medical attention. Bring this sheet and your biosafety protocol with you.

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Training Signature Page

This exposure control plan has been reviewed with me. I understand the risks associated with working with this agent, and understand it is my responsibility to follow the approved protocol and procedures to minimize risk to myself, my colleagues, and the surrounding UWM community.

Name	Signature	Date Reviewed (day/month/year)

Office Use Only

Date of Biosafety Review/ Approval:

Biosafety Officer Signature: